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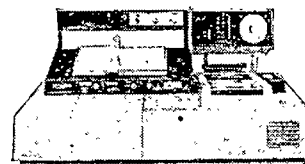
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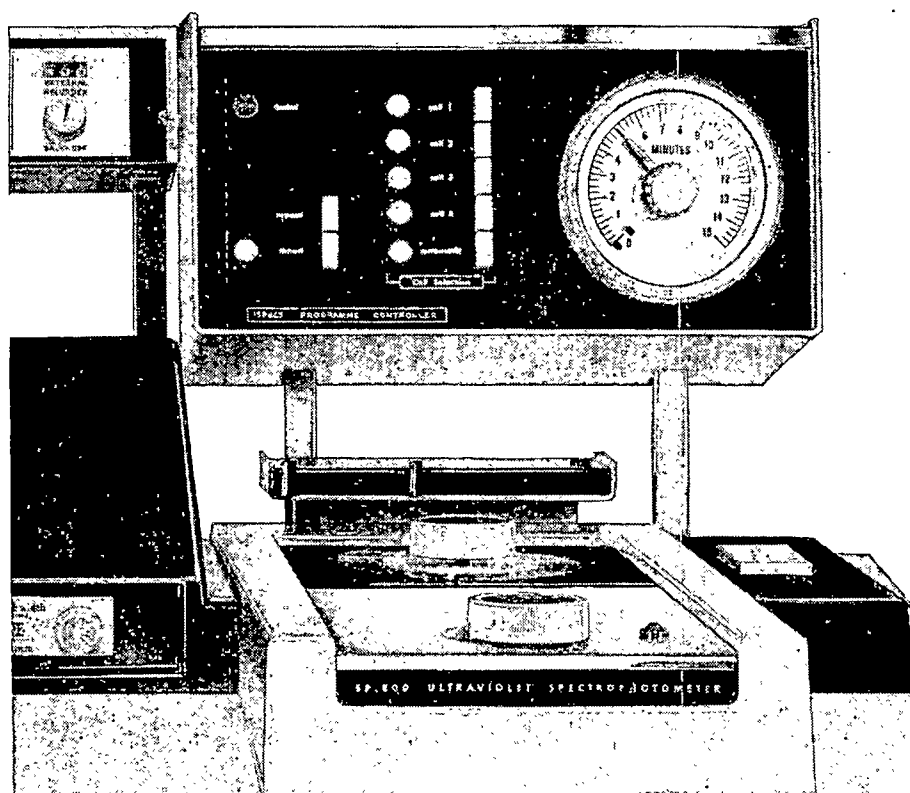
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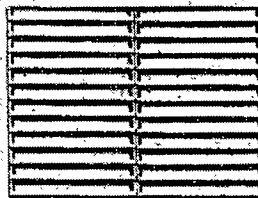
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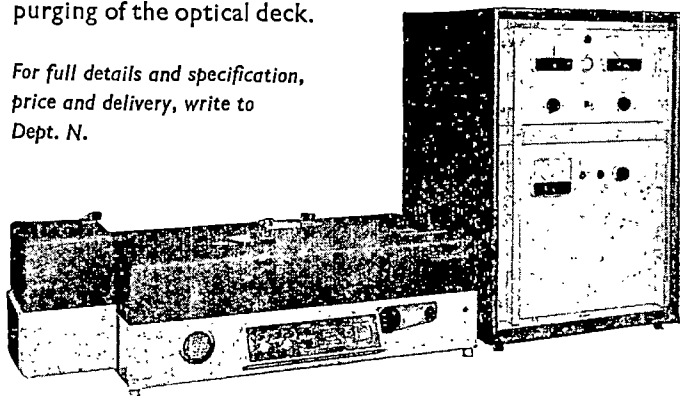


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## TELEVISION AS A SOCIAL INFLUENCE

THE Television Research Committee was appointed by the Home Secretary in July 1963, to initiate and co-ordinate research into the part which television plays, or could play, as a medium of communication and in fostering attitudes. The Committee was to investigate particularly the ways in which young people's moral concepts and attitudes develop, and the processes of perception through which they are influenced by television, in relation to other media of communication. It was also instructed to administer any funds which may be made available for such research, to select and clarify any relevant issues which might prove amenable to scientific enquiry, and to commission and co-ordinate the appropriate research projects. Research of this type is necessarily long-term and is a comparatively new development, and there are few experienced practitioners in Britain.

It will, accordingly, be some years before results of any new research projects are ready for publication, but in the meantime working papers aimed at assisting the Committee to clarify its own thinking will be published from time to time, which it is hoped will also encourage public discussion on the influence of television. The first of these, a survey by Mr. J. D. Halloran, the Secretary of the Committee, breaks no new ground but presents an informed summary of the effects of mass communication, particularly television, as they have appeared to the leading research workers in this field\*. It is limited essentially to source material and deliberately excludes studies on the use of television in formal education, the effects of advertising, or the use of television in propaganda and in psychological warfare. Mr. Halloran's main aims are threefold. First, to indicate the kind of knowledge about the effect of television on children which is empirical, verifiable, and which has been gathered and analysed systematically, and which may on occasion permit a generalization to be made. Secondly, to assist in mapping the problem areas. Thirdly, to provide an introduction to some of the theoretical frameworks in use and direct attention to some of the difficulties involved in the work. Besides seeing what generalizations, if any, can be made, he also endeavours to indicate some of the gaps in the research material, some of the areas not covered, and some questions which might be posed. Roughly half the booklet comprises appendixes dealing with the wider debate on mass media and mass culture, describing the work of the Committee for Research on Television and Children of the United States Department of Health, Education and Welfare, including the areas and topics suggested for research, and outlining the techniques and strategies for research into television and human behaviour proposed by the Television Bureau of Advertising in the United States.

These appendixes will give the general reader, as well as the specialist, some idea of the variety of approach which is being adopted, but the review itself is of some interest as a corrective of many loose assertions at present

made about the influence of television. It reminds us, for example, that the study by Dr. Trenaman and his colleagues of the Television Research Unit at the University of Leeds did not show that television had any major effect on the political life of the people during a three-week election campaign. We have, however, still to consider the impact over the years, and such evidence as is available suggests that influence is likely to be at its maximum when we are not aware of being 'got at' and when the context is one of which we generally approve.

Nevertheless, while the effects of communications can be many and diverse, operating at different levels and in different strengths, even when allowance is made for the part played by various mediating factors and the imperfection of the evidence, it would be wrong to assume that these effects are wholly good. All the evidence so far provided by detailed researches suggests that values are acquired, that a view of life is picked up by children watching television, and there are good theoretical grounds, largely supported by experimental and clinical evidence, for supposing that moral standards could be affected by television. Nor can the finding that children learn to like the programmes available to them, which ordinarily they would not select, be regarded without concern by those who consider that many programmes leave much to be desired.

The evidence seems fairly clear that television does not stimulate interest or broaden horizons to any greater degree than would happen in its absence. Another conclusion that deserves noting is that the viewer is more selective the older he is and the higher his cultural and educational level, and even when the positive correlation between parental and child viewing is discounted it still appears that the abler the child, the less he or she views. Several studies show that the heavy use of escapist material is associated with tendencies to anxiety, social maladjustment and frustration. However, while in consequence we can accept that this form of media content does not appear to be a prime cause of any particular way of life, Mr. Halloran reminds us that it does serve the psychological needs and reinforce the ways of life already characteristic of its viewers—the results of which are not always socially desirable or beneficial.

So far as passivity and dependence are concerned, there appears to be no evidence that television makes children passive and there is little to make us believe that violent programmes on television reduce the likelihood of violence in real life. Very little delinquency can be traced directly to the medium, but there are a number of situations in which violence might be encouraged and, although it would seem evident that heavy exposure to television is not a sufficient or crucial cause of delinquency, some work suggests that criminals are heavy users. On the whole the weight of evidence is behind the conclusion that the heavy dosage of violence in the mass media, while not a major determinant of crime or delinquency, heightens the probability that someone in the audience will act aggressively in a later situation. Here Mr. Halloran emphasizes the need for more research, and research which is not narrowly oriented. The role of mass media in

\* *The Effects of Mass Communication with Special Reference to Television: a Survey.* By J. D. Halloran. (Television Research Committee—Working Paper, No. 1.) Pp. 83. (Leicester: The University Press, 1964.) 7s. 6d. net.

this process of delinquency is undoubtedly important but it is only one aspect of the total situation.

Mr. Halloran stresses the importance of viewing the mass media of communication as functioning within the larger sociological perspective of culture, social structure and organization, and social groups. The socialization process is continuous, and is located in people and institutions, and it can be both deliberate and inadvertent. Neither its contents nor its methods are immune to the influence of mass communication, and while this represents only one aspect of the process it would be very surprising if it did not play some part in shaping our attitudes towards life, towards ourselves and towards others.

At present, conclusive evidence about the effects of mass communication is inadequate. We need to know much more about its content and the patterns of consumption and, Mr. Halloran adds, about the effect of acquiring social norms impersonally through the mass media as distinct from acquiring them through the primary groups. We need to know whether, or to what extent, the quality of the norms is changed in this process of transmission by the mass agencies; what degree of standardization is developing and what is the overall influence on the mechanisms of social control. Clearly, as he says, there are here many tasks for the research worker.

In the latter part of his survey Dr. Halloran is concerned to indicate some of the most promising lines for investigation, some at least of which are important in other connexions. He refers, for example, to the need to integrate research on mass communication into general research on child development. Communication, he reiterates, is not a one-way process, and there are consequences for group structures which should lead to the study of the larger social structure which transcends both the individual and the group. So far as research into mass communication is concerned, he suggests that it would be useful if

attempts could be made to compare the influence on message reception exerted by the primary groups on one hand, and by the larger social structure on the other, and to extend these attempts to cover different societies, sub-cultures and periods. Nor should it be forgotten that the communicator is also located within the structure of social relations and that his operations are influenced by that structural context.

Mr. Halloran thus presents the study of television not only as part of the study of mass communication but also as an integral part of the social system. It is in accord with this that, in the competition fostered in 1960-61 by the Television Bureau of Advertising in the United States, in which the awards were given for research strategies or plans, the highest award went to a programme of research into the television viewing, the norm-violating practices and the perspectives of adolescents, while the second award went to one on the application of scientific television methods to the analysis of perceptual feed-back in behaviour. While the gaps in our knowledge stressed in this first paper from the Television Research Committee fully justify the placing of generous resources at the disposal of the Committee, it is equally clear that such research should be carefully co-ordinated with social research in general. Whether in fact the Television Research Committee is appropriately placed under the Home Secretary is a matter for consideration. It would certainly appear difficult to justify a responsibility to any other Ministry than that responsible for social research in general, and, in view of the educational implications, the interrelations with educational research may call for reconsideration. Nevertheless, on the evidence of this paper alone the new Postmaster-General will be ill-advised to commit the Government too far in further extension of wave-lengths to independent television in advance of the findings of current and projected research of the type outlined in this paper.

## QUANTITY AND QUALITY IN SCIENCE AND TECHNOLOGY

IN a maiden speech on November 6 during the debate in the House of Commons on the Address to the Throne, Mr. N. Atkinson referred to the shortage of engineers and to Britain's reluctance to recognize that, in promoting technological change, engineers came first, technologists second and scientists third. Unless we could persuade more of our ablest young people to take up a career in engineering we would not have the supply of engineers needed to execute such a programme as that contemplated in the Queen's speech. This plea is also found in the Presidential address, "The Shape of Things to Come: a Challenge to Engineers", which Vice-Admiral Sir Frank Mason delivered to the Institution of Mechanical Engineers on October 28, in which he analysed at greater length the trends in secondary schools elucidated in 1962 by the Education Department of the University of Oxford in a report, *Technology and the Sixth Form Boy* (*Nature*, 199, 958; 200, 219; 1963).

Prof. I. D. Rattee has also expressed concern at those tendencies in a recent inaugural lecture, but from a different point of view (*Nature*, 204, 1017; 1964). Sir Frank Mason was concerned with the fact that the schoolboy, his masters and his parents do not think that

engineering is a worth-while occupation. In consequence, not only are we producing twice as many scientists as engineers instead of the reverse proportion which we need, but also the quality of entrants to engineering falls short of that of science students and of what is required. Admiral Mason makes some trenchant but justified comments on the situation. "Secondary Schools are ceasing to be places of education", and while he sees no hope of a rapid change, he recognizes that it is within the power of the universities, the entrance requirements of which have been an important factor, to rescue us from this ominous situation. Apart from this, it will be largely a matter of education, in which the engineer and the scientist can play an important part, if they make full use of their personal contacts.

Here Sir Frank touches one of the dominant notes of his address—professional responsibility. Only one passage in his address was concerned with the shortage of engineering students, but the note of professional responsibility struck in that passage recurs elsewhere. This is notable in his remarks on the dangers arising from university expansion, on the complementary roles of the engineer and the scientist and on the way in which the

engineering profession is meeting the challenge of to-day. Like Prof. Rattee, Sir Frank Mason is particularly anxious about the dangers of lowering standards in the unprecedented expansion of universities, particularly through the absence of sufficient teachers of the necessary calibre. He advocates not simply a critical appraisal of curricula or of teaching methods but of the methods of selection, and pointedly emphasizes the necessity of assessing the candidate's potential contribution to society. He regards with something more than disfavour the likelihood that possession of a degree will become an essential passport to every kind of job with any prospects—it would effect a disservice to both society and individual.

The warning is one we disregard at our peril. It is supported by Sir Gordon Cox's equally forthright insistence that a major problem of education is how to diminish the number of arrogant students who think that the world owes them a living beyond their real merits. To accelerate university expansion while retaining flexibility, and sufficient respect for individuals, and to pursue the quest for equality to the extent of denying opportunity to the gifted and embracing instead the mediocre and second-rate is the negation of responsibility and ultimately must be fatal to our economic development. Again, Sir Frank has the courage to question whether we are pitching our requirements for mathematics so high that we are losing many who would make good engineers; he gives a timely reminder of the importance and value of the sandwich system both to the universities and colleges of technology and to industry. He hopes that this problem will receive attention from the Industrial Training Boards and that care will be taken to prevent technical education in general from becoming too academic and theoretical.

Apart from this note of responsibility, Sir Frank's address derives much of its value from its balance and sweep. He recognizes the importance of the technicians, and while he has high hopes of the Council of Engineering Institutions he reminds the Council of its responsibility towards the technician. His suggestion here could well help the Council to overcome some of the difficulties into which it has run through the proliferation of organizations, and the problem of distinguishing between a professional and a lower qualification—a point emphasized by Mr. H. G. Conway in his presidential address to Section G of the British Association for the Advancement of Science at Southampton (*Nature*, 203, 951; 1964). The differentiation between technologist and technician is not always easy; there is a clear account of the distinctions made, and the balance maintained in the Royal Navy is apposite. Certainly, unless more attention is directed to this problem by industry and by the professional institutions there is real danger, as Sir Frank remarks, that in a few years we shall find that we have graduates doing work which really should be done by technicians: this situation would arise simply because we would have trained too many engineers and technologists and not enough technicians.

That is another aspect of the expansion of higher education which calls for much more attention than it has yet received. Elsewhere in his address Sir Frank Mason refers to the complementary roles of the engineer and the scientist, but from a different point of view from that of Prof. Rattee. Both, however, recognize the importance of research and particularly of securing the application of the results of research. Above all, however, Sir Frank recognizes the extent to which team-work is involved

and the necessity of training engineers who are not only professionally competent but are also capable of co-operating harmoniously and effectively with the accountant, the chemist, the metallurgist, the physicist, etc. He does not regard engineering as incapable of providing men with a broad outlook though he recognizes the need for some postgraduate study in management and welcomes the proposals to establish two new business schools.

Another interesting feature of Sir Frank Mason's address is the extent to which many of his remarks on, for example, the importance and functions of the Council of Engineering Institutions, the importance of engineering in our economy, or the relation between design and manufacture, anticipated those of Mr. R. M. Wynne-Edwards in his presidential address to the Institution of Civil Engineers the following week. The note of responsibility is sounded just as clearly in that latter address, even if Admiral Mason's final emphasis on the need for integrity as well as creative insight is much the more explicit. It is this interpretation of what is involved in responsibility and the idea of service that gives distinctiveness to the whole address. Sir Frank does not hesitate to affirm either his belief that man is a trinity or to point out where he believes man can renew his spiritual strength.

At this point Sir Frank's address links with the challenging address which Sir Gordon Cox delivered to the School of Pharmacy on October 14. Sir Gordon was discussing personal responsibility and technocracy and expressed some thoughts on the preparation of scientists for their responsibilities in a science-dependent society. Speaking in the context of the doubling every four years since 1945 of Government expenditure on civil science—an increase from about £7 million to about £170 million a year—and questioning whether young scientists appreciated the significance of these figures, as already noted, he felt there were too many who thought the world owed them a living beyond their deserts. Another major problem of education was how to diminish the number of those who lacked the faith to make full use of the talents they possessed. He believed that success in either direction would be attained rather through personal influence than through formal education.

This note of professional responsibility towards younger colleagues is the keynote of Sir Gordon's address and supplies its wider interest. With it he links the personal responsibility of every scientist to use his interest in spreading a real understanding of science among the population at large. Such a general understanding Sir Gordon sees as an essential condition if science and the scientist are to enjoy public confidence and support and the freedom which is essential to scientific advance and ultimately to technological advance as well. It is at this point that he emphasizes the value of the sciences, properly taught, as an educational medium, just as he stresses the need for the general public to understand the limitations as well as the nature of scientific method and the distinction between scientific and political decisions.

This is perhaps particularly important for informed public discussion on the organization of science and government where changes such as those now proposed for Britain are being made. Sir Gordon Cox does not overlook the vital importance of the gifted scientist. Scientific advance, notwithstanding all the help effective large-scale organization can give, depends above all on the creative and non-conformist individual. Organization and planning must strive to provide the conditions in which these few exceptionally gifted people can flourish and use their



creative powers most fruitfully as well as to ensure that they and their most fruitful fields of work will be identified at an early stage. Apart from this, Sir Gordon does not believe that educationally we need be too concerned with this group of exceptionally gifted people. The main task is so to organize our research and educational organization as to deserve success.

This is first a matter of attending to professional competence, and in this connexion too he emphasizes the influence which senior scientists can exercise on their younger colleagues. In this same context he is forthright about the importance of a command of good English. He has no truck with slovenly and imprecise writing, that goes with slovenly and imprecise thought. He has no doubt that new ideas are more likely to come if the old ones are formulated in precise language. That is a part of the problem of communication and its importance cannot be overstressed. Until the scientist accepts unhesitatingly that a command of good English is an essential part of his technical competence he is unlikely to be effective however conscientiously he addresses himself to the task of spreading a real understanding of science in the community. Moreover, the ease with which the results of his own scientific work are likely to be fully appreciated in industry or elsewhere depends on his eloquence.

If the challenge of all these addresses is more especially to the professional associations of scientists, engineers and other technologists and to their individual members, all three addresses point to dangers in the expansion of higher education which it would be most irresponsible to disregard. Preoccupation with numbers to the neglect of quality could thwart the whole purpose of that expansion. The Robbins Report is very far from having eliminated the need for hard thinking before even the scale of university expansion is finally determined. Even from the point of view of our economic growth, quality comes first, while from the point of view of the individual, failure to match the plans for expansion of scientific and technological education with plans to ensure provision of the requisite supply of supporting technicians and craftsmen could be as wasteful and untoward as the attempt to induce into an academic career in science or technology those whose aptitudes and desires lie elsewhere. Large institutions are not the complete answer and are certainly no insurance against frustration and disgruntlement if the broad policy is unsound and lacking in vision.

## INHERITANCE, CHROMOSOMAL AND OTHERWISE

### The Mechanics of Inheritance

By Franklin W. Stahl. Pp. xiii+171. (Englewood Cliffs, New Jersey, and London: Prentice-Hall Inc., 1964.) 21s.

### Extrachromosomal Inheritance

By John L. Jinks. Pp. xiv+177. (Englewood Cliffs, New Jersey, and London: Prentice-Hall, Inc., 1964.) 21s.

### The Cytoplasm in Heredity

By D. Wilkie. Pp. vii+115. (London: Methuen and Co., Ltd., and New York: John Wiley and Son, 1964.) 16s.

THESE are three very readable publications. That by Stahl is the exciting, concise, logical and arrogant, yet slightly insecure, product which we have come to expect when molecular biologists of high calibre start

from their immediate speciality and go beyond it. This one, in addition, is full of both very witty and feeble jokes. It is a very clear presentation of the physical basis of inheritance in bacteria and bacteriophages from the angle of the molecular biologist who works with them. The discontinuity in biological organization, that is, the dividing line between 'higher' organisms and others, is taken to be the presence of a "nucleus clearly differentiated from the cytoplasm". If by this is meant the presence of a nuclear membrane, I would certainly agree with this view, even though on its basis *Chlamydomonas* and yeast become 'higher' organisms. The book is at its weakest where—as in chapter 7, "Recombination in Higher Organisms"—it deals with processes of heredity in organisms other than phages and bacteria.

The tacit thesis is that, to learn the genetics of higher organisms, one requires the sort of epic effort which produced classical genetics: why not learn the easy way by applying to higher organisms the understanding gained from bacteria and bacteriophages? Of course, the answer is that microbial genetics is by no means simpler than that of higher organisms. In the analysis of crosses in higher organisms one seldom encounters, for example, as didactically intractable a system as that of  $F^+ \times F^-$  in *E. coli* or a situation as complex as that of an ordinary cross with phage. How would Mendel have fared if he had had to analyse a cross only after an unknown number of generations?

The genetics of bacteria and bacteriophages is simple if one already knows the principles from higher organisms. It contributes decisively, not in providing more easily the principles, but in giving a chemical basis to them. All told, this book is entertaining and useful. It is also recommendable for teaching, but only to sophisticated students.

The other two books, by Jinks and Wilkie, respectively, also make useful reading and can be used for teaching at an advanced level. The rather woolly treatment is the inevitable reflexion of the state of a subject—cytoplasmic inheritance—which has not yet had its Mendel let alone its Watson and Crick. Both books, and especially Wilkie's, are humble and they spare us feeble cracks, much as we may regret the price of not having any good ones.

Wilkie's book provides an effective summary of a small number of well-chosen examples of inheritance which are not interpretable in chromosomal terms. As to interpretations, his approach is cautious: he calls in reluctantly extrachromosomal entities endowed with genetic continuity only when every other known process appears inadequate.

In Jinks's book, in which the examples are drawn from a broader field, the mood is somewhat in the reverse direction. Yet, Jinks is careful in listing, and discussing the validity of, the criteria which should be satisfied before we accept extrachromosomal genetic structures as underlying any given case of heredity not explicable in chromosomal terms.

These three books are utterly different in outlook: Stahl is sold 100 per cent to the molecular biology of the year when he wrote; Jinks is 100 per cent a classical geneticist and steers clear of anything with a biochemical flavour; Wilkie takes an intermediate stand. Nevertheless, all three use terms such as 'genetic material', 'genetic continuity' and similar, which were indispensable in classical genetics but useless now.

We have come to recognize two quite different ways in which cell constituents are made. One involves step-by-step processes, with each step catalysed by a specific enzyme and almost no qualitative latitude in what the product of each step can be. The other involves the arrangement of low molecular weight substances of a small number of kinds—say four nucleotides or twenty amino-acids—into a precise sequence out of the myriads possible. The selected sequence is determined by a pre-existing sequence. The pre-existing and the new

sequences can be made of identical materials—as in the replication of DNA; or of different materials—as in the ‘translation’ of RNA into protein. For the former case the expression available is “template mode of replication”.

Cell division, and therefore ultimately heredity, involves stepwise as well as template processes, and among the latter replicatory as well as translationary processes. What we need from now onwards is an analysis of heredity—chromosomal or otherwise—in terms of these three types of processes, at least until they are the only ones known.

G. PONTECORVO

## A FARRAGO OF BIOLOGY

### The Evolution of Biology

By M. J. Sirks and Conway Zirkle. Pp. v+376. (New York: The Ronald Press Company, 1964.) 6 dollars.

“THE title of this book”, we read in the preface, “was chosen deliberately. It was never the intention of the authors to write a detailed history of biology. The book has now become a survey of the evolution of biology from its beginnings before the dawn of history to its most recent . . . advances.”

But “detailed” is a relative notion. To cram into some 350 pages not only the history of biology, but also some account of the knowledge of living things possessed by man before he made his first essays in scientific thought, should, we might have expected, have involved a strict adherence to what is of cardinal importance and a rigid exclusion of inessentials. In fact, what we are offered is another chatty and discursive little book—yet another ‘short history of biology’ loaded with a mass of varied information and not a little misinformation.

According to the preface, part of the book has already been published in Dutch, and inadequate translation may account for some of its oddities. For example, on p. 167, Leibniz is credited with the assertion that “the monads of animals are percipient but unconscious”. It is possible that the Dutch meant “percipient but not self-conscious”.

But there is much for which only the authors can be held responsible. Thus we are told that Buffon “was a writer rather than a scientist, a journalistic propagandist rather than an original investigator”. This is a false judgment: if the authors had not the time to examine Buffon’s admittedly voluminous writings, they could have obtained responsible accounts of them. We are given, of Lamarck’s theory of evolution, the notion based on the two misleading paragraphs so often quoted from the *Philosophie Zoologique*. This notion, that the inheritance of acquired characters was central to his theory, has more than once been shown to be erroneous.

I fail to see how Weismann’s hypotheses can be said to “form a perfect prototype of the theories to which . . . genetics still subscribes”. Weismann thought that “theoretically . . . one id would suffice for ontogeny” (*The Germ-Plasm*, London, 1893, p. 63); but he supposed the germ-plasm to consist of many, even very many ids. This is as though we were to believe that every fertilized egg contained twenty or a hundred sets of chromosomes each equivalent to the haploid set. Then, again, how can anyone say that Weismann and Hugo de Vries (even if the statement refers to 1892) “did not extend their discussions to the heredity characters themselves”? (p. 295).

The account given of ‘biology’ in antiquity is in places just as unreliable as that given of the biology of the eighteenth and nineteenth centuries. We are given (p. 29) to a year the exact dates of the birth and death of Hippocrates, and are told that through him “Greek medical knowledge became a systemized whole”; and although the expression “the writings ascribed to Hippocrates”

is used, the authors consistently write as though the whole Hippocratic corpus could be attributed with great probability to a single writer with a *floruit* about 420 B.C.

We are told (p. 31) that Empedocles “conceived of a definite evolution of living things”; whereas, of course, the most we could attribute to him would be a notion of synthesis of living forms under some sort of natural selection.

A great muddle is made (p. 36) of Aristotle’s neat classification of ‘souls’; and what is the uninitiated to make of the statement, “In plants, this soul was limited to the nourishment imbibed through the roots”?

To say (p. 38) that “The antithesis between *preformation* . . . and *epigenesis* . . . had already found an expression in the works of Aristotle” is a gross anachronism, and scarcely consistent with the statement (itself scarcely justifiable) that “the germ of the antithesis . . . between preformation and epigenesis” is to be found in Harvey’s theory of generation (p. 160).

A partial explanation of the weakness of this book is to be found in its list of secondary sources, which is most inadequate.

As a work of instructive entertainment the book has considerable merits, and it was possibly not intended to be judged as a work of scholarship.

J. S. WILKIE

## TECHNOLOGY OF COMMUNICATION

### Telecommunications

Vol. 1. By J. Brown and E. V. D. Glazier. General Editor: Prof. H. M. Barlow. Pp. xiv+370. (London: Chapman and Hall, Ltd., 1964.) 45s. net.

THE publication of this book is extremely puzzling—puzzling because there is no immediate explanation why authors of such prominence and leadership in the technology of communication should have produced a volume so lacking in original contribution, either of new material or of new points of view. Moreover, it is difficult to judge to whom the book is addressed; is it for the sixth-form schoolboy thinking of entering electrical engineering, or is it for the mature student interested in specializing? Perhaps the best that can be done here is to point out some of the bad aspects of the book and then to suggest improvements for a second edition.

The first chapter is written at schoolboy level. For example, although it is stated on p. 1 that “The reader is assumed to have a knowledge of electrical circuit theory, including alternating current theory . . .” on p. 3 it is explained that, in a pair of transmission-line wires the current flows along one wire, through the load, and back again along the other wire. Again a little later on, it is said that “radio . . . is an electromagnetic wave . . .”, equation (1) reads  $C=f\lambda$ , and on p. 6 it is stated: “1 Kilocycle/second (Kc/s) =  $10^3$  c/s”, etc. The chapter continues at this level, showing pictures of such things as the simple Morse-key telegraph, a telephone circuit and other diagrams that might appear in a *What Every Boy Wants to Know Encyclopaedia*.

It would have been much better to have omitted the whole of Chapter 1 and to have started the book at p. 24. There we are at a reasonable starting level; the distinction between (so-called) continuous signals and discrete signals is noted and Fourier theory is plunged into on p. 37. This omission is made more justifiable by the fact that, in spite of the schoolboy level of Chapter 1, the reader is faced with Fourier series equations and Fourier integral transforms, in the complex conjugate notation, as the second and third equations in the book, without any explanation by way of introduction whatsoever. The appendix dealing with Fourier theory is similar.

A further saving in size and cost of this volume might have been made by exclusion of the appendixes (72 pp.). These treat, formally, Fourier theory, convolution theorem, Laplace transforms, Heaviside's expansion theorem, and Hilbert transforms and Bessel functions. These subjects are already more than adequately treated in existing text-books. The volume would benefit by this exclusion, by directing attention more forcibly to the material in it which is less readily available elsewhere—for example, descriptions of various practical systems of radar, range-finding, direction-finding, etc.

On points of detail, the "simple deduction" on p. 204 of Shannon's capacity theorem (in statistical communication theory) is quite wrong and misleading. Again, on p. 152, the Gaussian probability distribution of 'white' noise is stated without any derivation at all; yet it could well be explained in terms of simple coin-tossing, if not rigorously derived, in a few lines. On the same page the statement appears that "this (Gaussian additive noise) . . . is appropriate for all noise phenomena of interest . . .". Alas, it is not. It is used so widely for reasons of simple mathematical tractability; more cases arise where the noise of interest is impulsive, or is cross-talk, or has other awkward forms. Several other statements of bare theoretical fact are made in the book, when simple explanations and justifications could easily have been given to assist enlightenment (for example, p. 216, the Hamming code).

This review may have seemed harsh, but with limited space it has been thought more important to criticize constructively than to praise blindly.

## RECENT RADIO RESEARCH

### Advances in Radio Research

Vol. 1. Pp. x+226. Vol. 2. Pp. xi+215. Edited by J. A. Saxton. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 50s. each.

**C**OMMUNICATION by radio waves may sometimes appear magically simple, as for example when Marconi first spanned the Atlantic, or in recent satellite relays of television broadcasts. It is, however, often attended by considerable difficulties, which have become the preoccupation both of international bodies concerned with organization and of research establishments concerned with the scientific details. There have for many years been powerful stimuli from civil and military needs, and the trends of modern research in such establishments as the Radio Research Station of the Department of Scientific and Industrial Research, and the Central Propagation Research Laboratory of the National Bureau of Standards can often be traced directly back to quite urgent practical questions. The research is nevertheless basic science, and the account given in these first two volumes of a new series has correspondingly given us a new text-book of radio research.

This collection of work is concerned basically with the propagation of radio waves through the terrestrial atmosphere. The troposphere, which is the lower part of the atmosphere, can both refract and absorb, and the extent of these aberrations is described respectively by Prof. A. W. Straiton (University of Texas) and by Dr. B. R. Bean (U.S. Central Radio Propagation Laboratory). Dr. Bean goes on to show that both troubles are at least predictable, so that, for example, the apparent position of a satellite as measured by a radio telescope can be corrected for refraction from meteorological measurements made on the ground. Prof. Straiton and Dr. Bean provide solutions to practical problems which are becoming commonplace in the age of space vehicles and of microwave communication.

Even without a troposphere, the inhomogeneous and curved surface of the Earth affects seriously the propagation of long radio waves. This has proved to be a difficult

mathematical field of research, which is now expounded by Dr. J. R. Wait (U.S. Central Radio Propagation Laboratory) in an article on electromagnetic surface waves.

A radio wave which has passed these hazards is often received against a background of noisy signals generated by atmospheric disturbances. The description of this thunderstorm noise by Mr. F. Horner (Radio Research Station, Slough) covers the source, its radiation properties and its effects on radio receivers at various places. Another natural hazard, the variability of the ionosphere, is dealt with by Dr. C. M. Minnis (Radio Research Station, Slough), who describes the correlation of ionospheric behaviour with various indices of solar activity.

Finally the scope is widened by the introduction of advanced modern techniques in an article by Dr. J. W. Findlay (U.S. National Radio Astronomy Observatory) on antennae and receivers for radio astronomy. Of particular interest here is the result of surveying the surfaces of two large radio telescopes at the National Radio Astronomy Observatory, Greenbank, by air-based photogrammetry.

These articles are without exception outstanding reviews, well presented, well illustrated and with a full list of references.

As scientific attaché in the British Embassy, Washington, D.C., Dr. J. A. Saxton has clearly found himself in a position to collect together the very best work in this field on both sides of the Atlantic. International radio only exists on the basis of international co-operation, and it is not the least of Dr. Saxton's merits that he has shown us how truly international is modern radio research.

F. G. SMITH

## A MODERN TREATISE ON CHEMICAL KINETICS

### Chemical Kinetics of Gas Reactions

By V. N. Kondrat'ev. Translated from the Russian by J. M. Crabtree and S. N. Carruthers. Translation edited by N. B. Slater. Pp. xiv+812. (London and New York: Pergamon Press, 1964.) 105s. net.

**T**HE investigation of the many aspects of chemical kinetics in the gas phase is a field so wide that, hitherto, up-to-date detailed surveys have usually been confined to limited branches of the subject. New work in this field is produced at such a rate that even monographs are quickly outdated. Prof. Kondrat'ev's *Chemical Kinetics of Gas Reactions*, which covers the whole of this subject, is as up to date in all the topics it deals with as can reasonably be expected, bearing in mind the time taken to produce a comprehensive work of such a length in the finished form. Prof. Slater, who has edited the translation, has pointed out that new material was added during the course of preparation and this has resulted in the presentation of published data up to about 1960. Formal treatment of basic theoretical machinery required for discussing theories of chemical kinetics has been kept to a minimum. The emphasis that has been adopted has been that of a detailed understanding of elementary reactions, and it is this attitude which is the essential strength of the book.

The book contains chapters on general kinetic rules for chemical reactions; the nature of the observations necessary for the elucidation of chemical mechanism; general theory of elementary reactions, bimolecular reactions, unimolecular and termolecular reactions; photochemistry; reactions in electric discharges; and a full discussion of chain reactions and combustion. The simple kinetic relationships and the approaches to the determination of reaction mechanism are well illustrated by recourse to experimental data. Modern techniques for examining elementary reactions including differential calorimetry, tracer techniques, isotope effects, flames,

electric discharges and flash photolysis are discussed in some detail. While full reference is made to the results of shock tube experimentation, a description of the shock tube itself, which is a very important technique and deserves as much space as many of the other methods considered, is omitted, perhaps for the reason that a number of monographs already exist on this subject.

The chapter on the general theory of elementary reactions illustrates the tone of the book. Of particular interest are the considerations of adiabatic and non-adiabatic processes, deviations from the Maxwell-Boltzmann distribution in chemical kinetics, the construction of potential energy surfaces and the transition state theory, the collision of three atoms and the quantum mechanical treatment of elementary processes including the 'Born method' and the method of distorted waves, and the role of the tunnel effect. The chapters that follow run smoothly from this, so that more than half the book is basically concerned with elementary processes. In more recent years, a large amount of work in combustion chemistry has been directed towards isolating and characterizing the elementary reactions, particularly of atoms and small free radicals, pertinent to combustion mechanism. While any general presentation on combustion must clearly be dominated by chain theory and its application to particular combustion systems, the culmination of such a discussion would be expected to be directed towards an understanding of the elementary reactions of which the overall combustion is comprised. Prof. Kondrat'ev maintains the general theme of his text here. The final two chapters on chain reactions and combustion, which comprise some 200 pages, could well stand as a monograph on their own.

With the exception of the photographic plates, which are poor in quality, the book is very well produced. There is a full list of references and a name index, but the subject index is sparse. It is hoped that these minor points will be rectified in later editions.

The large amount of work that has been carried out in recent years on the investigation of elementary processes, both from the theoretical point of view and experimentally by techniques such as shock tubes, flash photolysis and flow-discharge systems, finds its natural place within the appropriate chapters of Prof. Kondrat'ev's book; for this reason and many others *Chemical Kinetics of Gas Reactions* provides the research worker in gas kinetics with an integrated background of knowledge. It covers a very wide range, it is detailed, and it brings the reader to the forefront of thinking on chemical kinetics in its discussion of modern theories. The price of this comprehensive, lengthy and readable book is very reasonable indeed.

D. HUSAIN

## RESEARCH IN MOTIVATION

### Motivation

Theory and Research. By Prof. C. N. Cofer and Prof. M. H. Appley. Pp. 958. (New York and London: John Wiley and Sons, Inc., 1964.) 94s.

### Experiments in Motivation

Edited by Prof. H. J. Eysenck. Pp. viii+424. (London and New York: Pergamon Press, 1964.) 100s.

**M**OTIVATION is one of the basic psychological concepts which have rather different meanings for the man-in-the-street and for the psychologist. Pressed for a definition, both would probably say (or infer) that in studying motivation one's concern is to explain what causes organisms to behave in the way they do. Divergence occurs in relation to what may be called the level of explanation that is looked for, or accepted; in general the man-in-the-street is probably mostly interested in specific, or specifiable, behaviour acts—the psychologist

rather in explanatory principles, or what Drs. Cofer and Appley call "motivational constructs". As they say: "When we ask questions about the 'why' of behaviour we are seeking information about processes *not* directly observable in an individual's overt actions, or even from his verbalizations about his covert actions. The processes we must study are only indirectly inferable, painstakingly and tentatively, from the events available to direct observation or assessment".

That quotation is from the opening of Cofer and Appley's first chapter. The authors go on to point out that while problems of motivation have been variously "incorporated as subordinate parts of larger areas", or even ignored, the present-day trend is to treat them as "a separate set of problems worthy of independent focus". This is clearly the authors' own predilection—otherwise the book would not have been written. One might be inclined to argue this position, but in the event of this the scope of the research material surveyed is so wide that any implication of narrowing of interest implied by the word 'focus' proves unfounded. This breadth of scope, let alone the complex semantic overtones of the term 'motivation' itself, makes the subject-matter of the book coterminous with that of psychology at large, not excluding systems sometimes regarded rather as related disciplines, such as ethology and psychoanalysis.

A work of this magnitude clearly cannot be summarized; nor, indeed, does the present book lend itself to being read and assessed as a whole. The authors' most individual contribution is contained in their two introductory chapters, and in the concluding chapter, entitled "Toward a Unified Theory of Motivation". Their main concern in that chapter is to demonstrate, with frequent reference back to the main part of the book, that the concept of 'drive', described as a 'hydraulic-like mechanism' stimulated by deprivation states, lacks universality and so becomes a 'liability'. Nevertheless they feel that an equilibration model is in general terms satisfactory, and propose such a model based on the hypothesis of two mechanisms: an *anticipation-invigoration mechanism (AIM)* and a *sensitization-invigoration mechanism (SIM)*. 'Invigoration' is explained (in the former context) in these terms: "...behaviour displays an augmentation of vigour—an invigoration, so to speak—over the level of vigour that would be present in the same stimulus situation without anticipation". It is preferred to 'arousal' (although the terms 'anticipation-arousal' and 'sensitization-arousal' are also used) on the grounds that "it is perhaps not necessary to imply an intervening arousal state". Anticipation can only work, of course, after learning has occurred; cases in which motivated behaviour takes place without previous learning are explained in terms of *SIM*.

The above is an attempt—probably inadequate—to state the authors' views in a close paraphrase of their own words. It is perhaps a pity that they had not presented their case at greater length. As already suggested, the book makes rather heavy reading, and the same applies, to some extent, to the concluding chapter. However, the value of the book as a work of reference up to 1961 or 1962 ('spotty for 1963' is the authors' phrase) is not affected. Particularly remarkable is its extensive bibliography—author index covering nearly 100 closely printed pages.

While Cofer and Appley reject drive, Prof. Eysenck uses it as the basic concept on which the work reported in his *Experiments in Motivation* is based. The book is in two parts: Part 1, extending to nearly three-quarters of the book, reports eighteen experiments with human subjects; Part 2, four studies with animal subjects. Each part is preceded by a short introduction defining the characteristics of the populations from which the experimental ('high drive') and control ('low drive') groups were drawn. It should be noted that, in common with Prof. Eysenck's other compilations with similar titles, the scope

is limited to work carried out at the Institute of Psychiatry or under its auspices.

In fact, in the present instance the range of enquiry is even more than usually restricted, thus belying the claim on the fly-leaf that "the work will be of absorbing interest to all who are in any way concerned with the behavioural sciences, as well as to zoologists, geneticists and anthropologists". In the human experiments 'high drive' subjects were candidates for apprenticeship in the motor industry. The 'low-drive' subjects were apprentices already undergoing training with the same manufacturers. Both groups had the same general background, but they differed in mean age by about eighteen months, and since the 'low-drive' group had in fact passed selection its individuals were somewhat superior in respect of intelligence and other relevant abilities. In addition, the apprentices were aware of the purpose of the experiments, whereas the candidates may well have thought that they were part of the selection battery. No other criterion of drive level appears to have been applied. Is it, then, being tacitly assumed that the candidates' keenness to be accepted will carry over the test situation, while the comfortably secure apprentice will show indifference, even when taken into the experimenters' confidence? Maybe this distorts Eysenck's assumption, but perhaps it is not unfair to suggest that to generalize drive to this extent is to deprive the concept of meaning. In the circumstances it is not surprising to find Prof. Eysenck in his foreword claiming no more than "that there is a certain degree of congruence between theories and results, sufficient to encourage one to continue in this type of work, insufficient to make one feel that issues have been clarified even in the most rough and ready manner".

In the face of such disarming frankness one is almost ashamed to find oneself wondering why it had been considered opportune to publish this large and expensive collection of papers. There is no attempt to draw threads together: the reader is left to collate for himself the conclusions with which each individual chapter ends. Moreover, the book bears every sign of hasty production: much careless writing; innumerable examples of faulty proof-reading; injudicious proliferation of quantitative data (on p. 216 there appears an analysis of variance with 31 components, every one insignificant).

Nevertheless, the book does contain much of interest, particularly in Part 2, which might well have been published separately as a monograph on motivational aspects of rat behaviour. Here selectively bred strains (known as 'reactive' and 'non-reactive') provide experimental and control groups which can be quite explicitly defined, and although the linkage with emotionality, in the human sense, is not altogether convincing, the reader gets a clear impression of the purpose behind the work. One puts the book down with the impression that one has been looking through an enormous collection of journal articles, not all of which would have been accepted, although the animal papers would probably have fared better than the human.

B. SEMEONOFF

## SCIENTIFIC AND TECHNICAL LIBRARIES

### Scientific and Technical Libraries

*Their Organization and Administration.* By Lucille J. Strauss, Irene M. Strieby and Prof. Alberta L. Brown. (Library Science and Documentation: a Series of Texts and Monographs, Vol. 4.) Pp. xi+398. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1964.) 65s.

THIS handbook, by three stalwarts of the special library profession in the United States, is virtually a new and much revised version of *Technical Libraries:*

*Their Organization and Management*, published in 1951 under the sponsorship of the Special Libraries Association. Since the former work appeared, the face of special librarianship has undergone many changes which, in turn, have led to a substantial increase in the number and variety of publications on the subject. Nevertheless, there are still only two comprehensive manuals in the English language—the present volume and the Aslib *Handbook of Special Librarianship and Information Work*—which cover the field at all exhaustively, and the labour that has gone into bringing the earlier Special Libraries Association publication up to date will gain the gratitude of scientific library workers.

It is true that much that can be said about the organization and administration of scientific and technical libraries applies equally to special libraries serving other subjects. The authors have been wise, however, to restrict themselves, since theirs is essentially a practical work of day-to-day reference in which the examination of basic principles is subordinated to factual guide-lines for effective operation. Thus, for example, lists of names and addresses are included wherever possible for publishers, booksellers and subscription agencies. In these matters, while the book is clearly intended primarily for American conditions, its approach is perhaps less narrowly American than its predecessor, and it is good to see prominence given, not only to American publishers but also to some in the United Kingdom, France, Germany, Holland, Italy and elsewhere.

Some of the most effective chapters are those on stock selection, lay-out and equipment, and operating costs, the latter, as the authors recognize, being scarcely amenable to generalized treatment since the unique aspects of every situation make it a case in itself. Even so, they contrive to include range figures for both initial and annual expenditures which should prove a helpful guide to spending ratios on salaries, stock, equipment and services. The considerations to be borne in mind in making the best use of available space are well illustrated by useful diagrams, but rather more technical detail would have been welcome in the sections on lighting, flooring and air conditioning. Every aspect of stock is considered and, while books and periodicals necessarily receive minute attention, there are knowledgeable and thoughtful sections also on patents, standard specifications, translations—with a substantial list of organizations which supply translations—theses, reprints and company reports. The chapter on reference service, incidentally, contains a full and detailed section on patent searching, itself a highly specialized art.

In writing of cataloguing and classification and staff selection, the authors seem to be less at home. Old controversies about training and qualification are by no means dead and, in any event, no two scientific libraries can be counted on to need identical staff. Some imprecision may, therefore, be inevitable but managements, one of the four audiences envisaged by the authors, would certainly welcome a simple and more clear-cut tabulation. Cataloguing and classification are approached from a general, rather than a specifically scientific, library point of view, with the main emphasis on the Dewey and Library of Congress systems and with the U.D.C. relegated to "other general schemes" along with Bliss and Colon. What is inadequately explained is the nature of the many special problems encountered in scientific and technical libraries which frequently make conventional systems inappropriate—and the prodigious difficulties inherent in making a change if a conventional system proves to have been a wrong choice in practice. There is also no reference to the results of the International Conference on Cataloguing Rules held in 1963. In spite of these relatively minor shortcomings, the book is one to which American scientific librarians, and many in other countries also, will find themselves referring over and over again.

L. WILSON

**Synthetic Fibres in Papermaking**

Edited by O. A. Battista. (Polymer Engineering and Technology.) Pp. xi+340. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 105s.

THERE was a time when it was possible to draw a sharp distinction between paper and textiles, since they were always produced respectively by depositing individual fibres from a wet suspension and by weaving or felting fibres in the dry state. Moreover the raw material for paper was necessarily cellulose fibres, whereas textile fibres could be of animal, vegetable or mineral origin; or, more recently, synthetic. The increasing use of synthetic fibres in paper (and in the present work these also include glass, metal and ceramic fibres) has rendered the above distinction much less clear-cut. Indeed, if products containing more synthetic fibres than paper fibres are taken into account, then the traditional definition of paper would appear to call for revision. These new fibre combinations have resulted in products having striking wet- and dry-strength and electrical properties which make them of considerable interest especially for industrial uses. Applications to the textile, shoe, filter, abrasive, electrical packaging, clothing and household furnishing industries already exist, and the range will doubtless increase as manufacturing technique improves and, more important, as the production cost is reduced. This volume deals with these aspects of the subject, each type of synthetic fibre being the subject of a chapter by an appropriate expert. There are also chapters on web formation, on bonding, structure-property relationships, and on present and future markets.

A chapter on developments in Europe demonstrates the present big lead established by the United States, where the *per capita* consumption of paper is twice that of an average European country. It is claimed that this is the first "hard cover" book on the subject, and this and its American origin and list of authoritative authors commend it to those interested in this new and developing subject. By reason of the wide field of applications involved and the novel nature and possibilities of this branch of 'papermaking', it deserves a much wider public than the paper, fibre or textile technologist.

JULIUS GRANT

**Les Triterpénoides en Physiologie Végétale et Animale**

Par P. Boiteau, B. Pasich et A. Rakoto Ratsimamanga. Pp. xi+1,370. (Paris: Gauthier-Villars, 1964.) 310 francs.

THIS book provides the most comprehensive treatment of the properties of triterpenoids that is at present available. The coverage is unusually wide: it would appear that all references to the chemical, biological and physiological literature concerning triterpenoids published up to 1962 are included, and there are addenda which cover some of the publications of 1963.

The first chapter surveys the various structural types of triterpenoids and interrelates these in terms of their biosynthesis. Then follows a detailed summary of all known natural triterpenoids as well as their naturally occurring derivatives, such as esters and glycosides (saponins). This section also includes numerous indexes of trivial and systematic names presented in terms of common structural features, and lists are given in order of melting-points and specific rotations. The methods of isolation and identification are fully reported and the many methods of degradation which have been used in the structural investigation of triterpenoids are well reviewed. The use of mass spectrometry and optical rotatory dispersion measurement is not adequately covered owing to the very recent publication of much of this work.

The chemist concerned with natural products is often prepared to have his interest broadened, and this is encouraged by this book. Chapters 5, 6, and 7 are con-

cerned with the phytochemistry of triterpenoids, the part that they play in biological processes, and their biotransformation. In a book of almost 1,400 pages, divided into eight chapters, it is perhaps surprising that the last chapter should contain only fifteen pages. However, this is to be expected as it is entitled "Hypotheses of Perspectives".

The book may be likened to a good mixed grill: it certainly contains plenty of meat, but it could cause some indigestion! The sterling equivalent of the price is £22 11s. 0d.

W. D. OLLIS

**Les Oiseaux du Nord de l'Afrique de la Mer Rouge aux Canaries**

Par R. D. Etchécopar et F. Hue. Pp. 608. (Paris: Éditions N. Boubée et Cie, 1964.) 120 francs.

IT is appropriate that the French should give us a comprehensive work on the birds of North Africa, as their ornithologists have for long been active there, particularly in the western part. The authors are distinguished participants in this study, and they have the support of a well-known illustrator in Paul Barruel. The area covered comprises the whole width of the continent, and extends far enough south (approximately 21° N. lat.) to include most of the Sahara. Much of it is desert, but islands, coasts, mountains, and the valley of the Nile provide substantial diversity. The avifauna is essentially palaearctic in character; to have pushed the chosen boundary farther south would have brought in an increasing number of tropical elements. Most of the sedentary species in fact belong, exclusively or otherwise, to the Mediterranean basin as a whole; there are also a few species peculiar to the area, and a few that have infiltrated along the coasts from the south. To these are added the sea-birds and the very numerous migratory visitors from farther north. The book constitutes a valuable source of information on the occurrence and distribution of birds in North Africa, the admirably clear sketch maps giving the main facts for each species at a glance. It is at the same time a useful guide to identification in the field; and there are notes on general behaviour, reproduction (in more detail), and sub-species. No single publication has hitherto covered the whole of North Africa in these respects.

LANDSBOROUGH THOMSON

**Handbook of Mathematical Functions with Formulas, Graphs, and Mathematical Tables**

Edited by Milton Abramowitz and Irene A. Stegun. (National Bureau of Standards Applied Mathematics Series, No. 55.) Pp. xiv+1045. (Washington, D.C.: Government Printing Office, 1964.) 6.50 dollars.

THIS massive compendium is on the lines of the widely used *Funktionentafeln* of Jahnke and Emde, but is more up to date and extensive. There are 29 main sections, from mathematical and physical constants up to the special functions of pure mathematics, mathematical physics, and probability; each section has been prepared by an expert in that field. The text is mainly a collection of formulae, economically displayed on a double-column page, with emphasis on numerical utility. The tables aim at giving at least five-figure information, with intervals allowing for reasonably accurate linear interpolation; the introduction expounds recommended processes when more accurate interpolation is required. Bibliographies are short enough to be helpful, though a little more annotation would not have come amiss. The volume is an obvious item for even a small reference library, and we must thank the U.S. National Science Foundation for making funds available for the preparation of the book, thus enabling it to be put on sale at a price which is very small for its 1,000 closely packed pages.

T. A. A. BROADBENT



## THE SCIENCE OF SCIENCE FOUNDATION

By MAURICE GOLDSMITH  
DIRECTOR

THE 'science of science' is an awkward name but, as it describes accurately what is meant, it has become established. By the science of science we mean the examination of the phenomenon of 'science' by the methods of science itself.

It includes thus: the sociology of science; the psychology of scientists and of scientific work; the economics of science; the analysis of the flow of scientific information; operational research on science; the planning of science: that is, the study and formulation of quantitative observations; the study of the role of science in diverse types of societies; and the relation of science to technology.

The science of science begins to merit the name of science since, although some of the foregoing topics were investigated long ago, it is only now that quantitative treatments can be attempted. This has happened (a) because the volume of scientific activity is now large, taking perhaps 2 per cent of the national income of many countries, so that statistical investigations become meaningful; (b) because techniques of examining more complex phenomena have improved so that many biological, linguistic, economic and sociological topics can be examined quantitatively.

Studies of various aspects of science involve, as in any other branch of science, the collection of appropriate quantitative data; the construction of theoretical models exhibiting the behaviour features isolated from the system under investigation; the propounding of general laws or policies on the basis of the conclusions from examinations of the actual and of the idealized systems, and the comparison of the predictions of these laws with what is actually happening.

The science of science represents the self-consciousness of science. Already much is understood about how science operates. Since science is the factor which determines the rate of change of technology, it is being eagerly seized on by Governments, not only of emergent countries, which wish to increase their rate of development above the *laissez faire* rate. Recommendations need to be drawn up to prevent the repetition of earlier mistakes and to plan scientific growth. These can be found only by generalizing the results of investigations of the interaction of science and society.

Nevertheless, we would not wish the science of science to be just an applied science or the preserve of ministries for science. As in any other field, it will have to be attacked wherever it shows signs of yielding results which will be carried as far as possible for the sake of the understanding which they may give.

An excellent example of one topic in the science of science is Prof. D. J. de Solla Price's investigation of the output of scientific papers, in which he has shown that the rate of publication of papers has been doubling regularly every ten years. The number of scientists has been similarly increasing exponentially. This investigation has been very widely quoted as definite evidence for the feeling that there is a 'science explosion' (like the population explosion, although in this the doubling time is about forty years).

A Science of Science Foundation, accordingly, is being set up to encourage and promote the scientific investigation of national and international science policy and the interactions of science and technology and society.

This was announced in London on October 29 last, the occasion of the publication of the book, *The Science of Science*, a volume of tribute to Prof. J. D. Bernal. This year marks the twenty-fifth anniversary of the publication of his monumental work, *The Social Function of Science*, the first book to present the scientific analysis of science.

The Advisory Committee of the Science of Science Foundation at the moment consists of Lord Snow, Prof. Derek J. de Solla Price (Avalon professor of the history of science and medicine, Yale University), Prof. J. D. Bernal, Prof. Asa Briggs (dean of social studies, University of Sussex), Dr. Alexander King (director for scientific affairs of O.E.C.D.), and Mr. Gerard Piel (publisher of *Scientific American*).

The purposes of the Science of Science Foundation will include the following: To organize an annual lecture (plans are in hand to organize the first lecture in London in April 1965; it will be given by Prof. D. J. de Solla Price on "The Scientific Foundations of Science Policy"); to organize seminars, conferences, or other meetings connected with the aims of the Foundation; to support and initiate research work; to promote the collection of reprints, etc., on science policy and to make them available for analysis.

The resources of the Foundation consist of the royalties on the book *The Science of Science*, and gifts and bequests from other sources wishing to advance the objects of the Foundation.

The first director of the Foundation is Maurice Goldsmith, and the assistant director is Alan Mackay. The address is c/o The Ciba Foundation, 41 Portland Place, London, W.1.

The Ciba Foundation has also generously agreed to provide facilities for six seminars to be held in 1965, and shelf-space to initiate the science of science library.

## THE SCOTTISH RESEARCH REACTOR CENTRE

By DR. HENRY W. WILSON

Director, Scottish Research Reactor Centre, East Kilbride, near Glasgow

THE Scottish Research Reactor Centre at East Kilbride, some 11 miles from Glasgow, is the first of the university reactor centres to be set up in the United Kingdom and was formally opened by Sir John Cockcroft on November 13, 1963. The Centre serves the six universities of Scotland and Northern Ireland—the so-called 'Consortium'—and is financed by a grant made by the Department of Scientific and Industrial Research. This

grant covers running costs up to the end of the university quinquennium in 1967.

University reactors, ranging in power from a fraction of a watt to 1 MW or so, have been in existence for a number of years in the United States and several other countries. Among the reasons for such a facility are: (a) provision of beams of neutrons for physical and chemical investigations; (b) provision of a source of

irradiation for neutron activation and radioisotope production; (c) investigation of radiation effects; (d) provision of a neutron flux for reactor physics lattice structure analyses; (e) training facilities in the fields of reactor physics, nuclear engineering and radiochemistry. To these I would add that it can provide an excellent opportunity for scientists from different disciplines and, certainly in the case of East Kilbride, different universities to meet. On which of the above uses of a reactor are most important depends the final choice of reactor. Other factors are the amount of money available and the nature of the site on to which the reactor must go.

### Early History of the Project

The need for a reactor both for teaching and for research had been felt for some time by the universities in Scotland, and the first meeting was held in May 1959. It was considered that fullest utilization would be ensured by making a joint application by all the universities. Another meeting in December 1959, which was attended by Sir John Cockcroft and Sir Harry Melville in addition to the university representatives, considered among other things the question of where it should go. A site on the ground belonging to the National Engineering Laboratory was suggested and this is where the laboratory was eventually built. It has proved an excellent compromise, as will be made evident later. Another result of the meeting was the decision to make a submission to the Department of Scientific and Industrial Research for a grant to finance the scheme. A technical committee, formed of members from each of the five Scottish universities (the Queen's University of Belfast came into partnership some time later) and chaired by Dr. S. C. Curran, investigated the nature of the needs, considered the choice of reactor and was responsible for the general planning of the Centre. Visits were made to various reactor centres at home and abroad, and eventually the choice of reactor was narrowed down to two-tank type and two-pool type reactors. These were respectively the Hawker Siddeley *Jason*; the *UTR-100*, made by the Advanced Technology Laboratories division of Ideal Standard; *Consort*, designed and built by the Imperial College of Science and Technology and the General Electric Co.; and *Triga*, designed by General Atomics. The choice eventually fell on the *UTR-100* which, with modifications requested by the Scottish universities, seemed best to meet their very varied needs.

Application for a grant was made by the principals of the universities concerned to the Department of Scientific and Industrial Research on August 31, 1960, and in May of the following year it was indicated the submission had been approved along with that of the University of London and the joint application of the Universities of Manchester and Liverpool. However, because of the financial situation, the three schemes were stopped for the time being, and it was not until December 1961 that permission to proceed was given. The machinery was put into motion immediately, and work on the site commenced in the spring of 1962. By January 1963 the reactor hall was sufficiently advanced to allow construction of the reactor to begin. It became critical for the first time on June 24, 1963, and was handed over to the Consortium on September 3. The Centre came into use for teaching and research at the beginning of October, the official opening taking place, as indicated earlier, on November 13.

The remainder of this article will describe the Centre and the reactor itself, and this will be followed by an outline of the first year's utilization and experience.

### Location

The location of the Centre at East Kilbride has brought many valuable benefits. First, I should like to record the excellent co-operation which we have enjoyed with the

Director and staff of the National Engineering Laboratory who have helped us in many ways, particularly with regard to library, photographic, transport and restaurant facilities. The advantages also of building on an already prepared site are obvious—electrical, water, steam and drainage services were already to hand.

Geographically, it would be difficult to choose a more convenient site. A half-hour run by motor-car takes one into the centre of Glasgow, to which East Kilbride is also connected by rapid bus and railway services, while the journey to Edinburgh by car occupies about an hour and a half. The centres of St. Andrews, Dundee, Aberdeen and Belfast can be reached in three hours or so by road, rail or air as appropriate. Students from Glasgow and Edinburgh usually travel to and from the Centre each day by bus, while students from other universities are accommodated in a hotel in the district. This is a practicable arrangement since our courses are normally of one or two weeks' duration. A new hostel which is planned for East Kilbride will be of considerable use for students and research workers alike. Meanwhile, the latter reside in a hotel or sometimes, in the case of a longer stay, in lodgings.

### Reactor Centre Buildings

The layout of the Reactor Centre is shown in Fig. 1 and a general view from the west in Fig. 2. It will be seen that it consists of two main blocks. The reactor block contains a capacious reactor hall, control room, hot chemistry laboratory, hot metallurgical laboratory, plant room, isotope store, instrument wash room and solid radioactive waste store. Outside are two 600-gallon tanks for liquid radioactive waste. The reactor hall measures 80 ft.  $\times$  50 ft.  $\times$  29 ft. high and is served by a 7.5-ton crane which is used to transport the concrete shielding blocks, fuel element cask and for various experimental purposes.

A 1.5-in. pneumatic rapid transfer or 'rabbit' tube connects the hot chemistry and the metallurgy laboratories to the region of highest reactor flux ( $\sim 10^{13}$  neutrons  $\text{cm}^{-2} \text{sec}^{-1}$ ) and feeds into a revolving chamber which can take up to six samples at one time. A 2.25-in. rabbit tube for larger samples runs to the hot chemistry laboratory only. The transit time in each case is about 1 sec.

The hot chemistry laboratory contains stainless-steel fume cupboards and remote-handling equipment, and the metallurgy laboratory is equipped with three Lintott glove boxes. There will also be erected in the reactor block a Collins-type liquefier providing liquid helium for the use of the Centre and others in this area.

The front block, which is joined to the reactor block by the 'link' corridor, contains laboratory, office and other accommodation. The laboratories provide space and equipment for work in a number of different fields of science and technology. These include a radiochemistry laboratory which is used for teaching as well as for radiochemical investigations, neutron activation analysis and the like. There are also another smaller chemistry laboratory and balance room, counting room, physics laboratory, low background room, medical and biological laboratory, teaching laboratory (which can be converted to two laboratories by a folding partition), health physics laboratory, library, lecture room seating between 40 and 50 persons and fitted out with suitable still and motion-picture projection equipment, a conference room and several offices. The equipment in the counting room includes a 512-channel *Laben* pulse height analyser which can be connected to any laboratory in the Centre, including those in the reactor block, by means of coaxial cables terminating in a plug board in the counting room.

The library contains books on nuclear and reactor physics, nuclear engineering, radiochemistry and radiation chemistry, health physics and related subjects. Some two dozen journals are also taken in the foregoing fields. It

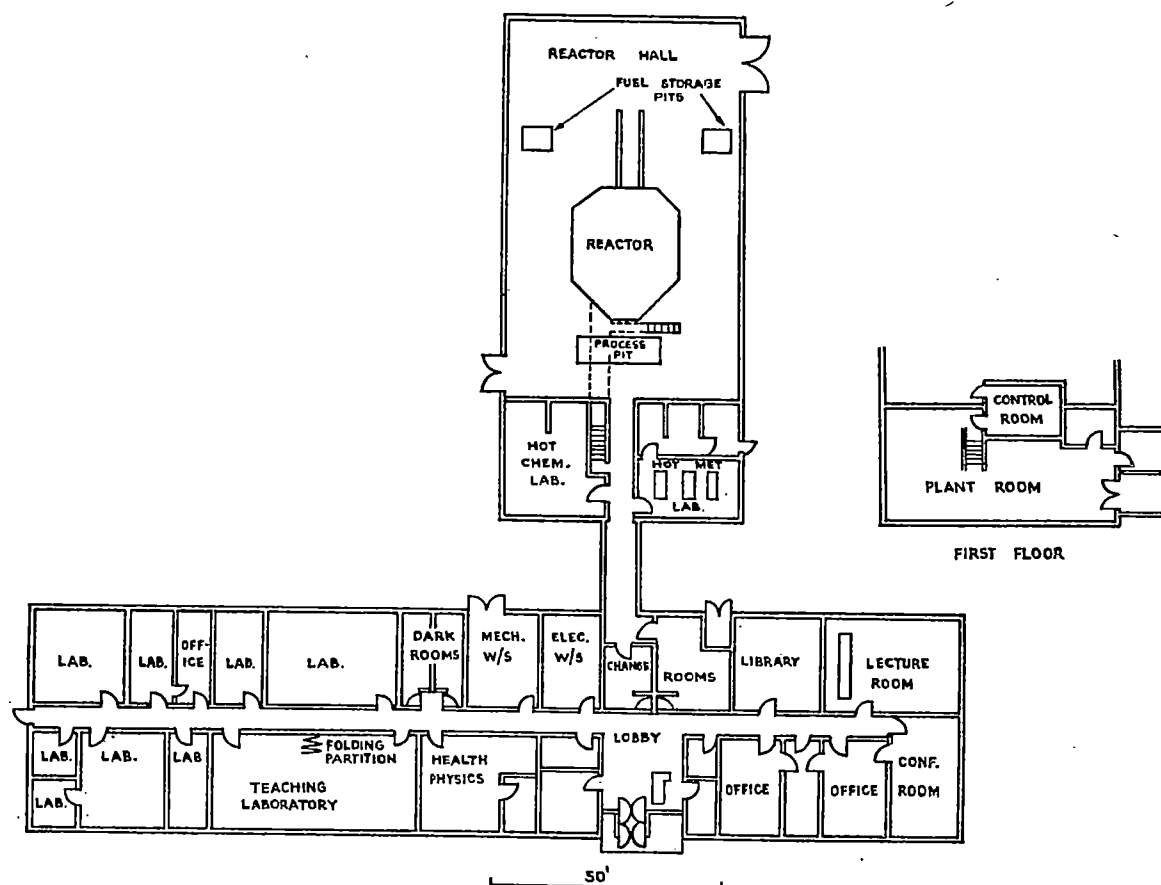


Fig. 1. Plan of the Scottish Research Reactor Centre

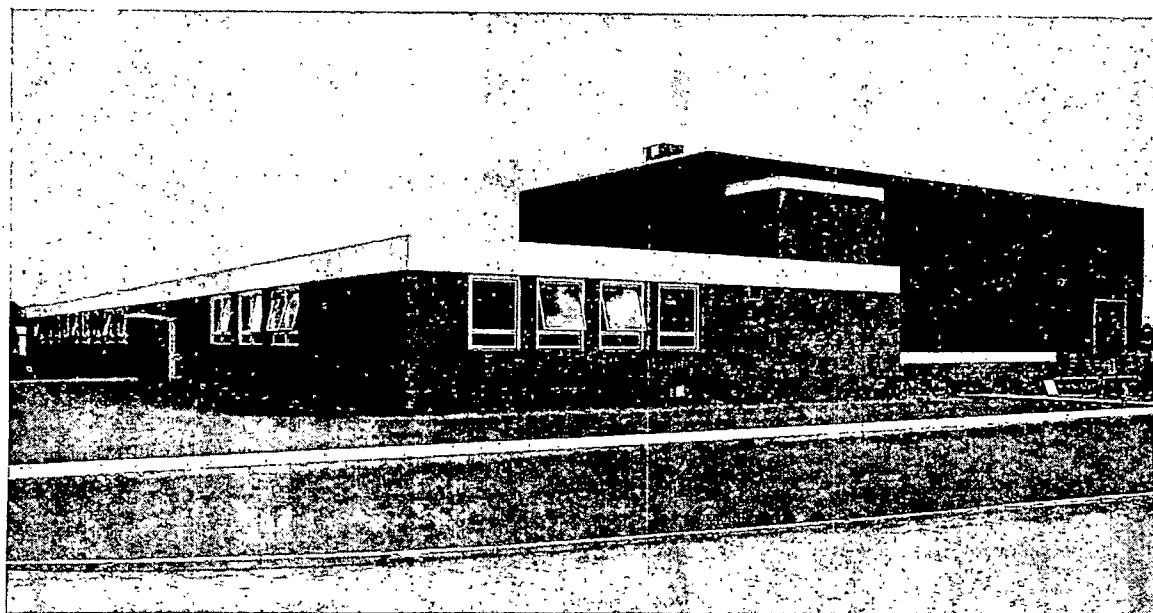


Fig. 2. The Scottish Research Reactor Centre

should be emphasized that, through the good will of the National Engineering Laboratory, we are able to make use of their much larger library some 200 yards away.

The laboratory block also contains two darkrooms, a mechanical workshop and an electronic laboratory. Thus it will be seen that an effort has been made to ensure that the research worker (or student) at the Centre will have all

the essential facilities. Although the Centre lies out of the Clydeside industrial area, a run of half an hour takes one into the centre of Glasgow, where most other requirements can be met.

An extension containing two rooms for research workers at the Centre, a technicians' room and a store is about to be built in the space to the left of the reactor hall.



### The Reactor

Fig. 3 shows the reactor, with the control room in the background. The principal features are shown in Fig. 4. The 12 fuel elements are arranged in two parallel rectangular tanks, 18 in. apart, contained in a 4-ft.  $\times$  4-ft.  $\times$  5-ft. graphite block. The flux between the fuel tanks is about  $10^{12}$  neutrons  $\text{cm}^{-2} \text{sec}^{-1}$  and is remarkably uniform over a large volume. The space is penetrated by two through tubes and five vertical holes, one of which measures 6 in.  $\times$  6 in. and can be reached from outside. This is much used for the irradiation of bulky or fragile samples, and it is planned to fit a facility to permit the insertion and removal of samples at full power. The other vertical holes measure 4 in.  $\times$  2 in. The rabbit tubes referred to previously also run into this region. There is also a 4-ft.  $\times$  4-ft.  $\times$  5-ft.-long thermal column which is penetrated by a third through tube plus 15 removable stringers. One of these (to which access can be gained from outside) goes into the core region, the others terminating at a lead gamma shield. Another thermal column, 30 in.  $\times$  30 in.  $\times$  40 in. long, runs to the foot of a water-filled shield tank, 5 ft.  $\times$  6 ft.  $\times$  18 ft. deep. A flux of  $10^9$  thermal neutrons or, with a fission plate, of fission neutrons is available for experimental purposes. The water provides adequate shielding even at 100 kW.

Each fuel element when full consists of 13 plates containing 22 g uranium-235 in the form of 90 per cent enriched uranium dioxide pellets in an aluminium matrix clad in pure aluminium. The reactor power is controlled by four boron plates. Two cores are provided. One, the low-power core, operates at powers up to one watt only and is used for teaching and certain research investigations. Further details of the design and nuclear parameters are available in Report ATL-D-869 "UTR-100 University Teaching and Research Reactor". A paper by J. A. Izatt *et al.* describing the design and commissioning of the reactor is also planned.

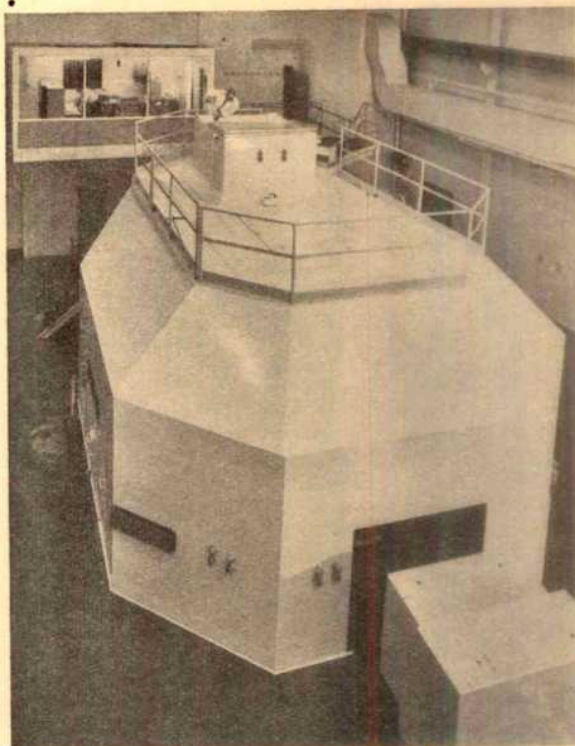


Fig. 3. The UTR-100 reactor at the Centre

The architectural design and construction of the centre and of the reactor were supervised by the Ministry of Public Buildings and Works. The reactor itself was designed by Advanced Technology Laboratories of the American Standard Co. and built in conjunction with

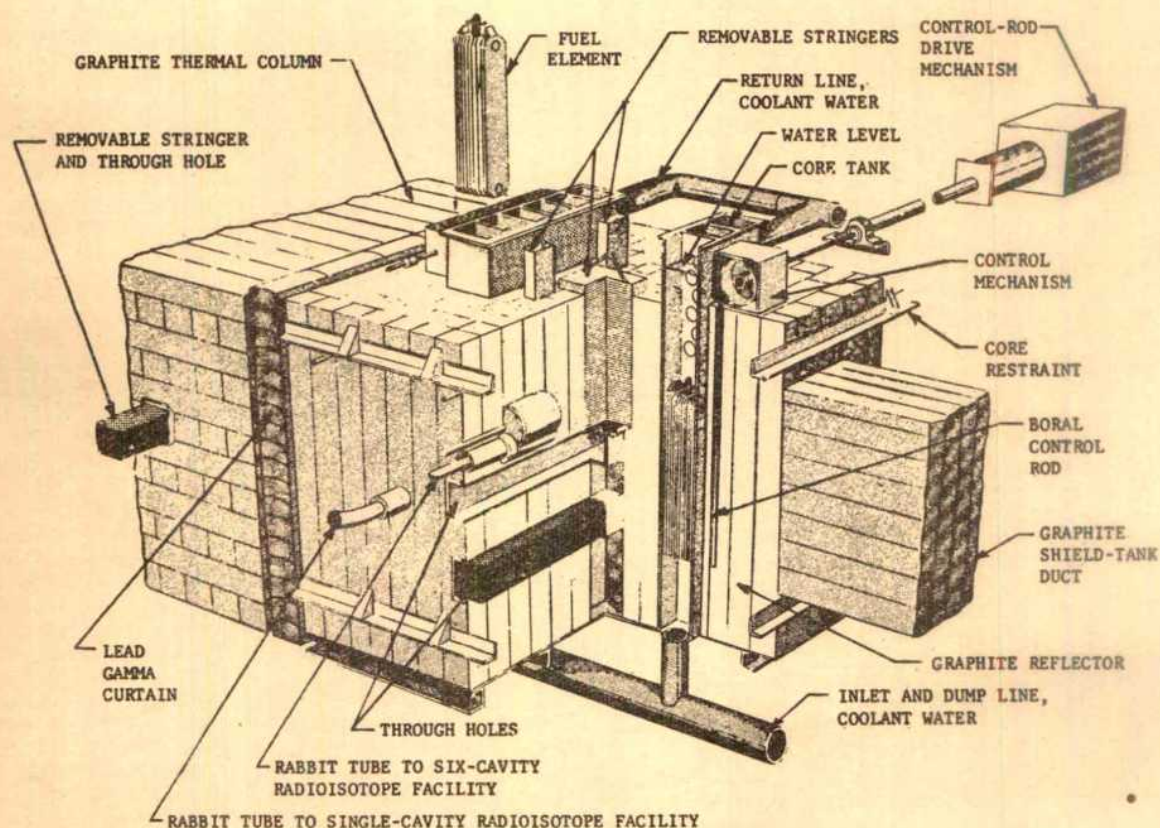


Fig. 4. Diagram of the Scottish research reactor (UTR-100) core

G. and J. Weir. Other contractors included Graviners (graphite and lead), J. Crawford and Sons (buildings) and a number of other firms. The cost of the reactor itself was £88,000 and the total cost of reactor, buildings, external services, furnishings and professional fee was £256,335. The original grant from the Department of Scientific and Industrial Research was for £458,300, which also covered running costs until 1967. The grant, which is administered by the University of Strathclyde, has been increased somewhat, principally to take account of increased staff salaries and the provision of other items including a vehicle for use by the Centre.

### Courses

During the first year of operation, that is, until September 30, 1964, 16 courses were held in five main subjects: reactor physics, nuclear engineering, health physics, radiochemistry, and uses of radioisotopes.

These were usually of one or two weeks' duration and were at undergraduate, postgraduate and extra-mural levels. One course had 50 students but, because of limitations of space and equipment, the optimum number is about 20. Students came from all six Scottish universities, Robert Gordon's College, Aberdeen, Heriot-Watt College, Edinburgh, and elsewhere. Two courses on the use of radioisotopes drew scientists from a number of fields. One of these courses was carried out in conjunction with the Western Regional Physics Department.

Held at the Centre this year also were a one-day symposium on the "Uses of Isotopes" (130 delegates were present), a neutron diffraction discussion group and part of a NATO summer school in "Activation Analysis" organized by the Western Regional Physics Department, the University of Glasgow and the Scottish Research Reactor Centre.

It should be emphasized that, although these courses occupied more than half the session, they did not interfere with the utilization of the Centre for research to any important extent.

### Research

It is convenient to consider research activities under different headings. First, the reactor is used to produce beams of neutrons or to study radiation effects as they occur. It is also used to produce radioisotopes, particularly short-lived ones, and to irradiate materials for subsequent study as, for example, in neutron activation analysis, radiation chemistry studies and so on. Finally, the Centre is used for research investigations because of other facilities which it possesses in addition to the reactor.

The first class of experiment involves the construction of special equipment in the reactor hall and beam tubes which is usually more expensive and more complicated than that associated with the second class. As a result the experiments take longer to organize and get under way. The second class more often than not makes use of equipment already available at the Centre or the experimenter's own laboratory. Some of the beam tube experiments planned or actually under way will be described briefly here.

(1) *Neutron diffraction.* A grant has been made by the Department of Scientific and Industrial Research to carry out crystal structure analysis by this method. The equipment is now available and is being assembled at the reactor. The groups primarily concerned in addition to the Reactor Centre staff are the crystal analysis groups at the Universities of Edinburgh, Glasgow and St. Andrews (Queen's College, Dundee). Interest has also been expressed by the Universities of Aberdeen and of Strathclyde. We should like to acknowledge the assistance given by the staff of the Atomic Energy Research Establishment, Harwell, in various ways.

(2) *Neutron focusing.* The very interesting suggestion of P. S. Farago<sup>1</sup> that slow neutrons could be focused and highly polarized by a hexapole magnetic field was followed by a grant application to the Department of Scientific and Industrial Research. This, if successful, will provide a valuable and, at present, unique tool in the fields of low-temperature and solid-state research.

(3) *Radiation chemistry.* An investigation of the effects of radiation on compounds of biological interest by mass spectrometry has been started by Dr. I. Reid and myself in collaboration.

(4) *Cold neutron research.* Prof. Allen and his group at St. Andrews plan to carry out work in this field. The neutron focusing equipment mentioned above would be of value here.

(5) *Noise and oscillator measurements.* These are being carried out by Mr. Izatt and Dr. Scobie, of the Centre staff.

(6) Prof. N. Feather, of the University of Edinburgh, and Dr. Scobie are engaged in the measurement of tritium produced in fission.

(7) *Other measurements.* Among those being considered are an investigation of creep properties by A. I. Smith and D. Murray of the National Engineering Laboratory, further fission investigations using the reactor and nuclear shielding examinations. Other experiments in progress or completed are concerned with flux profiles, control rod worths in various combinations, neutron lifetime and other reactor parameters.

Turning now to the second field of activity—radioisotope production, radiation investigations and neutron activation—we find that such experiments already begun or proposed come from a wide range of disciplines: chemistry, radiation chemistry, radiochemistry, biochemistry; solid state, nuclear and reactor physics; metallurgy, mechanical engineering, zoology, agriculture, medicine, dentistry, physiology and forensic science. Of these, about thirty-five separate projects have already started (and a few been completed) using the Centre's facilities in one way or another, and these have come from most of their fields mentioned here. It is not proposed to go into detail about these researches, but it is clear that the Centre serves a very useful function in this general field.

The third class of research at the Centre which does not make use of the reactor itself arises principally because of the existence of radioactive laboratories, high radioactivity handling facilities and specialized nuclear counting equipment. A prototype whole-body counter has also been developed which could be made mobile, and this would be used in collaboration with Scottish hospitals.

### Staff

The senior staff at the Centre include Mr. J. A. Izatt (deputy director and reactor supervisor), Dr. J. Scobie (reactor physicist), Dr. J. E. Whitley (radiochemist), Mr. K. Boddy (health physicist and safety officer), Dr. G. H. Maconochie (neutron diffraction studies), and myself as director. Other permanent staff include two reactor operators, four technicians (plus three more in process of recruitment), secretary, junior shorthand typist, two janitor-drivers and two cleaners. There are also an M.Sc. and four Ph.D. research students working directly under the direction of Reactor Centre staff and some more M.Sc. students are expected later in the session. These are, of course, in addition to staff and research students from other laboratories.

So many university, Government and other bodies and individuals have helped us that it is not possible, much as I should wish to do so, to name them here, although some have been mentioned earlier. I should, however, like to thank the members of the staff of the Centre for their enthusiastic co-operation and support.

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## INTERNATIONAL CONFERENCE ON EARTH SCIENCES

CECIL AND IDA GREEN BUILDING FOR THE CENTER FOR EARTH SCIENCES,  
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

By PROF. H. G. HOUGHTON

Head of the Department of Meteorology in the Institute

**A**N international conference on the Earth Sciences was held at the Massachusetts Institute of Technology, during September 30–October 2, 1964, on the occasion of the dedication of the Cecil and Ida Green Building. This building houses the Institute's Center for Earth Sciences, which is devoted to research and education in geology, geophysics, geochemistry, meteorology, physical oceanography and related aspects of the space and planetary sciences.

The conference consisted of five half-day sessions in each of which three papers were presented by invited speakers. The list of speakers and their titles gives an idea of the nature and content of the conference.

*The Earth's Environment*, Bengt Strömberg (chairman): The Sun and Solar Physics, by Leo Goldberg; The Moon, Planets and their Origin, by Gerard P. Kuiper; The Interplanetary Medium and Solar-Planetary Relationships, by Ludwig F. Biermann.

*Atmospheric Motions*, Robert M. White (chairman): Large-Scale Motions of the Atmosphere, by Edward N. Lorenz; Motions of Intermediate Scale: Fronts and Cyclones, by Arnt Eliassen; Atmospheric Turbulence, by William C. Swinbank.

*Dynamics of the Oceans*, C. O'D. Iselin (chairman): Long-period Phenomena of the Oceans Revealed by Chemistry, by Gustaf O. S. Arrhenius; Large-Scale Circulation of the Oceans, by Henry Stommel; The Spectrum of Waves, by Walter H. Munk.

*The 'Solid' Earth I*, J. Tuzo Wilson (chairman): Long-Term Mechanical Properties of the Earth and Internal Motions, by Gordon J. F. MacDonald; Seismological Information and Advances, by Frank Press; Composition and Phases of the Mantle, by A. E. Ringwood.

*The 'Solid' Earth II*, Sir Edward C. Bullard (chairman): Temperature, Heat Production and Thermal History of the Earth, by Francis Birch; Geochronology and Isotopic Data Bearing on Development of the Continental Crust, by G. J. Wasserburg; Mechanics of the Upper Mantle, by Walter M. Elsasser.

The principal speaker at the conference dinner was Prof. Lloyd V. Berkner.

It is clearly impossible to summarize these fifteen major contributions here, but it is planned to publish them in book form. Selection of highlights from such an extensive and outstanding array is certain to be a matter of personal choice that is best left to the individual readers of the forthcoming volume. I prefer to attempt to assess the present state of the Earth sciences as revealed by the contributions to the conference.

One of the most striking features was that, almost without exception, the speakers addressed themselves to the large-scale problems of the Earth. Much of this is

evident in the titles, and Munk, for example, devoted much of his attention to very-long-period waves in the ocean rather than to surface gravity waves. This seems to be one indication of the approach to maturity of the Earth sciences. These large-scale problems are not new, but they formerly appeared intractable. Attack on them has been made possible by new instruments and data resources and by the relative ease with which masses of data and computations may now be handled on high-speed digital computers. That these advances in knowledge due to technological developments is continuing was indicated by Kuiper's discussion of the lunar surface with the aid of the Mariner photographs and by Biermann's use of data from space probes to reveal the structure and motions of the interplanetary medium. Although it is true that we must observe before we can have a satis-

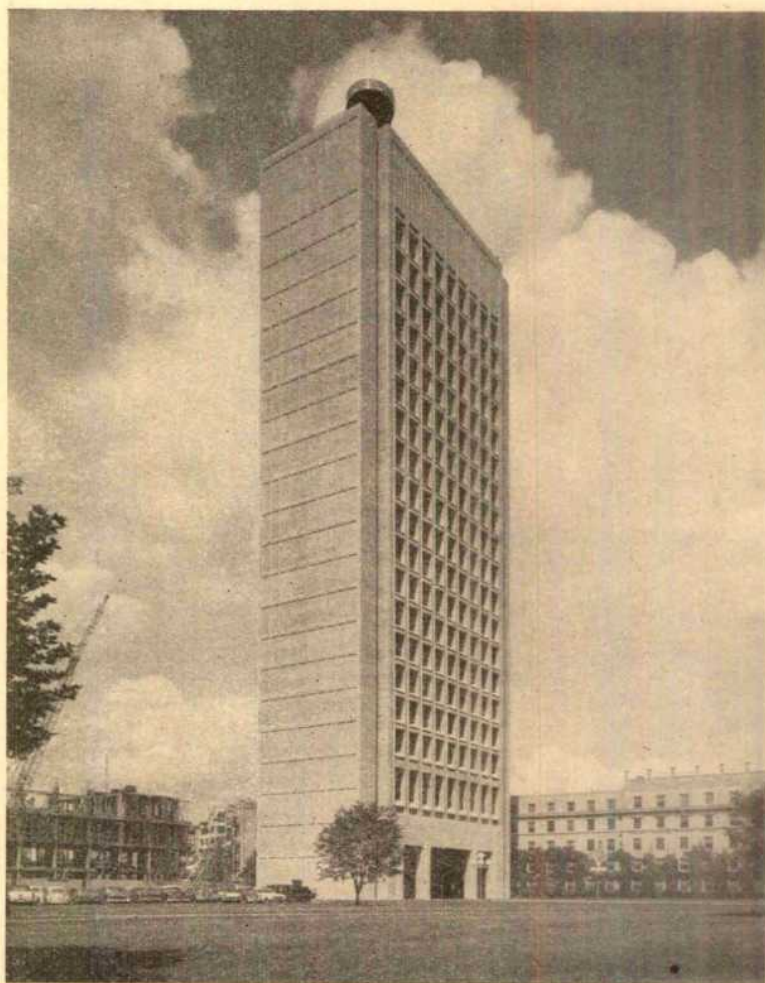


Fig. 1. The Cecil and Ida Green Building for the Center for Earth Sciences, Massachusetts Institute of Technology



factory explanation, it is equally true that observations by themselves seldom provide explanations. The very fact that hitherto unattainable observations are now being made has had a marked effect, part of which is psychological, in stimulating a new attack on the large-scale problems of the Earth.

Of equal importance has been the application of advances in the basic sciences of physics and chemistry to problems of the Earth. Among these, the application of radioactive isotopes to age determination has had a revolutionary effect on our understanding of the development of continental crusts as pointed out by Wasserburg. Similarly, the better understanding of chemistry at extreme pressures and temperatures and recent knowledge of Earth temperature gradients and heat flow has led to new insights into the composition of the mantle as shown by Ringwood and Birch. This, in turn, has led to the more cogent arguments on convection and continental drift presented by Elsasser and MacDonald. Indeed, in many of the pertinent areas of basic science it is the Earth scientists who are now making the most important contributions. An outstanding example is fluid dynamics and its close relative magnetohydrodynamics, which are basic to a wide variety of Earth science problems. This was well illustrated in the contributions of Goldberg, Biermann, Lorenz, Munk, Eliassen, Swinbank, Stommel and Elsasser. The effects of the essential non-linearity of the hydrodynamical equations were stressed by both Lorenz and Munk, and they, with other Earth scientists, are the leaders in this difficult area.

An interesting side benefit of the increasing dependence on the basic sciences in the exploration of the Earth is the greater ease of communication this has engendered. Particularly in their descriptive aspects, the several Earth sciences have their separate argots which make communication difficult. The common language of physics, chemistry and mathematics that was used by the conference speakers not only made all the papers fully intelligible to the audience of some four hundred but also clarified the essential interdependence of the Earth sciences.

There seemed to be a general feeling among the participants that the conference was an outstanding success.

In large part this reflected the stimulating nature of the papers presented by acknowledged leaders in their fields. Beyond this there was the intangible feeling that the conference was a symbol of the emergence of the Earth sciences as mature fields of science with great promise of further important developments in the near future.

The climactic feature was the dedication of the Cecil and Ida Green Building on the afternoon of October 2 (Fig. 1). The principal speaker at the dedication was Roger R. Revelle, the well-known oceanographer. This handsome and striking 20-storey tower was the gift of Cecil H. Green and his wife of Dallas, Texas. Mr. Green is an alumnus of the Massachusetts Institute of Technology and was one of the founders of Geophysical Services, Inc., which, in turn, led to the creation of Texas Instruments, Inc. Mr. Green was a pioneer in the development and application of geophysical methods for oil prospecting and is an Earth scientist himself. He is a life member of the Institute's Corporation, and both he and his wife have a long-standing interest in education.

The Green Building is constructed of poured exposed concrete with load-bearing walls and is 277 ft. in height—the tallest building in Cambridge, Massachusetts. Each floor contains a free area 48 ft. wide and 93 ft. long between the service rooms, stairs and elevators at either end. This space can be divided in any way desired with simple non-load-bearing partitions. Most of the building is devoted to laboratories, workshops and offices, but there are a number of special features. These include the 300-seat McDermott Hall, the Lindgren Earth Sciences Library, faculty and student lounges, class rooms, seminar rooms and a roof laboratory containing three radar systems and other atmospheric probes. The building houses the Institute's Center for Earth Sciences, which comprises the academic departments of Geology and Geophysics and of Meteorology together with other members of the Institute's staff who are interested in any aspect of the Earth sciences. The aim of the Center is to direct attention to the interdependence of the Earth sciences and their common dependence on the basic sciences through a closely integrated programme of research and higher education.

## ORGANIC GEOCHEMISTRY

By DR. G. D. HOBSON

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**O**RGANIC geochemistry has numerous aspects, and, like many other fields of investigation, its beginnings are obscure. Work in this field has gone on for a long time; the name, however, is of fairly recent origin. The early work was on the composition of coals and crude oils, as well as on the nature of the organic matter of soils. Interest has developed from the determination of bulk elemental composition, to attempts to identify types of compounds or structures, and even specific compounds. Patience, coupled with powerful new techniques and complex new instruments, has led to striking advances in knowledge, especially on the nature of the compounds and structures present. Great as the advances have been, a vast amount of work remains to be done, the problems ranging from the purely academic to the definitely applied.

### Materials

Not only are rocks and oil and natural gas considered, but also substances dissolved or suspended in water. The organic matter may be 100 per cent of the raw substance examined, or it may be a mere trace; it may be of recent origin or even hundreds of millions of years old; it may

be comparatively stable under atmospheric conditions, or readily subject to alteration after collecting the sample, unless carefully preserved until laboratory examination begins.

Weathering, which can extend to considerable depths in some areas, may affect the organic matter in rocks. Ancient rocks at outcrop may become contaminated to a small extent by organic matter of recent origin. Petroleum and aqueous solutions of organic substances can move through permeable rocks, and so be found in rocks other than those in which they were formed or in which they first accumulated.

### Mechanisms and Conditions

Identification is not the only aim of organic geochemistry; there are important cases where explanation is the ultimate goal. Consequently, substances have been subjected to various treatments, the rate of change and the products being examined.

During investigations of mechanisms and allied matters a variety of problems arise. What degree of simplification should there be? What parameters should be investi-

gated? In some cases the choice of a suitable organic substance, or starting point, is reasonably simple; in others there is decidedly less certainty about what it should be. The experiments and auxiliary investigations may lie mainly within the realm normally attributed to chemistry, yet in this type of examination in particular, team-work is imperative, both in planning and interpretation, with an adequate contribution from the geologist. A chemical or a physical determination can be a full answer to some questions; in other instances the significance and meaning of the determination have to be decided against a background of data and ideas which may be assignable to some other branch of science.

Sometimes the probable range of physical conditions in Nature's laboratory can be estimated with moderate accuracy; but when igneous intrusions have played a part the maximum temperature and pressure may be much less certain, with the former exceeding the values attained in a sedimentary rock merely by depth of burial. Although temperatures and pressures, such as are applied in Nature, can usually be attained experimentally, time cannot be matched.

Except when igneous activity is involved the temperature and pressure to which the organic matter is subjected will rise slowly in Nature. Rocks now at outcrop have returned to a low pressure and temperature. Some rocks will have been subjected to more than one phase of rising pressure and temperature.

The environment of the reactions in Nature includes inorganic matter, which is often far more abundant than the organic matter, and commonly it is possible for fluids, with dissolved matter, to enter and to leave the reaction site. Water is invariably present, and in many cases the inorganic mineral matter is fine-grained and contains clay minerals. Soils and recently deposited sediments have micro-organisms, and may also be worked in some measure by larger organisms in the course of burrowing or feeding. Low-intensity radioactivity may exist in the deposits.

The components of living organisms vary in their resistance to change when incorporated in sediments on death, and change is also dependent on the sedimentary environment.

### Some Methods and Results

Fractionation is often the first step in characterization. For coals, petrographic examination and painstaking picking may be the preliminary operations<sup>13</sup>; whereas with other materials solvents or chromatographic techniques may be used. Beyond this stage the resources available, the state of knowledge and the nature and amount of the material available determine the extent to which identification of specific compounds can be made<sup>6</sup>. An example of what can be achieved by skill, patience and extensive resources is the examination of the Ponca City crude oil in which, over a period of more than thirty years, 234 compounds (230 hydrocarbons and 4 sulphur compounds) have been identified; these compounds account volumetrically for nearly half the crude oil<sup>12</sup>.

A desire to process large numbers of samples or relatively intractable materials may lead to the use of an empirical approach and the use of methods which measure something not very clearly specified in every case, yet which, in some senses, give quantitative data. Alternatively, a relation may be observed between the amount of organic material of interest and some other more easily measured parameter, and thereafter measurements of the latter parameter are used to build up a picture of the amounts of the organic material present in what is believed or assumed to be the same type of rock. In one area, radioactivity and other logs have been made in boreholes in an oil shale series in order to estimate the yield of oil,

thereby avoiding the costly operation of taking cores which have afterwards to be assayed, a somewhat time-consuming process for detailed coverage<sup>8</sup>. Elsewhere the nitrogen content, or the organic carbon content, has been used, with a multiplying factor, to estimate the total content of organic matter in rocks<sup>16</sup>.

In sinking wells for oil and gas, coring is normally slower and more expensive than drilling, and it is impracticable to recover cores without the risk of some disturbance of any liquid or gaseous organic content. Moreover, the cores, on reaching the surface, have to be preserved by special means, if further changes are to be avoided before laboratory examination can be undertaken. Logging techniques have been developed which give an almost continuous record of the low-molecular-weight paraffins in the rocks penetrated, either by detecting the hydrocarbons which have entered the drilling mud from rock broken up by the bit, or which have escaped into it later from rock fragments<sup>21</sup>. In addition, rock fragments brought to the surface by the drilling mud may be separated and subjected to sub-atmospheric pressure to release gas which is detected and can be analysed chromatographically.

Recently the production of hydrocarbons from behenic acid by heating at 200° C in the presence of water and a clay mineral has been reported<sup>19</sup>, and alterations to hydrocarbons and crude oils by radioactivity in the presence of limestone, quartz sand or clay minerals, with and without water, have been investigated<sup>1</sup>. Radiolysis increased the amount of resins and asphaltenes in crude oils. There have been renewed investigations of the rate of alteration of organic matter in rock on heating<sup>1</sup>.

Particular types of organic matter have been used as indicators of conditions or sources. Thus the presence of porphyrins has been taken to point to anaerobic conditions of deposition and to only moderate temperatures in the subsequent history of the rock<sup>4</sup>. Some compounds or types of structure may not, however, be quite such good pointers to the kind of source organism as was once thought to be the case. Thus, porphyrins with the phyllo spectrum are derived from chlorophyll, but those with the aetio spectrum may be from the haemoglobin series, or from plants<sup>7</sup>. Electron-spin-resonance measurements on coal and carbonized coal samples have been used to estimate the temperature attained by a coal in the vicinity of an igneous intrusion, and similar measurements have provided support for the view that the origin of fusinite involves carbonization<sup>9</sup>.

Investigations of the ratios of the stable carbon isotopes of organic matter in recent sediments have shown trends in values in going from freshwater to marine deposits<sup>14</sup>. This kind of observation may be capable of indicating environment in ancient deposits. Recently an extensive compilation of data on carbon isotope ratios has been made, together with a summary of the hypotheses put forward to explain the ratios<sup>15</sup>.

Bacteriostatic substances have been detected in coals, and the preservative effects of materials in peat bogs<sup>4</sup> have been revealed spectacularly by the discovery in them of corpses of considerable antiquity. The occurrence of coal deposits is controlled by factors different from those which apply in the case of oil. Accordingly, geochemical investigations may not be directed towards the discovery of new deposits, but rather towards the better utilization of coal and to purely academic problems.

Sometimes the amount of organic matter in a sediment, and not its exact nature, is of practical importance. When the Oxford Clay is used for brick-making by one process the amount of organic matter markedly affects the quantity of coal required in the kilns.

The boundaries of organic geochemistry are diffuse, a description which applies also to other subjects. It may, therefore, be in order to consider certain aspects of carbonate sedimentation in which organisms may be, or

are known to be, involved. In addition to shell and skeleton building, pelleting activities and burrowing can lead to some measure of mixing of organic matter with carbonate minerals, and it has been suggested that the rapid multiplication of diatoms may lead to the precipitation of calcium carbonate from sea-water as 'whittings'<sup>17</sup>. There are probably other instances, or types of rock, in which the effective organic agent or substance is not apparent in the deposit.

During recent years, American oil companies, especially, have paid considerable attention to organic geochemistry. Fundamental investigations have been undertaken<sup>2,8</sup>, because these may well contribute eventually to the more effective search for new oil fields. In Europe, too, there has been similar, yet decidedly less, activity<sup>10</sup>. Universities and research institutes have also carried out investigations in organic geochemistry, the departments involved varying from place to place. Some problems require materials from distant places, or samples that are expensive to obtain. Other investigations can be made on materials that are decidedly easier to acquire. The facilities needed for effective work depend on the problem, and range widely in cost and complexity. Scope exists for many more teams to enter this fascinating field of investigation, and it is to be hoped that in the cases of university departments detailed and fundamental work

will be given preference, even though the facilities may be more limited than in major industrial organizations.

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## RELATIONSHIP OF BIOLOGICAL REGULATORY MECHANISMS TO LEARNING AND MEMORY\*

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**T**HERE have been a number of recent suggestions concerning the possible involvement of various biological regulatory mechanisms in the process of memory storage. These proposals have considered such reactions as nucleic acid synthesis<sup>1-3</sup>, protein synthesis<sup>4,5</sup>, enzyme induction<sup>6,7</sup>, and antibody formation as possible models for the biological basis of this process. The specificity of these proposals has tended to obscure their underlying agreement that a general biological regulatory mechanism has probably been adapted in the nervous system to mediate its function of memory storage. Stated in this manner, this proposition seems almost self-evident. Since such widely differing cells as bacteria and mammalian liver cells share similar regulatory mechanisms, why should not all mammalian cells, including neurones, be governed by the same processes of adaptation and control? The major difference between neuronal and other tissues is the necessity, not only for regulation of each individual cell, but also for precise regulation of subtle intercellular relationships which establish the neuronal circuits which control behaviour. It seems reasonable to assume that such regulation makes use of the same biological processes that are used for the control of other specialized functions in other tissues.

This article attempts to relate our present understanding of biological regulatory mechanisms to the problem of memory storage. The emphasis here, like that in a recent paper by Dingman and Sporn<sup>8</sup>, will be on a general approach to the problem. Nevertheless, a specific point of view will at present be favoured—that memory is based on the development of new synaptic connexions, and that protein molecules are involved in this process. Experiments relevant to this notion will then be considered in the context of this framework.

The proposal that learning and memory are based on the development of new functional synaptic connexions has been maintained since the demonstration of the nature and significance of this structure<sup>10</sup>. From the point of view of this discussion, a feature of synaptic morphology which should be particularly noted is that it is consistent not only with the presence of many functional interneuronal connexions, but also with the existence of 'potential' connexions. Thus, of the hundreds of synaptic knobs in close proximity to a given neurone<sup>11</sup>, only some may be involved in functional synapses. Other synaptic knobs may not, however, be in sufficient 'contact' with the post-synaptic neurone to participate in its activation, but may, because of their proximity to the post-synaptic neurone, be considered to be 'potential' connexions. Small changes may be made in such 'potential' connexions in the process of learning, and the resultant functional connexions may well be the basis for memory storage. It is important to realize from this that the change which is the basis of memory may not necessarily be effected by a major process which builds up a whole neuronal connexion. Rather, it may be due to so subtle a process as the alteration of a single critical molecule with consequent conversion of a 'potential' synaptic connexion into a functional one. Whether by subtle or major change, however, a new synaptic connexion seems crucial for memory; and the molecular mechanisms considered in this report will all be oriented towards explaining how this could be brought about. From this point of view, the molecule involved in memory is not believed, in itself, to contain the specific information which has been stored. Rather, the new synaptic connexion which is established by this biological alteration is felt to be the repository of the psychic event.

Before considering the specific molecular mechanism which may be involved, several more general aspects of the problem will be briefly noted.

\* Substance of a lecture given at the symposium on "Mechanisms and Disturbances of Memory", 72nd Annual Convention of the American Psychological Association, September 7, 1964.

The first of these is related to the overall mechanism by which a new synaptic connexion might be effected. A number of investigators have felt that repeated activation of a neurone might lead to the formation of synaptic connexions with other neurones on which its processes terminate<sup>10</sup>. Support for this comes from the observation that use of a neurone may lead to biochemical alterations in the nerve cell body<sup>1</sup> and also may stimulate the outgrowth of terminal neuronal processes<sup>12</sup>. Sharpless<sup>13</sup> has recently questioned this concept on the grounds that use of many of the same neurones occurs in both learning and extinction with different behavioural results. This criticism seems, however, to be really directed at our ignorance of the mechanisms of reinforcement and extinction. Our understanding of these phenomena is too meagre to answer questions about the nature of the neuronal circuitry between stimulus and response. But the biologist has often done useful work by tentatively ignoring phenomenological complexities and focusing rather on what he can readily envisage and explore. In this case, synaptic connexions seem a likely basis for memory, and activation seems to lead to biological alterations which could be related to the formation of these connexions. Although we do not understand reinforcement, we need in no way be deterred from making plausible proposals based on what we do understand about biological regulatory mechanisms.

A second feature which should be noted is the apparent existence of an interval between a learning experience and its permanent deposition in the nervous system. This is suggested by experiments in which retrograde amnesia has been produced by administration of electroconvulsive shock following learning. The interval following learning during which a convulsion can produce amnesia has varied in different experiments, from hours<sup>14</sup> to seconds<sup>15</sup>. Beyond this period, the memory is considered to have been 'consolidated', that is, deposited in a more permanent form. Since the delay between experience and 'consolidation' seems so variable, its duration has not as yet provided a clue to the type of biological process which might be operative during this period. That there may be different mechanisms for 'short-term' and 'long-term' storage is, however, suggested. Our commitment to the concept that memory storage is based on the development of new synaptic connexions extends to all phases of this process. But the specific reaction involved may well be different at different times after experience, so that the quest for a single change as the sole basis of memory might prove to be naïve.

In recent years, two major processes which regulate cellular function have been discovered. The first, which will be referred to as 'biosynthesis', has been primarily studied in bacteria, but a great deal of evidence suggests that a similar process is operative in mammalian cells. Enzyme induction is an example of this, but there is no reason for believing that the synthesis of proteins with non-enzymatic functions is not also regulated in a similar manner. In the case of enzyme induction, the substrate of a particular enzyme stimulates the biosynthesis of a specific molecule of messenger RNA<sup>16</sup> the structure of which is dictated by the inherited DNA of the cell. This RNA contains the information for the biosynthesis of a specific protein which can, in turn, react enzymatically with the substrate. The cell, in response to the substrate, thereby makes a protein which can utilize or otherwise alter the substrate. A similar process is believed to be involved in the regulation of mammalian liver cells by adrenocortical steroid hormones<sup>17</sup>. In this case, the hormone may stimulate the biosynthesis of a number of different enzymes which in turn alter some aspect of the metabolic activity of the cell. It should be clear that 'messenger' RNA synthesis is believed to be involved in enzyme induction because its rate of synthesis controls, in some systems, the rate of specific protein synthesis.

In mammalian systems it remains possible, however, that the rate-limiting step in protein synthesis may not always be the synthesis of new 'messenger' RNA. Therefore, even if protein synthesis is needed for memory storage RNA synthesis may or may not be involved as a necessary antecedent event.

A second regulatory mechanism will be referred to as a 'molecular rearrangement'<sup>18</sup>. Here a specific substance, whether substrate or hormone, or other 'regulatory compound' causes a structural alteration in a specific protein molecule, thereby altering its function. A marked functional change is, thus, produced—not by the biosynthesis of a new protein, but rather by the structural alteration of a pre-existing protein. As in the case of biosynthesis, there is no reason to believe that this regulatory mechanism is confined to altering enzymatic activity. Structural or receptor functions of protein molecules could indeed be regulated by a similar mechanism, but have been more difficult to investigate since they cannot be isolated and functionally evaluated as readily as can an enzyme.

It should be noted that both these regulatory mechanisms act on proteins. It is because of our familiarity with these processes, and because of the myriad of structural, enzymatic, receptor and regulatory functions of proteins, that at present it seems most likely that this class of molecule is involved in the cellular alteration which constitutes memory. This does not exclude the participation of other classes of molecules in this process. New or pre-existent RNA may be required for new protein synthesis. Lipids, or other molecules, may be required to stimulate or maintain a change in protein structure. For that matter, processes which are at present unknown may, of course, be involved. But the participation of proteins seems, on the basis of our present understanding of biological regulatory mechanisms, to be extremely likely.

If we are prepared to consider that memory storage is mediated by the same general regulatory mechanisms as are other adaptive alterations in cellular function, it seems reasonable, from the foregoing considerations, to maintain that the new synaptic connexion may be formed either by biosynthesis or by molecular rearrangement of a protein. The stimulus for such a change may be a neurotransmitter substance, or it may be a material released during neuronal activation which does not function as a neurotransmitter. The resultant change may occur either in the pre-synaptic neurone or in the post-synaptic neurone, or in both. The post-synaptic neurone may seem a more likely site because its dendrites and cell membrane are physically closer to the regulatory processes of the neuronal nucleus than are the axonal terminals of the pre-synaptic neurone. But it remains quite possible that the new connexion is based on an alteration in the axon terminal which is not dependent on control by the nucleus. Formation of a new functional synapse may or may not be based on an increased proximity between the processes of the neurones involved, since functional contact between these may be produced, not only by physical closeness (produced by biosynthesis or by 'stretching' due to molecular rearrangement), but also by alterations in transmitter or receptor functions. The change may be in an enzyme which controls the synthesis of, release of, degradation of or permeability to a transmitter substance. The change may also be in number or configuration of receptor proteins. Some of these possibilities are listed in Table 1. Both the suggestions of others and other unemphasized mechanisms are represented in this tabulation. So far, there is no basis for choosing between the alternatives listed.

One of the puzzling aspects of a memory is its permanent deposition within a nervous system which, except for its presumably immutable neurone DNA, is apparently being degraded and re-synthesized at a fairly rapid rate. The



Table 1. SOME WAYS A NEW SYNAPTIC CONNEXION MIGHT BE ESTABLISHED

Neurone changed		Mechanism	Likely molecule
Pre-synaptic	Proximity increased	Outgrowth of axonal process	
	Proximity same	Increased synthesis of transmitter	
		Altered release of transmitter	
	Proximity increased	Outgrowth of dendritic process	Protein ('biosynthesis' or 'molecular rearrangement')
Post-synaptic	Proximity same	Increased synthesis of receptor	
		Increased sensitivity of receptor	
		Increased permeability to transmitter	
		Decreased degradation of transmitter	

fact that the molecules of the nervous system are subject to the process of 'turnover' has necessitated the notion that the molecular change in memory can reproduce itself, that is maintain its permanence by some form of self-replication. This has suggested to Schmitt<sup>3</sup> that an RNA molecule, which could conceivably direct the synthesis of identical RNA molecules, is involved in memory storage. Such a conclusion appears unnecessary. Instead 'self-replication' may be explained by the 'trophic' properties of a newly established connexion. It is well known, from investigations of the behaviour of cells in tissue culture, that removal of a cell from its normal neighbours may result in profound morphological and biochemical changes. Intercellular 'contact', therefore, seems of great importance in regulating cellular activity. A similar mechanism may be responsible for maintenance of a new synaptic connexion, once it has been established. Thus, the greater 'contact' between the pre- and post-synaptic neurones produced by the initial event in memory deposition may stimulate one, the other, or both of these cells to repetitive biosynthetic activity which results in the maintenance of the connexion. This 'maintenance phase' may require a different type of biochemical process than that required for establishing the connexion. Indeed, although initial establishment of the connexion could be based on either biosynthesis or molecular rearrangement, it seems likely that maintenance of the connexion involves some type of biosynthetic activity.

A number of different experiments have been considered to approach the identification of the specific biological regulatory mechanism involved in memory storage. These will be briefly evaluated in the light of the preceding discussion. One type of investigation has sought to implicate RNA in this process by observing the effect of yeast RNA on learning. It has been shown that injections of yeast RNA enhance learning and memory in old people with cerebral arteriosclerosis<sup>19</sup> and accelerate acquisition of a conditioned avoidance response in rats<sup>20</sup>. Such experiments have not as yet, however, contributed to our understanding of the molecular basis of memory storage since the manner in which the injected RNA works is at present obscure. Even if we assume that a portion of the nucleotide degradation products of the injected material cross the blood-brain barrier and enter the brain, they could operate in such diverse ways, as increasing the pool of adenosine triphosphate or guanosine triphosphate which participate in many reactions, or influencing the formation of such important compounds as uridine diphosphoglucose or cytidine diphosphotriglyceride which are required for sugar or lipid metabolism, respectively. To argue that they are acting by influencing RNA synthesis seems unfounded. The interpretation of these results is rendered even more

uncertain by the finding (Cohen and Barondes, unpublished) that injection of mice with highly purified yeast RNA does not influence their learning to avoid shock by escaping through a white, rather than a black, door.

A second approach was taken by Hyden and Egyhazi<sup>21</sup>, who have reported alterations in the composition of nuclear RNA in vestibular neurones following learning of a balancing task. This interesting observation underscores previous demonstrations of altered RNA metabolism in activated neurones<sup>1</sup> which has suggested that such alterations may be related to the learning process. It does not, however, successfully distinguish a general response due to activation from a presumed specific response due to learning.

A third approach to this problem has adapted inhibitors of protein or RNA synthesis to examine the role of these processes in learning and memory. RNA synthesis from a DNA template can be completely inhibited by actinomycin D (ref. 22). Administration of analogues of the precursors of RNA, such as 8-azaguanine, may lead to the incorporation of these compounds into RNA, thereby causing the RNA to function abnormally. Administration of puromycin can markedly inhibit the biosynthesis of protein<sup>23</sup>. Each of these inhibitors has been used in an attempt to identify the participation of protein or RNA synthesis in learning and memory.

Dingman and Sporn<sup>24</sup> injected rats intracisternally with 8-azaguanine and found that this resulted in impaired acquisition of a swimming maze. This finding, and that of Chamberlain, Rothschild and Gerard<sup>25</sup>, who investigated the effects of this compound in a somewhat different situation, indicate only, as suggested by Dingman and Sporn<sup>24</sup>, that RNA synthesis may be required for normal neuronal function. They do not imply that RNA synthesis is specifically required for memory storage. This latter possibility is questioned by the experiments of Barondes and Jarvik<sup>26</sup> in which actinomycin D failed to influence conditioning of a passive avoidance response, despite the fact that cerebral RNA synthesis was inhibited by 85 per cent of animals injected with this compound. Interpretation of the experiment with actinomycin D is, however, limited since memory had to be tested within hours after training, to avoid the period of irreversible toxicity which followed shortly thereafter. Because of this, participation of RNA synthesis in the long-term 'maintenance' process of memory storage may not have been tested by this experiment, although at the time the animals were tested the learned response was no longer susceptible to the retrograde amnesic effect of electroconvulsive shock. The only conclusion which we can reach about the experiments with inhibitors of RNA synthesis is that there is at present no definite evidence that this process is specifically involved in memory storage.

Flexner, Flexner and Stellar<sup>5</sup> have reported that when mice were injected intracerebrally with puromycin their memory of the solution of a maze learned days or weeks previously was obliterated. This experiment suggests that protein synthesis is required for memory storage, despite the previous finding<sup>4</sup> that subcutaneous injections of puromycin given before, or one day after training, had no such effect. The long period after learning during which puromycin is effective suggests that protein synthesis may be required for the 'maintenance' of the new synaptic connexion. It does not, however, indicate that protein synthesis is necessary for the initial event in the formation of the new connexion which can still, on the basis of what is at present known, be explained by the process of molecular rearrangement.

It should be clear from this discussion that we are now in a preliminary phase of investigation of the complex problem of the biological basis of memory storage. It is anticipated that our growing understanding of biological regulatory mechanisms will prove indispensable

to further investigations in this difficult interdisciplinary area.

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## OBITUARIES

### Prof. W. H. Pearsall, F.R.S.

PROF. W. H. PEARSALL, who died on October 14 at the age of seventy-three, was a versatile botanist and a man of whom it was said that he had become a legend in his own lifetime.

He was educated at Ulverston Grammar School and the University of Manchester. After service in the First World War he joined the staff of the University of Leeds, eventually becoming reader in botany. In 1938 he was appointed professor of botany in the University of Sheffield. From 1944 until his retirement he was Quain professor of botany in University College, London. He continued his association with the College as emeritus professor and honorary research associate. In 1940 he was elected a Fellow of the Royal Society, which he served in many ways, including two terms on its Council. In 1963 he was awarded the Linnean Society's Gold Medal.

Pearsall's father was an amateur botanist and ecologist and a lover of the Lake District. It was during holidays there that Pearsall began to acquire his unique knowledge of the district. He and his father began the study of the aquatic macrophytes of Esthwaite Water and other lakes in 1913. After the War they made a thorough investigation of the planktonic algae. Pearsall was also mapping the vegetation of the fen at the head of Esthwaite Water. More than forty years later he made his last, unpublished map. The North Fen, now a Nature Reserve, is the best-known fen of its kind in Britain. From these investigations came a series of classical papers about the development of the English Lakes and their vegetation, together with his interest in the chemistry of underwater soils and post-glacial history. Meanwhile he was also carrying out laboratory investigations on plant physiology which were both valuable in themselves and threw light on the ecology of the plants concerned. He did notable work on the growth of *Chlorella*, which he also used as an ecological tool to elucidate the growth of algae in lakes. The work he did on the physiology and ecology of aquatic plants has been a constant source of inspiration to workers at the Freshwater Biological Association and elsewhere.

Pearsall edited the *Journal of Ecology* for several years, and, at the time of his death, was still one of the editors of the *Annals of Botany*. He was also a trustee of the Society of Experimental Botany. He exerted special influence on the growth of the Freshwater Biological Association, the Nature Conservancy and the Institute of Biology.

A founder member of the Freshwater Biological Association, he acted as honorary director during 1931-37 and chairman of Council from 1954 onwards. Few have done as much as he did to bring the Association from its humble birth in a period of economic depression to its

present position as one of the most famous bodies of its kind in the world. His influence on the members of the staff extended into every aspect of their work, including the ecology of fish, in which, perhaps, he had a special interest from his love of fishing. In his artistic appreciation of Nature—he was a good painter in water-colours—lay part of his wonderful insight into the broader aspects of ecology. He was a natural choice as a Charter Member of the Nature Conservancy. The unique position this body holds in the world to-day is, to a large extent, the result of the untiring efforts he made on its behalf. Characteristically his influence was felt both in the sphere of high policy and among the younger scientists who joined the Conservancy. He also made a notable contribution to the protection of wild life in Africa by his *Report on the Ecological Survey of the Serengeti National Park, Tanganyika* (1957). His influence on the Institute of Biology, of which he was chairman in 1957-58, was also profound, while he was the first vice-president of the Council for Nature. He was consulted officially on innumerable biological matters, but it may not be generally known how often he was also consulted unofficially.

Pearsall was the most understanding and generous of friends. He was always good company, and many will remember with affection the twinkle in his blue eyes which heralded the start of one of his stories. To be with him in the field, especially on the hills he loved so much, was pure joy. Something of this can be felt when reading his *Mountains and Moorlands* (1949). His influence on younger workers was immense, notably in the period when he was at University College, London. He could be obstinate, even exasperating; yet one of his most wonderful characteristics was that it was out of disagreement that some of the most fruitful researches of his colleagues were likely to come—much to his delight. Their regard for him grew all the time, irrespective of whether they agreed with his ideas or not. Moreover, his hypotheses were by no means always incorrect; they might equally be too novel for people to appreciate their value at first.

It is no wonder that Pearsall was so highly respected and widely loved, or that he will be so sorely missed. His wife and two sons who survive him will receive sincere sympathy in their loss from a host of friends and colleagues.

J. W. G. LUND

### Prof. Vincent Nechvile

PROF. V. NECHVILE, whose death was reported in July of last year, was born in Prague on April 10, 1890. Like many other astronomers of his generation, Prof. Nechvile entered our science as a mathematician. Soon after his doctorate at the Charles University (as a pupil and later assistant of the late Prof. Karel Petr), his career was interrupted by four years of the First World War, most of



which Nechvile—the gentlest of persons—spent in the uniform of a cavalry officer. His scientific career did not really blossom out until, in the post-war decade, a grant from the French Government enabled him to spend several years at the Observatoire National de Paris, for it was there that Nechvile developed all his interests, sympathies, and inclinations to which he remained faithful throughout his scientific life.

Under the influence of Prof. Andoyer, Nechvile's mathematical interests turned to the restricted problem of three bodies; and he was the author of the well-known transformation of co-ordinates in the elliptic case (that is, when the two finite masses describe elliptic orbits) which has become the basis of most subsequent work on this subject. Collaboration with George Willis Ritchey (the creator of the large reflectors at Mount Wilson, who was also working in Paris at that time) led Nechvile to study geometrical optics. His work on the theory of the Ritchey-Chrétien catoptric systems constitutes a fundamental contribution to the subject (alas, but little known, as most of it was published in Czech).

However, Nechvile's principal work at Paris was in the field of stellar proper motions. In the 1880's, the Henri brothers secured more than a hundred negatives with their prototype of the 'normal astrograph' of the *Carte du Ciel*, with sufficiently long exposures to record the positions of stars down to the 14th magnitude. At the encouragement of Prof. Deslandres, then director of the Paris Observatory, Nechvile repeated these photographs with the same instrument after a time lapse of more than 40 years; and from the combined material derived proper motions of almost 3,800 faint stars in certain areas distributed widely all over the sky—probably the largest homogeneous measurements of proper motions of faint stars then available.

This work, which earned Nechvile the Lalande Prize of the French Academy of Sciences, paved the way for an academic career at home. On his return to Prague in 1930, Nechvile became docent of astronomy at Charles University (followed by a recommendation to honorary professorship in 1939), which together with the position of astronomer at the Czechoslovak National Observatory, he held until his retirement in 1960. During his years in Prague, Nechvile's scientific interests continued in the same fields; but increased teaching and administrative duties at the Observatory (Nechvile became acting director during a part of the difficult years of the Second World War) left him but little time for research. This was especially true in post-war years, which for him became a time of increasing solitude. He never married (twice death intervened to deprive him of the prospective companions); his family dispersed (his only brother found his final resting place in England), and so did many of his pupils—of whom I had the privilege of being one. He died in Prague last summer, aged seventy-four, alone,

so that even the exact day when death claimed him remains unknown.

Vincent Nechvile will be remembered with warm affection by all who knew him, as the type of man—so increasingly rare in these days of competitive life—to whom Horace's epithet "*integer vitae scelerisque purus*" can truly be applied. Always kind and gentle, he instinctively shied away from any situation which could have compromised his principles; it is doubtful if he ever harmed anyone, or made a single enemy in his lifetime. Although of somewhat frail health, his strikingly youthful appearance did not desert him until almost the end; and as such he will live in the memories of all who remember him from the pre-war years.

ZDENĚK KOPAL

### Prof. W. Klüpfel

In the death of Prof. Walther Klüpfel on September 16, 1964, at the age of seventy-six, Germany lost an outstanding geologist.

Klüpfel was born on May 28, 1888, in Heidelberg, Germany. He received his training in geology at the University of Heidelberg, where he graduated as a Ph.D. He was appointed as lecturer in the Geological and Palaeontological Institute, University of Giessen, Germany. In later years he held the chair.

During the First World War, Klüpfel was sent to France to work on the water-supply for the German Army. This was resumed during the Second World War, which led him to Jersey, Channel Islands, then occupied by the German Forces. There he remained for three years, during which time he did much valuable field-work in the Island. After the War, he was transferred to the University of Marburg, Germany. The University of Giessen had been heavily bombed.

Since 1921, and in the following years, Klüpfel's research work on volcanic formations convinced him that there is a fundamental difference between Pre-Quaternary volcanoes and Recent and Diluvian volcanoes. His extensive investigations and results were published in Germany. One of these papers was translated into English: "On the Old Type Volcanoes and the New Type Volcanoes and their Origin" (1941).

On his retirement from the chair, with his great devotion to geology, Klüpfel continued his field-work, especially in Jersey. In 1962 he once again visited the island.

Geology to Klüpfel was a whole life's work. He was an exemplary teacher, both in the field and as a lecturer. He was very much liked by his students, although very exacting; he could be most humorous, laughed heartily, and had many sterling qualities. I will remember him with gratitude, affection and admiration.

He died at his home in Giessen, Germany, and is survived by his second wife.

M. CASIMIR

## NEWS and VIEWS

### The Royal Society: Vice-presidents

THE President of the Royal Society, Sir Howard Florey, has appointed the following vice-presidents for the year ending November 30, 1965: Lord Fleck, treasurer of the Royal Society, formerly chairman of Imperial Chemical Industries, Ltd.; Sir William Hodge, physical secretary of the Royal Society, Master of Pembroke College and Lowndean professor of astronomy and geometry in the University of Cambridge; Prof. A. A. Miles, biological secretary of the Royal Society, director of the Lister Institute and professor of experimental pathology in the University of London; Sir Patrick Linstead, foreign secretary of the Royal Society, rector of the Imperial College of Science and Technology; Prof. A. H.

Cottrell, Goldsmiths' professor of metallurgy in the University of Cambridge; Prof. B. Katz, professor of biophysics in University College, London.

### Director of the Bedford Institute of Oceanography, Dartmouth, Nova Scotia: Dr. William L. Ford

DR. WILLIAM L. FORD, chief of personnel at the Canadian Defence Research Board, has been appointed director of the Bedford Institute of Oceanography at Dartmouth, Nova Scotia, and will assume his post on April 1. Formerly Dr. Ford was scientific adviser to the chief of the Naval Staff, and besides being chief of personnel at the Defence Research Board he was a member of the defence research management committee. As director of the Bedford

Institute he succeeds Dr. W. N. English, who recently accepted the position of superintendent of the electronics wing in the Canadian armament research and development establishment at Val Cartier, Quebec. Dr. Ford has carried out research in both the Atlantic and Pacific Oceans. He attended Victoria College and the University of British Columbia, where he obtained his B.A. in 1936 and M.A. in physical chemistry the following year. He continued his postgraduate studies in the same field at Northwestern University in Illinois and obtained his Ph.D. in 1940. Following a four-year period in the Nylon Research Laboratory of the Du Pont Company in Wilmington, Delaware, Dr. Ford directed his attention to oceanographic research in 1944. Until 1948 he carried out research at the Woods Hole Oceanographic Institution. During the atomic experiments at Bikini in 1946, he was a member of the oceanographic team concerned with the drift of radioactive waters at the Bikini site. In 1948 Dr. Ford joined the Naval Research Establishment of the Defence Research Board in Halifax. During 1952-53 he attended the National Defence College in Kingston, and then assumed the position of director of scientific services for the Royal Canadian Navy. He was superintendent of the Pacific Naval Laboratory at Esquimalt from 1955 until 1959, when he returned to Ottawa to assume the position of scientific adviser to the Chief of Naval Staff. In 1963 he was appointed chief of personnel.

#### Chemistry in the University of St. Andrews:

Prof. J. M. Tedder

DR. J. M. TEDDER was recently appointed to the newly created Roscoe chair of chemistry at Queen's College, Dundee, in the University of St. Andrews. He was educated at Dauntsey's School, Wiltshire, and Magdalene College, Cambridge. In 1948 he joined Prof. M. Stacey's group at the University of Birmingham, where he carried out research on organic fluorine compounds. He was appointed University Research Fellow in 1950 and gained his Ph.D. degree a year later. After a further year at Birmingham he continued his studies in the same field with Prof. A. L. Henne at Ohio State University. In 1953 he returned to Britain as an Imperial Chemical Industries Research Fellow at the University of Edinburgh, and in 1955 he was appointed lecturer in chemistry at the University of Sheffield. His research at the University of Sheffield was very productive, and in 1963 he was appointed reader in organic chemistry. Dr. Tedder's early investigations were concerned with trifluoroacetic acid and the use of its anhydride in various organic reactions. His investigations have covered several important areas of organic chemistry and his main contributions have been concerned with the chemistry of diazonium compounds and the examination of gas phase free radical reactions. He pioneered the direct route for the synthesis of diazonium salts and used this method for the preparation of novel acetylenic diazonium salts. He has also been concerned with directive effects in free radical substitution reactions, and recently he has directed his attention towards the factors governing orientation of free radical addition processes.

#### Botany in the University of Liverpool:

Prof. V. H. Heywood

DR. V. H. HEYWOOD, reader in the Department of Botany in the University of Liverpool, has been appointed to the newly established second chair of botany in the University. Dr. Heywood, who is thirty-six years of age, was educated at George Heriot's School, Edinburgh, and the University of Edinburgh, where he graduated B.Sc. with first-class honours in botany in 1949, and was awarded the Class Medal and the MacGillivray Prize in Zoology. He gained a D.Sc. degree in 1964. In 1949 he was awarded a Carnegie research scholarship and a Cross Trust research scholarship at Pembroke College,

Cambridge, where he graduated Ph.D. in 1953. From 1952 until 1955 Dr. Heywood held the post of "Professor agregado" in systematic botany at the Instituto Botánico, Madrid, being appointed special lecturer in experimental taxonomy and technical assessor, Instituto Nacional de Investigaciones Agronomicas, Madrid, in 1955; in the same year he was appointed lecturer in botany in the University of Liverpool. He was promoted to a senior lectureship in 1960 and to a readership in 1963. Dr. Heywood has made a number of expeditions and visits to Spain as well as to Greece, Anatolia and Jugoslavia. He is a member of the Council of the Systematics Association, a member of the Publications Committee of the Botanical Society of the British Isles, secretary of *Flora Europaea* Editorial Committee, and a member of the executive and secretary general (during 1961-63) of the International Organization of Biosystematists.

#### British Museum (Natural History): Appointments

Dr. J. P. Harding

DR. J. P. HARDING has been appointed keeper of the Department of Zoology at the British Museum (Natural History) in succession to Dr. F. C. Fraser, who retired from this post at his own request on November 30 in order to resume his researches on the Cetacea. Dr. Fraser will remain on the staff of the Museum. Dr. Harding, who has been a deputy keeper in the Department of Zoology since 1961, was educated at Torquay Grammar School; University College, Exeter (now University of Exeter); the University of Cincinnati, and King's College, Cambridge. Prior to joining the Museum in 1937, he was for a year an assistant naturalist in the Ministry of Agriculture and Fisheries. Dr. Harding is a carcinologist specializing in the Entomostraca.

Dr. R. H. Hedley

DR. R. H. HEDLEY has been appointed a deputy keeper in the Department of Zoology in succession to Dr. Harding. Dr. Hedley, a graduate of the University of Durham, joined the Museum in 1955 and is a specialist in the biology and cytology of the Foraminifera, with a special interest in the distribution of inter-tidal forms. He held the New Zealand national research fellowship in 1960.

#### Charles Hatchett (1765-1847)

CHARLES HATCHETT, son of a wealthy coach-builder of Long Acre, London, was born two hundred years ago on January 2. Later he was to become noted as the discoverer of what he named 'columbium'—a discovery which he announced in a paper to the Royal Society of London in 1801. He detected it in a mineral (columbite), using part of a specimen collected in New England by Governor John Winthrop the younger (1606-76)—himself a chemist and physician—and sent to Hans Sloane by Winthrop's grandson (the specimen is at present in the British Museum (Natural History)). In 1802 Anders Gustaf Ekeberg (1767-1813) announced the discovery in several minerals of a new metal to be called 'tantalum'. In 1809 William Hyde Wollaston analysed both columbite and tantalite and considered the metals columbium and tantalum to be identical. This opinion, from a chemist of great authority, was generally accepted until 1846, when it was refuted by Heinrich Rose. As tantalum had been named after Tantalus, mythical king of Phrygia (to whom all things were elusive), Rose decided to rename columbium 'niobium' after Niobe, daughter of Tantalus and goddess of tears. Niobium is the name now preferred in Europe and the one which has been accepted by the International Union of Pure and Applied Chemistry; however, the term columbium is still generally accepted in the United States. Hatchett had not in fact isolated the metal, and this was not done until 1864 by C. W. Blomstrand. A versatile chemist, Hatchett worked on, *inter alia*, the nature of lac, magnetic properties of minerals, substances of vegetable origin and (with Caven-

dish) on the durability of the gold coinage, and, in his decade of inspired research from 1796 until 1805, published, through the Royal Society, papers on the analysis of mineral waters, dental enamels and various other topics. Yet, as the historian Thomas Thomson lamented, his "lucrative business weaned him from scientific pursuits"; his withdrawal from chemistry was much regretted by his contemporaries. Apart from his chemistry and coach-building, Hatchett was active in the Literary Club, in which Davy, Wollaston and Blagden also assured representatives among scientists, and also in the Animal Chemistry Club, where Davy, Brodie and Brand discussed work on physiological chemistry.

### The Royal Society of Edinburgh

BESIDES details of its administration, recipients of its awards, meetings and lectures, and election of Fellows, the *Year Book of the Royal Society of Edinburgh* contains information on the contributions which many distinguished past and present Fellows have made to the growth of science and technology (Pp. 136. Edinburgh: Royal Society of Edinburgh, 1964. 20s.; 3.50 dollars). One outstanding Fellow, for example, was Sir William Wallace, who was managing director of Brown Bros. and Co., Ltd., Edinburgh, the marine engineers, from 1916 until 1957. It is reported that "In 1917 he was on board the submarine K. 13 when she sank during trials in the Gareloch, and was instrumental in saving the lives of many on board by blanking off a hydraulic pipe with a two-shilling piece inserted in a pipe coupling". From about the date of William Wallace's appointment as managing director, Brown Bros. and Co., Ltd., has been continually employed by the Admiralty on the development and manufacture of a variety of equipment: in the early 'thirties the company obtained an order to manufacture catapults to an existing design, followed by further development to suit the changing requirements of the Service. The early 'thirties also saw the start of a new venture, when Sir Maurice Denny, of William Denny and Bros., Dumbarton, collaborated with William Wallace in developing the Denny-Brown ship stabilizer: the first was fitted on a cross-channel ship in 1936, followed by a number of similar installations on a large number of H.M. ships during the Second World War, and then, the first of many 'big ship' installations, in the *Chusan* in 1952. Another outstanding record in the *Year Book* is that present developments in management education were foreshadowed by Prof. William Oliver's appointment to the part-time lectureship in organization of industry and commerce at the University of Edinburgh in 1919. During the thirty-three years in which Prof. Oliver was in charge of the Department many important developments occurred. The subject, which was originally confined to the degree of Bachelor of Commerce, was added to the curricula of the degrees of B.Sc. in engineering, technical chemistry and mining, as well as the certificates and diplomas of social study, administrative law and practice and public administration.

### Institute of Navigation

THE annual report of the Council of the Institute of Navigation for 1963-64 covers the year ended June 30, 1964, and records an increase in membership from 2,061 to 2,245 during the year (Pp. 18. London: The Institute of Navigation, 1964). The fourth tripartite conference, organized jointly with the Institut Français de Navigation and the Deutsche Gesellschaft für Ortung und Navigation, was held in Eastbourne in May. As a result of general agreement that immediate measures should be taken to enable ships to navigate through heavily congested areas more efficiently and with more safety, a working group was set up to determine and to propose solutions for the areas in which the problem was most acute. A working group had already been set up dealing with the Dover Straits, and the work of this committee was brought to a satisfactory

conclusion in the year, with 3,513 replies in favour of introducing some form of routing in the Dover Straits in addition to improving the navigational marks and other aids. The report of the working group after consideration by the Maritime Safety Committee in January 1962 is receiving further examination by numerous Governments. The report also refers to the lack of sufficient definite support for Sir John Cass College, London, to start the proposed course in the science and technology of navigation, leading to the award of the College diploma. The course has now been shortened by various arrangements to cover the same subject in 30 weeks instead of 60, but it still appears that many organizations will find it difficult to release employees long enough to take it.

### International Union against Cancer

THE first report of the Director of this Union, which was founded as a voluntary organization in 1935, covers the work of the years 1962-63 (*Activities Report, 1962-63*. Pp. 47; *Manual of the International Union Against Cancer*. Pp. 94. International Union Against Cancer: Geneva, Post Box No. 400, 1964). The president during these very active and productive years was Dr. Delafresnaye, who has now retired and is succeeded by Dr. Alexander Haddow. The Union owes much of its success during these two years to Dr. Harold F. Dorn, who has been its general secretary for 10 years. One of the improvements effected by him was the establishment of a central office in Geneva required for the administration of the Union's fellowship programme instituted in 1960 at the request of the Eleanor Roosevelt Cancer Foundation. The director's report discusses the work of this central office and the other work of the Union; the work of the Commission on Cancer Research and its related Committees and that of the Commission on Cancer Control and the Union's relations with the World Health Organization. The report concludes with a bibliography on various aspects of cancer research, a list of the members of the Union belonging to 67 countries and financial statements for the years 1962 and 1963. The *Manual* describes, in both English and French, the origin of the Union, its present organization and its activities.

### Population Statistics

IN the most recent issue of the Registrar General's *Quarterly Return for England and Wales* a new set of statistics showing seasonally adjusted quarterly figures of birth occurrences and birth rates are given (Quarter ended June 30, 1964. No. 462, second quarter 1964. Pp. 24. London: H.M.S.O., 1964). These figures make it clear that the recent slight decline in the birth rate was temporary; the seasonally adjusted rate of 18.6 for the second quarter is the highest since 1947 and would indicate that there may be approximately 870,000 births in 1964, compared with 856,000 in 1963. The natural increase for the first half of 1964 was approximately 165,000. Infant and neo-natal mortality have continued to fall. The expectation of life shown by the abridged life table, which is normally published in the second quarterly return, is very slightly lower than twelve months ago, mainly as a result of the higher death rates in the exceptionally severe winter of 1963.

At the same time as this *Return* was published, the *Population Bulletin* of the Population Reference Bureau Inc., a private American organization, has devoted its September issue to the new pattern in American fertility (Vol. 20, No. 5, *New Patterns in U.S. Fertility*. Pp. 113-140. Washington, D.C.: Population Reference Bureau, Inc., 1964. 50 cents). In the United States, too, fertility is rising rapidly, and the recent slight decline in births is probably due only to a shift in the age distribution of women of reproductive age. As the larger birth cohorts of the 1950's begin childbearing themselves, the number of births is likely to go up sharply. The *Bulletin* article deals

with some of the social factors which influence fertility, and directs attention to the growing importance of the gap in the fertility between different religious groups, which is accompanied by a narrowing of income, educational and occupational differentials. It seems clear that relative affluence in the two richest countries of the world has led to a resurgence in population growth. Unfortunately, much less research on the subject has taken place in Britain than in the United States, and a detailed assessment of fertility changes, such as is provided by the survey in the *Population Bulletin*, is not possible for Britain at the moment.

### Mental Health in Britain

THE recent *Supplement on Mental Health* to the Registrar General's *Statistical Review* for 1960 is the last of a series of reports on this subject, which have appeared since 1949, to be issued by the General Register Office (Pp. vii + 191. London: H.M.S.O., 1964. 15s. net); future reports will be issued from the Ministry of Health. The report contains the usual data on admissions, discharges and lengths of stay in mental and mental-deficiency hospitals, classified by sex, age and diagnosis, which one has come to expect in this publication. There are two principal innovations. Admissions and discharges by age and sex are given for a sample of 20 individual mental hospitals, selected so as to give adequate coverage of different size groups, as size is considered to have an influence on the movement of patients. In the second place, there is a decrement table, showing the number and percentage of patients admitted in 1959 who remained in hospital, died or were discharged at various periods up to one year of their date of admission. These data are given for each sex by three age groups, and it is hoped in subsequent years to break these down further by marital status and diagnostic group.

### Day Centres for Children

THE series published by the World Health Organization entitled *Public Health Papers* is intended as a medium for the publication of occasional papers prepared as contributions to the study of particular health problems. No. 24 of the series is entitled *Care of Children in Day Centres* (Pp. 189. Geneva: World Health Organization; London: H.M.S.O., 1964. 7 Sw. francs; 12s.; 2.25 dollars). The publication consists of ten chapters by eight authors on various aspects of the subject and includes a contribution from the International Labour Office and articles on day centres in Poland and Paris. It should be useful to everyone who is concerned with the care of children.

### South African National Parks

*Koedoe*, the journal for scientific research in the National Parks of the Republic of South Africa, No. 7 (1964), contains fifteen papers, mostly on zoological subjects, both vertebrate and invertebrate, with two each on botany and geohydrology. The Parks present a magnificent opportunity for research, and the National Parks Board of Trustees is to be congratulated on publishing this valuable journal incorporating the results of work carried out under its auspices. The present issue contains several systematic and faunistic papers, and an interesting ecological study of the plant communities of the sandveld flora of the northern section of the Kruger National Park by H. P. van der Schijff. F. C. Eloff contributes new observations on the predatory habits of lions and hyenas, and an unusual paper by K. Babich gives an account of animal behaviour with respect to tourists in the Kruger National Park.

### Precise Measurement in Engineering

A 16-MM FILM entitled "Precise Measurement in Engineering" has been prepared by Film Workshop, Ltd., for the Department of Scientific and Industrial

Research through the agency of the Central Office of Information. Modern mass-production techniques demand accuracy in the design and manufacture of components in order that randomly selected parts may be interchangeable. The film examines the significance of precision measurement to the engineering designer and to the industry as a whole. In dealing with present industrial techniques of inspection, it shows how factory floor gauges are checked against factory standards which are, in turn, checked against standards maintained by the National Physical Laboratory. The film, which runs for 28 min., shows the principles and practice of precision measurement, and will be of special interest to production engineers, designers and quality control inspectors. Engineering students in particular should find the animated sequence on interferometry most valuable. Applications for loan should be addressed to the Central Film Library, Government Building, Bromyard Avenue, Acton, London, W.3, the Scottish Central Film Library, 16-17 Woodside Terrace, Charing Cross, Glasgow, C.3, or the Central Film Library of Wales, 42 Park Place, Cardiff.

### Steam Curing of Concrete at Atmospheric Pressure

IN the production of precast concrete products, steam curing is a method increasingly used for the purpose of accelerating the hydration of the cement. Some advantages of this process are that it permits earlier removal of the product from moulds and pallets and allows earlier transfer of prestress in the case of pretensioned prestressed units; the use of steam keeps the concrete moist, at the same time raising its temperature. Steam curing, otherwise known as 'damp heat' or 'low-pressure steam curing', is practicable up to a maximum temperature of 100° C at atmospheric pressure; at higher temperatures pressure must be raised appropriately to keep the steam saturated. This subject is discussed in some detail in a paper by P. W. Keene entitled "Concrete Cured in Steam at Atmospheric Pressure" (*R/Bou* 112, National Building Research Institute, South African Council for Scientific and Industrial Research, Pretoria. Reprinted from *Prestress*, 13; December 1963). Mr. Keene finds that "Maximum early strength is obtained by delaying the application of steam until a certain time after casting, the length of this period depending on the total period available for steam curing. Under optimum conditions it is possible to obtain within one day at least 50 per cent of the compressive strength that would be obtained at 28 days by curing at normal temperature". Of the other characteristics involved, tensile strength and modulus of elasticity at early ages are increased, but to a lesser extent than the compressive strength; there is a reduction in initial drying shrinkage and creep; permeability is increased, but there is a tendency for frost resistance to be reduced; bond with plain steel reinforcement is reduced; ultimate load of pretensioned prestressed units may, in certain circumstances, be reduced; and corrosion of steel reinforcement is increased if calcium chloride is present. The application of steam curing is restricted to products made with certain types of cement; some cements lose strength at high temperature, for example, high alumina cement products above 25° C, supersulphated cement above 40° C. This paper is an excellent summary of present-day knowledge of the effects on concrete of steam curing at atmospheric pressure.

### Gemstones in New South Wales

THE Geological Survey of New South Wales (Department of Mines) is in the process of issuing a Summary Report Series covering the mineral industry of that State, to replace the valued *Mineral Industry of New South Wales* (1928) now long out of print. Of the 45 reports projected in this new series, many of which are apparently not yet published, No. 18 on *Gemstones* (compiled by G. Rose. Pp. 60. Sydney: Government Printer,



1960. 4s.) is of particular interest to the general reader of the subject. The minerals dealt with, in the order listed in this report, are: opal; diamond; sapphire and ruby; emerald and beryl; topaz; turquoise; garnet; spinel; titanite; zircon and quartz. In the introduction it is noted that: "New South Wales opal is world famous and has constituted 88.6 per cent of the value of all gemstones produced in this State. This must give opal the rank of our premier gem" (see also *Nature*, 204, 1151; 1964). To be used as gems, the quality of all these minerals must be of the highest standards; "... unfortunately the Australian diamonds and sapphires are generally poor when compared with overseas material". This does not detract from their industrial value for other purposes, however. The general treatment of each of the other minerals described follows an orderly scheme of information on composition and properties, varieties (where applicable), history and mode of occurrence, and occurrences in New South Wales. In a short section under the heading "Miscellaneous", it is recorded that: "Several other minerals occurring in New South Wales have been used for semi-precious stones. Alunite, azurite, chlorite, fluorite, jet, malachite, prehnite and rhodonite may be included in this group". This report concludes with a useful list of references, chiefly to literature concerning New South Wales; it also includes a small-scale folding map of the State depicting, in colour, areas and 'spot' localities where opal, diamond and sapphire are separately exploited.

### Phosphate Deposits of the South-West Pacific

In 1955 a programme of exploration for island phosphate deposits, to supplement the dwindling reserves of Ocean, Nauru, and Christmas Islands, was agreed on by the Australian and New Zealand Governments, and a noteworthy report resulting from these investigations has recently been published (*A Survey of Phosphate Deposits in the South-West Pacific and Australian Waters*. By W. C. White and O. N. Nairn. 173 pp. *Bulletin* No. 69, Bureau of Mineral Resources, Geology and Geophysics, Commonwealth of Australia, 1964). The Ocean Island phosphates discovered can be grouped into the four classes of (1) avian guano deposits of modern and recent date; (2) phosphatized sands (phosphatic guano) on cays and atolls, resulting from the decomposition of guano under warm arid conditions and subsequent metasomatic replacement of underlying carbonate sands; (3) ferruginous-aluminous phosphates, often oolitic, on elevated limestone islands, formed under lagoonal conditions from an admixture of phosphatized sediments and guano residues with transported terra rossa clay derived from limestone weathering; (4) high-grade phosphate rock on flat-topped oceanic islands, occurring between pinnacles of dolomite, with a more complex history. Of the new finds, those of significant size are restricted to Bellona Island in the British Solomon Islands Protectorate, but their potentialities do not materially augment the resources previously known which, at the present rate of production, will be exhausted in about thirty years. Under ideal conditions where arid islands are adjacent to regions with an upsurge from depth of cold, phosphatic, oceanic water, providing the nutrient for extensive marine life on which birds subsist, the rate of deposition of modern avian guano deposits can be as high as 8-10 cm annually. In addition to valuable discussions on the genesis of these phosphates, this well-written memoir provides a review of geological and topographical conditions in the Solomons, the islands off Papua and New Guinea, the Gilbert and Ellice Islands, Ocean Island and Nauru, Christmas Island, the Fiji archipelago, and the islands of the Northern Territory of Australia.

### International Conference on Thermal Analysis

THERMO-ANALYTICAL techniques, and particularly thermogravimetry and differential thermal analysis, are

becoming increasingly common in research and in industry, and are now being applied in fields quite distinct from those for which they were developed. The techniques, too, have developed differently in various countries, depending on the outlook of the research workers and the apparatus available. Consequently, it has been considered by many interested parties that a broadly based international interdisciplinary convention should be organized at which problems, questions of interpretation and results can be discussed. Moves in this direction have now been made by a small international committee (consisting of Prof. R. Barta, Czechoslovakia, Prof. L. G. Berg, U.S.S.R., Prof. L. Erdey, Hungary, Dr. R. C. Mackenzie and Dr. J. P. Redfern, Great Britain, Dr. C. B. Murphy, United States, and Prof. T. Sudo, Japan), which is at present organizing the "First International Conference on Thermal Analysis", to be held in Aberdeen, Scotland, during September 6-9, 1965. The main theme of the conference will be "Thermal Techniques and their Applicability" and circulars giving details are now being distributed. These, together with any further information, can be obtained from Dr. R. C. Mackenzie, the Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, Scotland, or from any other member of the committee.

### The Chemical Institute of Canada

DR. ROGER M. BUTLER of Sarnia, Ontario, has been elected chairman of the Chemical Engineering Division of the Chemical Institute of Canada. Dr. Butler received his degrees from the Imperial College of Science and Technology, London. From 1951 until 1954 he was assistant professor of chemical engineering at Queen's University, Kingston, Ont. Since 1954 he has been in Sarnia with the research department of Imperial Oil on process development and more recently as senior research chemist for new projects. The Chemical Engineering Division is the engineering arm of the Chemical Institute of Canada, Canada's national organization of chemists and chemical engineers. Dr. Butler succeeds Dr. W. J. M. Douglas of McGill University, Montreal. Other new officers elected were: *Vice-chairman*, Dr. D. B. Robinson, University of Alberta, Edmonton; *Secretary-Treasurer*, Dr. N. E. Cooke, Canadian Industries, Ltd., Montreal.

### Announcements

MR. FREDERICK D. MCCARTHY, formerly curator of anthropology, Australian Museum, Sydney, has been appointed principal of the Australian Institute of Aboriginal Studies, Canberra, and took up his duties on November 16. The Institute was set up by the Commonwealth Government of Australia to record information about the Australian Aborigines and their culture by promoting research through universities, museums and individual field workers.

THE twelfth annual conference of the Western Spectroscopy Association will be held in Asilomar, California, during February 4-5. The programme will include sessions on: spectroscopy with lasers; molecular vibrations; molecular spectroscopy of planets and stars; non-radiative energy transfer. Further information can be obtained from M. W. Windsor, Quantum Chemistry Department, TRW Space Technology Laboratories, One Space Park, Redondo Beach, California.

AN international symposium on "The Impact of Mendelism on Agriculture, Biology and Medicine", organized by the Indian Society of Genetics and Plant Breeding, will be held at the Indian Agricultural Research Institute, New Delhi, during February 15-20, to commemorate the Silver Jubilee year of the Society and the centenary year of the publication of Mendel's classic paper. Further information can be obtained from Dr. A. T. Natarajan, Division of Botany, Indian Agricultural Research Institute, New Delhi 12.

## EDUCATION FOR BUSINESS AND MANAGEMENT

THE debate on education for business and management which Lord Alport opened in the House of Lords on November 18, in which Lord Snow made a distinguished maiden speech as Parliamentary Secretary to the Ministry of Technology, greatly transcended any previous debate in Parliament this session, and was, moreover, free from the pettiness which has marred most of those in the House of Commons so far. Lord Snow did not attempt to claim all the credit for advances in management education for the Government, but, apart from his tributes to the work of Lord Franks, Lord Robbins, Lord Nelson of Stafford and Lord Normanbrook, for example, generously acknowledged the foundation laid by the previous Government and stressed the need for close co-operation between Government, industry and the academic world, without concern as to who received the credit. After mentioning the support which Lord Nelson had secured from industry for the two new business schools, Lord Snow stressed the imperative necessity for us to use with intelligence and imagination every scientific resource we had and he put management studies among the twelve or twenty most important tasks we had to tackle for survival within the next two or three years. Besides the two new institutions there were courses at various universities or colleges of advanced technology leading to a postgraduate degree, courses at regional colleges of technology leading to diploma in management studies, and finally a large amount of management education within firms themselves. Lord Snow declined to say that any one method was more important than the others: all would be needed. Probably less than 3 per cent of managers required no training, and apart from perhaps 20 or 30 per cent who would not be improved, the rest would be greatly improved by training. The next problem was to find the teachers, and here much more part-time help from industry was essential. Industry, too, had to make appropriate dispositions to utilize those who had been trained; finally, there was the vital problem of effective selection.

Most of these points had been put in questions by Lord Alport, but Lord Snow did not reply to his question about the need for increased research facilities. This aspect was specially emphasized by Lord Bowden later in the debate, who thought it was even more important that industry should co-operate in this field than that it should co-operate in teaching. Unless research workers from a business school could go into industry and investigate industrial problems on the shop floor the subject would never advance. Lord Bowden illustrated his point by referring to two investigations carried out from the Manchester College of Science and Technology within the past two or three years. The first, with the active support of the Ministry of Health and the collaboration of the hospitals concerned, was into a group of hospitals of similar size, appearance and scope and revealed remark-

able differences in the skill with which hospitals could retain and train nursing staff and in the time taken for a patient to recover from similar conventional operations. Another survey, of the attitudes of workpeople to general problems of innovation and change, indicated variations between factories which greatly exceeded those within a factory, and the differences in attitude of mind were strongly correlated with the communications system within the factory.

Lord Bowden maintained that such problems of the pathology of a hospital or factory were much more easily handled by investigators with the impartial reputation that a business school or a university could ensure and he believed that in this way such institutions could make an important contribution in efficiency.

Earlier in the debate, the Bishop of Chichester, supported by Lord Kilmuir, had emphasized the personal aspects and particularly the importance of people, alike in research and in training for management: there was a moral aspect which could not be disregarded. Lord Plowden put his emphasis on co-operation between industry, the universities and the Government, while Lord Nelson of Stafford stressed the importance of making the most of existing experience, of securing an adequate supply of trained teachers, of effective co-ordination between the various types of courses, and of the Government ensuring that adequate grants were available for the students who came forward. Lord Chorley, who referred to the Beveridge Experimental School of Economics, thought the failure of the earlier schemes was worth studying and that the pioneer work of Lord Beveridge was not sufficiently appreciated. Lord Geddes of Epsom, commenting on the increasing need for managers to be scientifically minded, raised an important point about the use of scientists as administrators: it could be a serious situation if scientists could not easily enter the managerial class if they were competent. Lord Aberdare stressed the need for first-class and imaginative teachers in the business schools and supported Lord Bowden's remarks about constant interchange between the academic and the industrial world. Lord Shackleton, in concluding the debate for the Government, was also studiously fair in acknowledging what had already been done under the previous Government, but was unable to offer at this stage any firm assurance about grants for students, and while he undertook that the Government would continue to co-operate he could say little on co-ordination, and the responsibility for management education, he said, would lie with the Secretary of State for Education and Science, and, for non-university courses in Scotland, with the Secretary of State for Scotland. Although the Ministry of Technology, the Board of Trade, and the Ministry of Labour might also be concerned, management education should properly grow out of the educational system.

## SCIENTIFIC AND INDUSTRIAL RESEARCH IN NEW ZEALAND

THE annual report of the Department of Scientific and Industrial Research, New Zealand, for the year ended March 31, 1964 (Pp. 87. Wellington: Government Printer, 1964. 3s.), records a gross expenditure of £2,607,112, or £2,538,794 net, of which about 42 per cent is estimated to be on agricultural research and 27 per cent on industrial research, including grants totalling £200,577 to incorporated research associations. Rather less than 10 per cent of the total expenditure is for scientific

services, mainly for other Government Departments and for industry. The major items of expenditure were £279,253 on the Dominion Physical Laboratory, £264,265 on the Dominion Laboratory, £165,908 on the Geophysics Division, £138,061 on the Soil Bureau, £132,616 on the Geological Survey, £122,414 on the Grasslands Division, £116,471 on the Antarctic Division, £111,524 on the Plant Diseases Division, and £94,458 on the Oceanographic Institute. The staff increased during the year from 1,229



to 1,287, the latter figure including 472 professional, 552 technical, 182 clerical and 81 others.

The Director-General, directing attention to the applied research which constitutes most of the Department's work, notes that the experimental measures for controlling yellow dwarf virus disease of barley are estimated to have saved £112,000 in one season. He also stresses the importance of the fundamental research on which this applied research is based, instancing the discoveries of the Fruit Research Division relating to plant cell division and the advances in understanding of wood shrinkage and aurora made by the Dominion Physical Laboratory. In a geochemical laboratory of the Geological Survey and University of Otago investigations at high temperatures and pressures have shown that greywackes and argillites constituting many New Zealand mountain ranges lose their chlorite minerals and begin to grow other platy minerals like mica when heated below their melting point at pressures up to 1,000 atmospheres, and the growth of the platy minerals can be oriented in one plane to give schistosity similar to that in large areas of Otago rocks. Laboratory research has also shown that temperature is the most important factor in the conversion of lignite into bituminous coal.

Sheep grazing trials in the Manawatu on New Zealand short-rotation and on Arika and Italian ryegrass indicate that cellulose content is an important controlling factor in the nutritive quality of ryegrass. The Grasslands Division has demonstrated the practicability of high-stocking rates on steep hill country at Te Awa and has found that heavier stocking rates may limit the useful life of ewes by increasing the wear on the sheep's teeth. Movements of mineral constituents through the soil have been demonstrated by the use of radioisotopes, and recent work at the Soil Bureau has shown that severe iron deficiency in *Crotalaria*, a tropical legume used as a green manure crop, can be corrected by use of iron chelate, which is stable under alkaline conditions. Rabbit population investigations have emphasized the importance of natural predators of rabbits in control in the wild, and

field experiments have confirmed the wide existence of grass-grub populations tolerant to DDT. A new crown-rust resistant strain of 'Onward' oat has been bred by the Crop Research Division and released as 'Onward (1963)', and the Division is also testing potato hybrids for processing for export. The Fruit Research Division has now succeeded in isolating from immature sweet corn seeds a natural cell-division stimulant, or kinin, which has been named 'Zeatin', and its synthesis is now being attempted.

Systematic astrolabe observations were recommenced in January 1964, and the Dominion Physical Laboratory has shown that very low-frequency signals travelling over long distances can sometimes suffer such severe phase distortion as to render them useless for navigational purposes. A small-scale process for steel castings has been further simplified by using a charge composed almost entirely of steel scrap and adding silica and manganese as ferro-alloys to produce the heat of reaction. The economics of the process are satisfactory and it now has possibilities for the average New Zealand foundry.

The main programme of geothermal investigation at Ngawha has been completed, but prospects of useful resources within economic depths do not appear hopeful. In investigations of the optimum sulphate content of cements a method has been developed for determining the degree of dehydration of gypsum, and the Wheat Research Institute, from theoretical investigations of the loss of steam from bakers' ovens and the development of an instrument for rapidly measuring the concentration of steam in the oven, has devised important designs for bakers' ovens and a number of travelling ovens have been built to this design. Reports from the various branches are included in the report, to which lists of publications during the year are appended. The sixth annual report of the Ross Dependency Research Committee is also appended, as well as brief reports on grant-aided research in the Cawthron Institute, Lincoln College, Massey University College of Manawatu, and the University of Canterbury.

## SCIENTIFIC POLICY IN BELGIUM

**THE** *Inventaire du Potentiel Scientifique de la Belgique* — *Année 1961*, issued by the National Council for Scientific Policy (Pp. 303. Brussels, 1964) is in three parts. The first sets forth the reasons for the survey, its principal characteristics and the methods used. The second, which occupies roughly one-half of the report, contains the statistical information and appendixes, while a third part, occupying about one-third of the whole, lists the units participating in the survey. The survey estimates the number of effective full-time personnel in 1961 as 22,504 engaged in 1,644 scientific units, and of these 6,703 were of university status, 2,451 held higher technical diplomas and 13,350 were technicians, craftsmen and others. Excluding those in the sector of teaching, inter-university centres and learned societies, the expenditure of these units was about 4,312.9 million francs. Of these 1,644 units, 1,284 were concerned with the exact sciences (628 with technology) and 180 with the social sciences, and another 180 with the other research sciences; the numbers of those with a university degree thus occupied are 5,433, 821 and 448, respectively. Again, in 81.5 per cent of those in the field of education, 71.6 per cent of those in the public sector, 55.7 per cent of those in private industry, 61.4 per cent of industrial or agricultural research associations, 50 per cent of other private establishments and in all the inter-university centres research is an important activity, the percentage of university graduates engaged in such activities being estimated at 85.5, 73.9, 86.6, 71, 56.3 and 100, respectively.

The four universities employed 80 per cent of the scientific units and 88 per cent of the personnel with university qualifications in the educational sector; 56 per cent of the latter was in the faculties of science and medicine. Ministerial departments included 64 per cent of the scientific units and 59 per cent of university graduates in the public sector; 45 per cent of the latter being employed in the Ministries of Education, Agriculture and Defence. Although private enterprise and the industrial and agricultural research associations only include 16 per cent of the scientific units, they include 58 per cent of university graduates engaged in research in this sector, 17 per cent of these being in the research associations. Firms with more than 1,000 employees with research departments numbered only 10 per cent of firms engaged in such activities and employed 49 per cent of the university graduates in this sector.

Nearly half the scientific units and more than half the university graduates engaged in such activities are employed in the province of Brabant, those of Liège and East Flanders coming next with 15 per cent each, mainly in the educational sector in East Flanders. In the exact sciences, mathematics accounts for 54 per cent of the units and 62 per cent of the personnel. Those engaged in technology employed 20 per cent of university graduates engaged in scientific work in industry, but in the educational field only 14 per cent of the scientific units and 14 per cent of university graduates were concerned with technology. In the exact sciences 25.9 per

cent of units and 33.7 per cent of personnel were engaged exclusively or mainly in fundamental research, compared with 62.1 per cent and 54.1 per cent, respectively, for applied research.

Nearly 10 per cent of the scientific units employed no persons with university qualifications and nearly 90 per cent of the units employed not more than 10, while about a quarter had no technicians or trained auxiliaries. Some 60 per cent employed no one with technical diplomas and nearly half the remainder employed only one such person. In the educational sector, 24 units employed some 15 per cent of the university graduates, mainly in the

faculties of science and medicine, while in the public sector 13 per cent were employed in 3 per cent of the units in this sector, especially at the Centre for the Study of Nuclear Energy. Excluding the educational sector in the exact sciences, about half the units spent less than 1 million francs on research in 1961, and half these not more than 700,000 francs. In the public sector, including research associations, half the units spent not more than 3 to 4 million francs, while ten private enterprises, responsible for 60 per cent of the expenditure on such activities by private enterprise, spent more than 50 million francs and 5 of those 100 million francs.

## REGIONAL ADMINISTRATION IN FRANCE

**A** PAMPHLET, *The French Administrative Reform*, issued by the Press and Information Service of the French Embassy, describes the structures of the Departments, of the Paris region, and of the other Regions as modernized by the decrees of March 14, 1964 (Pp. 30. London: Ambassade de France, Service de Presse et d'Information, 1964). The reforms, which will be implemented over a period extending to at least 1965, are based on the results of experiments conducted during two years in five test departments and in the pilot regions of Upper Normandy and Burgundy. The basic principle consists in making the Prefect once more solely responsible for the State service; the sole representative of the Government in the Department, he is the sole co-ordinator of public activities, with the exception of the military services, judicial services, assessment and collection of taxes, payment of public expenses, valuation of national property, factory inspection and supervision of the application of the social loans in agriculture. To ensure that the Prefect is fully informed of all important aspects of life in his Department, a central correspondence service will provide photographic copies of incoming letters, while copies of outgoing letters will also be submitted. Delegation of power is provided for certain highly technical services but delegation of signature allows more freedom and flexibility to heads of services.

A similar principle has been applied to the regions but only to external services concerned with economic matters, public investment and equipment, those which manage Government stocks or participate in programmes

involving national public funds. Such State services in the regions will be placed directly under the Prefect of the Region and cease to be directly under the Paris ministries. The Prefect will accordingly now have a role of initiation and decision in the economic field and a simple function of co-ordination in the administrative field. He will be assisted by an enlarged inter-departmental administrative conference and by a new commission on regional economic development. The new commission will be consulted when the Government prepares future development plans, after the draft has been submitted to Parliament and again after the final planning project has been discussed by Parliament and the operative sections relating to each region have been defined.

This regional reform does not apply to the Paris region, the administrative districts of which are being subdivided to give smaller units while a single assembly will replace the General Council and the Municipal Council, retaining on the whole the powers of the present Municipal Council and assuming those possessed by the General Council of the Seine. The suburbs are to be divided into Departments of normal status, while the pilot prefectures will permit construction of national administrative cities in each chief town. Provision is also made for a system of administrative and economic co-ordination, including a regional assembly or council, the composition of which is not yet fixed. Additional financial resources will be provided and it is anticipated that the new organization should be functioning smoothly by 1968.

## THE BUILDING RESEARCH STATION

**A**T its headquarters in Bucknalls Lane, Garston, Watford, Herts., the Building Research Station (Department of Scientific and Industrial Research) had its 'Open Days, 1964' during September 22-25, when members of the public, including Press representatives, were accorded an unrivalled opportunity of seeing something of the inside workings of this long-established and important organization. Displays and hourly full-scale demonstrations illustrating some of the more important activities of the Station and many items of present-day research were made available to the visitors. In the department concerned with building operations and economics, the theme is research into the efficiency and economy of building operations, development of new techniques, examination of methods of management and organization, and factors influencing costs. Mechanical engineering deals with research applied to production and handling of building components on site and in the factory, and investigations in the engineering services requisite to completed buildings. Environmental physics is concerned both

with factors controlling conditions in buildings and with the subjective reactions of the occupants to these conditions. The section devoted to user requirements and design deals with building design and occupants' requirements in relation to housing and to some special building types. Research in the materials department ranges from the constitution and properties of building materials to investigation of their behaviour in use, including laboratory-scale tests to pilot plant trials. Structural engineering is concerned fundamentally with loadings, with the behaviour both of components and of full-scale and model structures, and with the engineering properties of building materials.

The soil mechanics department was the first to be set up in Britain. It has done pioneer work on the engineering properties of soils and their relationship to the structural behaviour of foundations, tunnels, retaining walls and earth dams. A section devoted to mathematics provides a service for the rest of the Station, and present-day work, reflecting projects in three other departments, includes the

theoretical basis for modular design, the theory of micro-wave techniques for moisture measurement, and the theory of sound transmission through lightweight partitions. Building practice is concerned with external contracts, technical enquiries, co-ordination of work on British Standards and Codes of Practice, building legislation, etc. The tropical building section is concerned with special problems of housing, building and planning in tropical and subtropical countries. Apart from these instructive demonstrations, facilities were available for providing technical information, in the form of leaflets giving background information and details of the researches involved in many of the exhibits; the technical enquiry service was in operation, dealing with questions

on the technical aspects of building construction; the Station library was open to visitors and various official publications of the Station were displayed. In this latter connexion an important publication has since appeared entitled *Developments in Roofing* (Building Research Station Digest 51 (Second Series), October 1964; pp. 6, 4d.). This Digest discusses the properties, construction and laying of roof decks and the weatherproof coverings applied to obtain satisfactory performance. It also deals briefly with the properties of some new roofing materials. This is an excellent and up-to-date summary of both conventional and unconventional roofing practices (excluding tiling) and a copy should be available to architects and all others concerned with modern building construction.

## STRUCTURE AND INTEGRATIVE FUNCTIONS OF NERVOUS SYSTEMS

**D**URING the Southampton meeting of the British Association, Section D (Zoology) discussed some recent progress in our knowledge of nervous systems, particularly the relationship between structure and function. The first two speakers were concerned with invertebrate animals, many of which continue to provide preparations and information which is of value in furthering our understanding of basic nervous mechanisms. The second two speakers presented data from two fields of study which have greatly advanced our knowledge of the mammalian brain during recent years.

Dr. E. A. Robson (Department of Zoology, Cambridge) began appropriately with a quotation from Sherrington's classic *The Integrative Action of the Nervous System*, in which he said "in the multicellular animal . . . it is nervous action which *par excellence* integrates it, welds it together from its components, and constitutes it from a mere collection of organs an animal individual. This integrative action in virtue of which the nervous system unifies from separate organs an animal possessing solidarity, an individual, is the problem before us . . .". In discussing coelenterate nerve-nets, Dr. Robson pointed out that these animals are without a central nervous system and yet show a remarkable degree of integration in their behaviour. Histological studies of the nervous systems of jelly fish and sea anemones by Schäfer and the Hertwigs in 1878 had shown the existence of neurones as separate units. Their work, in fact, provided early support for the neurone theory, which was only established later from data on the vertebrate nervous system. Dr. Robson went on to review the classical work of Pantin and his co-workers which, by means of electrical stimulation and mechanical recording techniques, had made it possible to interpret the behaviour of coelenterates in terms of the properties of neuromuscular and interneural facilitation. Thus it had provided valuable physiological support for the view that the nerve net is synaptic. The distinction was made between through-conducting systems responsible for the protective response of a sea anemone and the swimming beat of a medusa, and the more localized responses which are co-ordinated by a net in which the number of synapses to be crossed appears to be greater.

In addition to these reflex responses, coelenterate behaviour is also characterized by inherent activity, first studied in detail by Batham and Pantin using the sea anemone *Metridium*. This animal shows rhythmic expansion and shortening which are usually synchronized with dawn and dusk, but may persist in total darkness for at least three weeks. The interaction of this and other autonomous phases of activity with responses to environmental stimuli can produce impressive sequences of behaviour—well illustrated by the observations of Ross on the transference of *Calliactis* from one whelk shell to another.

More recent advances in our knowledge of the coelenterate nervous system have resulted from the application of electrical recording methods to these animals. This was first achieved by Horridge, who recorded a nerve impulse in the through-conducting system of the jellyfish, *Aurelia*. More recent work has shown that a variety of electrical phenomena can be recorded using micro-electrode techniques. With electrodes in the foot of *Hydra*, Passano and McCullough recorded a constant background of potentials which arose from this region. They could be excited by light and were possibly endogenous to the endodermal nerve net. They also observed bursts of impulses which were associated with spontaneous longitudinal contractions. These arose from the hypostome and were initially inhibited by light and possibly occur in the endodermal nerve net. Other potentials were associated with attachment of the tentacles during locomotion and other tentacular movements. Clearly there is considerable electrical activity in the nervous system of *Hydra* and exactly what mechanisms are involved is only now being investigated. It suggests that the behaviour of *Hydra* is controlled by a hierarchy of pacemakers which are in turn influenced by sensory stimuli. Josephson has found a similar picture in the marine hydroid *Tubularia*, where rhythmical potentials often not directly related to visible behaviour are also observed. The application of these methods has revealed new physiological problems, since, for example, it appears that there are sometimes more than two separate conducting systems, although only two separate nerve nets, ectodermal and endodermal, have been recognized structurally. Dr. Robson suggested that other structures such as muscle fields may have a role as conducting systems.

It seems probable that, as with other invertebrates, the ability to 'tune in' to the nervous activity will give us far more detailed information about the integrative mechanisms where previously our information was based largely on the overt behaviour and responses of the animal. The application of similar techniques has very recently led to the recording of the electrical signs of nervous action in the remaining major group of animals in which it had not been achieved—the Echinoderms, which also rely a great deal upon a nerve net.

Dr. G. M. Hughes (Department of Zoology, Cambridge) explained that the title of his talk, "Electrophysiological Anatomy of Invertebrate Nervous Systems", had been chosen to emphasize the way electrophysiological techniques developed, for the investigation of detailed mechanisms of nervous function is of value in establishing both gross and micro-anatomical features of nervous organization. Thus, in the crayfish they have shown that the areas innervated by a given segmental nerve are not confined to a single segment. Furthermore, the segmental pattern of the sensory innervation of the abdomen is out of phase with the segmentation of the ganglia supplied by the

sensory nerves. Electrophysiological studies are of particular value not only in establishing the anatomy of the system but also because they give information about the functional connexions between neurones. In experiments on the abdominal cord of the dragonfly larva and crayfish, use has been made of the separation of synaptic regions in different ganglia by splitting the connectives between ganglia and so recording the effect of inputs from different segments. Several types of sensory-interneurone connexion have been established; in a common one a given interneurone makes connexions with homologous sensory inputs entering several ganglia. Impulses initiated in these separate synaptic regions will collide following simultaneous stimulation of inputs to different ganglia. Such interneurons produce different patterns of impulses depending on the particular way the sensory field is stimulated.

The sea hare, *Aplysia*, has provided many useful neurophysiological preparations, one of which has recently been found during electrophysiological studies of the anatomy of the two largest cells on each side of the nervous system. One of these cells (LGC) is in the left pleural ganglion and the other (RGC) on the right side of the abdominal ganglia. These two cells send axonal branches into many of the nerves which innervate the foot and parapodia.

Stimulation of any of these nerves is followed by the recording of an antidromic spike in the cell soma and, conversely, an impulse may be recorded in the axon when the soma is stimulated. It has recently been shown that both cells innervate not only ipsilateral regions but also send branches to the contralateral nerves. Direct stimulation of the RGC in whole animal preparations does not lead to any muscular contraction. They are probably homologous cells whose different positions in the CNS result from the processes of torsion and detorsion during development. Functionally, a direct synaptic connexion between the two cells has been established from the RGC to the LGC but not vice versa, and both cells have several common interneurons. Both giant cells show a remarkable degree of habituation in their response to synaptic inputs, there being a marked fall in the response between the first and second stimuli.

Other *Aplysia* neurones have shown that conduction between two axonal branches is not always equally possible in both directions. This provides a possible mechanism whereby only a part of the total output from a given neurone might be excited by a given input. In this way a single neurone could sub-serve the functions usually ascribed to several neurones. Some recent observations have also suggested that similar mechanisms may be involved in the separate function of portions of neurones in arthropod central nervous systems. Such devices, together with variations in synaptic properties, will be of great value as integrative mechanisms. They also suggest how animals with relatively small numbers of neurones may be able to accomplish more complicated feats of integration than might have been supposed had each neurone functioned in an all-or-none way and as a single unit according to the neurone doctrine.

In concluding the discussion of the first half of the symposium, the President of the Association (Lord Brain) emphasized how, despite many advances in the techniques being used for these investigations, the experimental methods were based on those established by the classical studies of Sherrington.

Dr. J. de C. Downer (Departments of Psychology and Anatomy, University College, London) prefaced his paper on "The Role of Brain Commissures in Interhemispheric Transfer of Training" with a point of view which is generally accepted by students of brain and behaviour, namely, that the ability of an animal to learn depends on the structure and organization of the brain. Hence an obvious technique is to remove specific regions of the brain and to test the ability of the animal to retain some previously learned solution and, if lost, its ability to

re-learn. Results of such investigations have proved extremely difficult to interpret but have demonstrated quite clearly that the vertebrate brain, as with some invertebrate nervous systems, is characterized by the ability of one part to take over the function of another following its ablation. From some classical experiments, Lashley concluded that sub-cortical learning does not occur when the cortex is present and that it can only take place following cortical damage.

Dr. Downer went on to describe experiments in which he had made use of the split brain preparation introduced to this type of study by Sperry and Myers. Their experiments clearly showed that when the visual input is restricted to one-half of the brain (by cutting the crossed optic nerve fibres and occluding one eye) visual information normally crosses from the half receiving the input to the other half via the large commissures. This provided a function for the largest of these (corpus callosum) which had long been conjectural. In the primates, however, it does not connect the visual receiving areas across the midline and there is little evidence to suggest that information can pass from the visual association area to the primary visual receiving area. There seemed a distinct possibility that the visual association area might contain the residual memory store. Experiments designed to test this hypothesis led to the surprising results that, after ablation of this region, a monkey continued to be able to transfer a previously learned ability, and make a given visual discrimination even when the input was confined to the untrained and damaged side. However, such an animal was unable to transfer new discriminations learned through the intact side of the brain. Evidently the memory trace must lie outside the association area removed, but in its absence a new memory trace could not be established through the transfer training technique. However, by restricting the input to that eye which fed into the damaged side of the brain, it was found that the transfer of visual learning from the damaged to the intact side of the brain could still occur.

Using a second group of monkeys, an investigation was made of the role of other neocortical areas in the above experiments (optic chiasma sectioned). All the neo-cortex of the untrained half of the brain was removed except for the primary visual receiving area and motor cortex. When new visual discriminations had been learned on the intact side they did not transfer to the damaged side. The latter could still be trained but usually required about five times as many trials. After training the damaged side it was unexpectedly found that the learned response transferred to the intact side. These experiments showed, therefore, that visual learning does not transfer from the intact half of the brain to that from which most of the cortex outside the primary visual receiving area had been removed. However, if the side sustaining the ablations is trained, the learned response does transfer to the intact side.

A plausible explanation is that when the intact side is being trained the neo-cortex is primarily involved in the learning process. Transfer to the damaged side cannot occur since the neocortical commissures have degenerated. But when the damaged side is trained, sub-cortical structures, probably the hippocampus, sub-serve the learning process and learning transfers to the opposite side via the hippocampal commissures which remained intact in these experiments.

The final talk in the symposium was given by Dr. E. G. Gray (Department of Anatomy, University College, London), who dealt with the relationship between structure and function of the nervous system at a sub-cellular level. He pointed out that during the past ten years electron microscopy has completely changed our understanding of the fine structure of the brain and the all-important transmission regions, the synapses. Before this time, light microscope preparations of silver-stained brain tissues had shown synapses as small bulbs or ring-shaped structures. But the electron microscope has shown that these struc-



tures are fine bundles of protein neurofilaments within the axon and its presynaptic terminal expansion. The light microscope did not show their true shape or the various organelles present at the synapse itself. Most pre-synaptic terminals do not contain neurofilaments and are not observed in silver preparations. Consequently there was a considerable underestimation of the number of synapses in a given region. In particular, the apparent absence of typical synapses in the cerebral cortex caused much embarrassment to theorists on cortical function. However, abundant synaptic contacts in the cortex have now been revealed by the electron microscope.

Electron microscopy has also revealed the presence of numerous synaptic vesicles in the pre-synaptic terminals and it is believed by some that these contain chemical transmitters (acetylcholine at some synapses) which are released into the synaptic cleft by an impulse in the pre-synaptic fibre. Recent work has revealed regularly shaped dense structures at the synaptic membrane and these may function as focal points or pores for the liberation of transmitter substances. Most vertebrate and invertebrate synapses show this chemical-release method of synaptic transmission, but certain specialized synapses depend on direct electrical transmission from pre- to post-synaptic elements without involving a chemical-release mechanism.

In some cases these synapses appear to differ from chemically transmitting synapses in that there is a fusion of the pre- and post-synaptic membranes.

Much morphological variation of pre- and post-synaptic components can be observed with the electron microscope. Especially interesting is the occurrence of numerous spinous outgrowths, once regarded as artefacts in Golgi-method impregnation material, which can be seen to have synaptic contacts at their tip. A peculiar organelle, 'the spine apparatus', occurs within the spine cytoplasm of neurones of the cerebral cortex. Comparable apparatus is not known from other non-mammalian nervous tissue and at present we have no information about its function. Future advances may make it possible to distinguish excitatory from inhibitory synapses and perhaps to detect the sort of structural modifications which probably take place at synaptic membranes during the process of learning.

The whole symposium was concluded by a very profitable discussion. It had certainly given a broad view of the integrative action of the nervous system. Furthermore, as Sir Wilfrid Le Gros Clark pointed out in his final remarks, it had demonstrated the value of the integration of anatomy, zoology and physiology.

G. M. HUGHES

## ACCURACY OF SEA GRAVITY SURVEYS: COMPARISONS OF SHIPBOARD AND SUBMARINE GRAVITY VALUES

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**S**HIPBOARD gravimeter surveys are the latest tool of marine geophysics<sup>1</sup>. The accuracy of any particular survey is very difficult to assess, and very often can only be estimated by intelligent guessing because of the uncertainty of the navigational correction (Eötvös correction)<sup>2,3</sup>. When sufficiently close to land, the ship's position can be determined either by using direct fixes on shore marks or by using precision electronic positioning devices (Decca). An estimate of the accuracy of the shipborne gravimeter can be obtained in this case either by evaluating the gravity differences at track intersections<sup>4</sup> or by comparing shipboard values with those obtained by means of gravimeters placed on the bottom of the sea<sup>5,6</sup>. The latter technique is the only one which provides a means of comparing shipboard values with reference values which are at least an order of magnitude more accurate. However, the bottom gravity measurements can be made only in the depth of water normally found on the continental shelves. Yet the largest part of oceanic survey is carried out over abyssal depths and a method is required for checking the sea gravimeter measurements obtained over the deep ocean.

If a buoy can be placed in deep water and a close survey is carried out within radar range of it<sup>7</sup>, then the accuracy of the sea gravimeter may be evaluated by comparing the gravity values obtained on different tracks at their intersections<sup>8</sup>. This is a special case, and for most oceanic surveys the only available means of checking is to attempt to re-occupy previously established submarine pendulum stations. This article describes the checks during the first two years of operation of a Graf-Askania sea gravimeter by the Department of Geodesy and Geophysics, University of Cambridge.

The method of submarine gravity measurements<sup>9,10</sup> has been established for more than thirty years and the root mean square error of a single station observation has been estimated as  $\pm 3.4$  mgals (ref. 11) or as  $\pm 3.6$  mgals (ref. 12). The main difficulty in using submarine stations

as check points is due to the inherent problem of locating a ship at sea: our methods of celestial navigation have not changed for at least a hundred years. If the weather conditions are ideal and the drift due to currents and wind can be allowed for, then the ship's position can be estimated with an accuracy of 1–2 miles. Viewing conditions are seldom ideal even when the sky is not completely overcast, thereby increasing the error in the estimated position. In the past the location of submarine stations was usually chosen with particular geophysical problems in mind: to investigate the crustal structure over the continental slope, the seamounts, deep trenches or mid-ocean ridges. In each case the horizontal gradients are large, and small error in the position of either the submarine or ship makes comparison between their respective gravity values meaningless. In spite of all the foregoing arguments, re-occupation of submarine stations gives a worth-while check, and should be attempted whenever the research vessel's track can be conveniently arranged to pass near a known submarine pendulum station.

The eastern Atlantic is a particularly good area for checks of the shipborne gravimeter because of the large number of submarine stations which are fairly uniformly distributed. Forty-seven of these stations were re-occupied during the 1960 cruise of R.R.S. *Discovery II* and the 1961–62 cruise of H.M.S. *Owen*<sup>13</sup>. Thirty-eight comparisons were available for evaluation and they are presented in Fig. 1 and Table 1.

The details of comparisons are given in Table 1 so that other investigators can make further checks if there is an opportunity to do so. The serial numbers in column 1 have been arbitrarily assigned. The distances, given in column 5, between the reported position of the submarine station and the nearest approach of the ship's track have a probable error of two miles due to navigational uncertainties in position. The gravity values are the free air anomalies and the three values given for the ship are 10 min (1.5–1.8 nautical miles) readings around the

Table 1. COMPARISONS BETWEEN SHIPBORNE GRAVIMETER AND SUBMARINE PENDULUM GRAVITY VALUES

Part 1: Atlantic Ocean, R.R.S. <i>Discovery II</i> , 1960								
Serial No.	Station No.	Position	Ship-submarine (miles)	Gravity anomaly (mgal)			Difference ship-submarine	Notes
				Submarine	Ship			
1	W2829	N 45° 12' W 17° 49'	4	3	27	28	25	
2	W2832	N 46° 57' W 16° 28'	5	6	17	17	11	
3	W2833	N 47° 00' W 15° 46'	3	-2	17	20	22	a
4	W2834	N 47° 08' W 15° 03'	6	2	7	6	9	
5	W2836	N 47° 44' W 12° 49'	8	10	9	14	18	
6	W2837/8	N 47° 54' W 12° 21'	10	-6	4	9	15	b
7	W2841	N 48° 37' W 9° 40'	4	65	66	70	72	
8	W2842	N 48° 45' W 9° 03'	8	11	27	27	27	
9	W2843	N 48° 58' W 8° 12'	8	6	36	33	31	
10	VM492	N 45° 21' W 11° 50'	1	4	2	4	8	
11	VM731	N 45° 31' W 13° 16'	6	30	42	40	38	
12	VM836	N 45° 29' W 13° 20'	4	29	46	45	42	
13	VM427	N 47° 44' W 9° 21'	7	-75	-62	-60	-57	c
14	VM727	N 47° 42' W 9° 25'	10	-60				
15	VM488	N 48° 08' W 7° 18'	5	42	48	48	47	
16	VM489	N 47° 34' W 8° 19'	7	0	54	54	52	d
17	B9	N 47° 24' W 14° 11'	3	14	15	14	13	
18	B10	N 47° 07' W 15° 05'	6	12	6	9	12	
19	B11	N 46° 59' W 15° 59'	0	37	31	36	41	
20	B12	N 47° 45' W 13° 21'	7	13	10	10	10	
21	B6	N 48° 57' W 8° 38'	6	0	10	11	13	
22	B7	N 48° 40' W 9° 37'	2	58	64	66	70	
Part 2: Atlantic Ocean, H.M.S. <i>Owen</i> , 1961								
23	VM489	N 47° 34' W 8° 19'	4	0	44	45	43	d
24	VM841	N 47° 15' W 9° 09'	2	-29	-29	-26	-22	
25	VM490	N 46° 49' W 9° 30'	1	7	21	6	-3	
26	VM483	N 44° 42' W 10° 25'	7	11	29	21	14	
27	VM482	N 43° 47' W 11° 34'	1	-65	-60	-61	-61	
28	VM481	N 42° 37' W 11° 54'	2	123	121	125	129	
29	VM479	N 40° 42' W 12° 11'	7	-25	-19	-20	-21	
30	VM811	N 38° 04' W 13° 56'	2	66	59	58	55	
31	W2815	N 36° 38' W 13° 55'	3	47	38	38	37	
32	VM475	N 36° 30' W 12° 42'	5	119	93	82	73	
33	W2814	N 36° 28' W 11° 42'	3	295	229	267	294	e
34	W2813	N 36° 27' W 11° 20'	2	160	300	250	200	e
35	W2812	N 36° 19' W 10° 13'	4	-65	-57	-54	-49	
36	W2811	N 36° 14' W 09° 18'	2	-40	-29	-38	-43	
37	W2810	N 36° 13' W 08° 34'	2	52	33	42	53	
38	W2809	N 36° 01' W 06° 34'	1	-61	-45	-46	-45	
39	W2808	N 35° 58' W 05° 55'	1	-87	-72	-78	-84	
Part 3: Indian Ocean, H.M.S. <i>Owen</i> , 1961-62								
40	H272	N 12° 35' E 45° 08'	1	-35	17	-9	-12	f
41	H271	N 12° 56' E 47° 24'	2	24	14	17	13	
42	H270	N 13° 00' E 48° 55'	2	-23	-22	-17	-13	
43	H269	N 13° 13' E 51° 44'	4	14	39	24	13	
44	H267	N 13° 12' E 53° 32'	2	0	-2	2	9	
45	VM20	N 7° 53' E 65° 58'	1	-33	-31	-34	-31	
46	H263	N 8° 12' E 72° 42'		9				
47	VM22	N 8° 06' E 72° 48'		-34	-18	-14	-9	g

a, W2833 is 13 miles from B11 but 39 mgal lower. b, 12 mgal difference for a repeat measurement. Depths at two stations 2 miles apart were 4,237 m and 3,672 m. c, Two submarine stations close together. d, See discussion in text. e, Large local gradient due to topography. This value ignored in calculation of root mean square error. f, Near the shelf break; large gradient. g, H263 in 625 fathoms and VM 22 in 1,020 fathoms of water. The depths corresponding to three ship values given were 870, 810, 780 fathoms respectively. If both submarine and shipboard gravity values are plotted against depth, they all fall on a straight line.

nearest approach. From these three readings horizontal gradients along the ship's track can be estimated.

All the shipboard measurements were made with the Askania-Graf GSS 2 sea gravimeter serial No. 11. In 1960 this gravimeter was mounted on a prototype gyro-stabilized platform built for Dr. A. Graf by the Anschütz G.m.b.H. of Kiel, Germany, and owned by the Deutsches Forschungsgemeinschaft. The measurements since 1961 have been performed with the gravimeter mounted on the

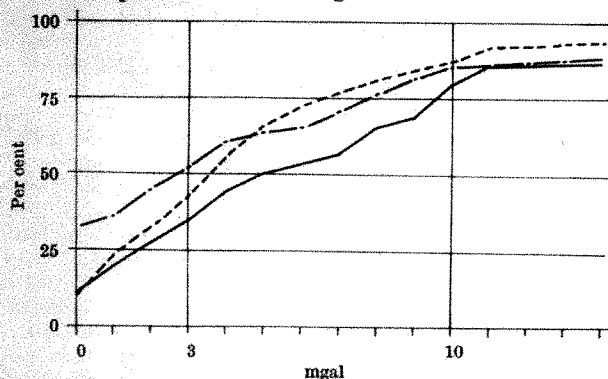


Fig. 1. Cumulative percentage error for shipborne sea gravimeter measurements. —, Differences as given in Table 1, — — —, differences if ship track adjustment up to 10 min allowed; . . . , ref. 4

new Anschütz platform<sup>14</sup>. The navigation during all cruises was by celestial fixes only. The positioning of H.M.S. *Owen* was superior to that of R.R.S. *Discovery II*; in the former ship the navigating officer was fully engaged in estimating the ship's position while in the latter ship navigation was done by the officer on watch.

The more accurate results of the 1961 cruise are probably due to both the advanced design of the platform and the better navigation. The root mean square error for 1960 comparisons is  $\pm 12.3$  mgals while the root mean square error for 1961 comparisons is only  $\pm 7.1$  mgals. Considering all the differences given in Table 1, 13, or 34 per cent, were less than 3 mgals and 30, or 79 per cent, were less than 10 mgals. If the differences are adjusted by allowing a 10-min error in the ship's track then 14, or 37 per cent, checks were within 1 mgal; 34, or 90 per cent, were within 10 mgals. The shipboard values are known to have an uncertainty of 3-5 mgals due to difficulties in evaluating the Eötvös correction<sup>5</sup>. The conclusion is, therefore, that there is no evidence for large systematic errors (as suspected by the theory of cross-coupling<sup>15</sup>), and if the means of navigation of research ships at sea could be improved shipborne gravimeters would be more accurate than the submarine pendulums.

Fig. 1 illustrates the percentage error which might be expected from a large number of measurements. Two curves are given for submarine checks. One curve is



calculated on the basis of the estimated nearest approach of the ship's track to the submarine station. The other curve is based on the 10-min adjustment of the ship's track and selection of the shipboard gravity value within 10 min of the nearest approach which gives the smallest difference. For comparison, a percentage error curve for track intersection comparisons off the coast of Southern California is also shown in Fig. 1. (This is curve 2 from Fig. 2 of ref. 4, which is the most extensive evaluation published and refers to a LaCoste and Romberg sea gravimeter.)

Before concluding this discussion, it might be useful to consider the assumptions made in estimating the accuracy of submarine gravity values<sup>11,12</sup>. This accuracy is based on the evaluation of known experimental errors. However, submarine measurements are isolated values and the possibility of a gross error cannot be discounted. The source of such an error might be an undetected malfunction of a part of the equipment or a blunder in calculation. An isolated erroneous value might not look odd and therefore would not be questioned. The particular case in point is Vening Meinesz Station No. 489. This station was approached within 7 miles by R.R.S. *Discovery II* (Ser. No. 16) and within 4 miles by H.M.S. *Owen* (Ser. No. 23). While the two shipboard values agree within 7 mgals, they are both more than 45 mgals higher than the submarine value. The reasonable conclusion is that the submarine value is in error and one is left with a question: How many of the extreme differences in Table 1 are due to unsuspected gross errors in the submarine gravity measurements? It will not be possible to answer this question until the accuracy of shipborne gravimeters can be ascertained by independent tests.

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## GEOCHEMICAL APPLICATION OF SPARK SOURCE MASS SPECTROGRAPHY

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**A**NALYTICAL methods capable of determining elements in geological materials in the concentration range from 0.001 to 10 parts per million have become necessary for the examination of many geochemical problems. The distribution of elements such as thallium, caesium, hafnium and the rare earths can provide sensitive indexes of fractionation processes in the Earth's crust<sup>1</sup> and rapid methods of analysis are urgently needed to determine their abundances in a wide variety of rocks and minerals. The geochemistry of some thirty additional elements is virtually unknown, but doubtless many interesting problems remain to be uncovered. Ehmman<sup>2</sup>, for example, has suggested that the rare element, iridium, would be a better indicator of meteoritic contribution to terrestrial materials, such as impact glasses, than the commonly used nickel. In summary, methods for the determination of about half the chemical elements are required.

The growing industrial demand for materials with impurity concentrations of the order of a part per million, as in semiconductors<sup>3</sup>, has led to the development of mass spectrographs using spark excitation of solid samples. Impurities added from reagent chemicals are thus avoided and the material is analysed with a minimum of processing.

The success of these instruments in determining a wide range of trace elements in a variety of materials<sup>3-7</sup> encouraged the application of this technique to the analysis of geological materials, and a spark source mass spectrograph (type M.S. 7) manufactured by Associated Electrical Industries, Manchester, was installed in this Department

in July 1964 for this purpose. General descriptions of the instrument have been given by several workers<sup>3-7</sup>. A vacuum spark is used to produce a beam of positive ions, largely because it provides roughly equal sensitivity for all elements. The source parts are constructed of tantalum. Ions produced in the spark discharge are accelerated through a series of slits by a potential difference of 20 kV. A double focusing electrostatic and magnetic analyser system, based on the design by Mattauch and Herzog<sup>8</sup>, is used. This design compensates for the wide spread of ion energies produced in the spark source and brings ions to focus in one plane enabling a photographic plate to be used to detect them.

A full description of the instrument and the analytical technique will be published elsewhere; a brief account is as follows: the powdered-rock sample is mixed with an equal weight of National Carbon SP-1 graphite powder, and briquetted in a small laboratory press to form a rod 20 mm × 2 mm × 2 mm. This is trimmed to a cylindrical form with a diamond-tipped tool in a small lathe, to remove possible surface contamination. Two of these rods are clamped in the electrode holders in the source. Since only a few mg of sample are consumed for the longest exposures, careful attention to mixing and sampling procedures is required. To achieve the requisite detection limits pressures of the order of 10<sup>-6</sup> mm mercury (torr) are required in the analyser region. A spark voltage of 25 kV is applied and graded series of exposures are recorded, the pulse length and repetition rates of the spark being increased for larger exposures. Because of the instability of the spark discharge, exposure is measured

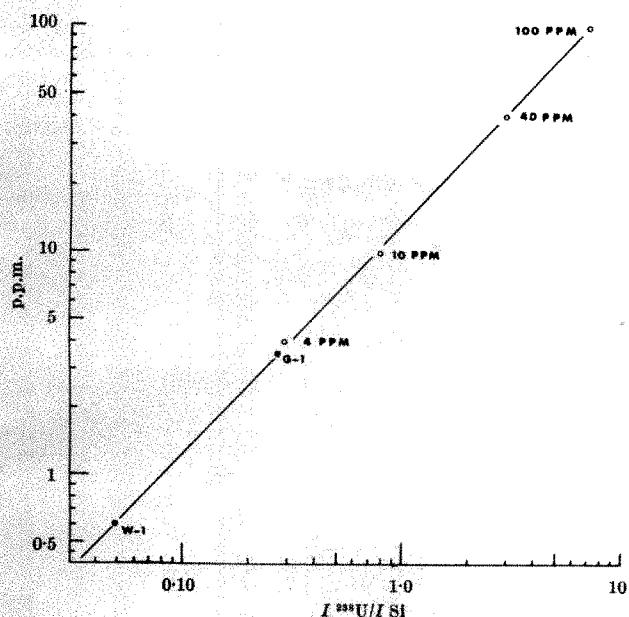


Fig. 1. Intensity-concentration graph for the determination of uranium using  $^{235}\text{U}$  and  $^{28}\text{Si}^+$  as internal standard. The points labelled 100 p.p.m. refer to standards containing that amount of uranium

in terms of the total integrated ion current. The instantaneous ion current is of the order of  $10^{-12}$  amp for short exposure conditions, rising to about  $10^{-9}$  amp when large exposures are recorded. The shortest exposure is commonly  $10^{-12}$  coulombs and the longest  $3 \times 10^{-6}$  coulombs. The time varies from a few seconds for the shortest exposures to 2-3 h for the longest. Mass spectra are normally recorded in the range 7-240 mass units, enabling recording of nearly all elements during a single exposure. The photographic plates used are Ilford Q-2, and these are developed in Kodak 'D-19' developer for 2 min and fixed for 1 min in Kodak rapid liquid fixer. These Schumann-type plates are very sensitive to abrasion and require careful handling and storage. The density of the mass lines is read using a Jarrell-Ash microphotometer. Densities are converted to intensities using a plate calibration curve constructed from the densities of  $^{171}\text{Yb}$  and  $^{173}\text{Yb}$ , which have an intensity ratio of 1.52. The photographic calibration curve is an elongate S shape with a small linear portion. Use is made of a seidel function to obtain low-intensity values. Background intensities are subtracted from line intensities where necessary, although the line to background ratio is commonly better than 0.9. Silicon is used as an internal standard and the intensity values of other lines are ratioed to that of the line at mass 9 $\frac{1}{2}$  due to  $^{28}\text{Si}^{3+}$ . Work is proceeding on the use of other internal standards.

The intensity values are plotted against concentration for known standards. Initial tests were carried out using well-established values for elements in the standard granite G-1 and diabase W-1. Although these indicated the general feasibility of the method, reliable values for many of the elements of interest are too few<sup>9</sup>, and artificial standards using the purest oxides where available were prepared, using a base of high purity quartz. A typical intensity versus concentration graph is shown in Fig. 1, for uranium.

The results for a number of elements based on similar graphs are given in Table I for G-1 and W-1. Comparison of these results with the wide scatter of values reported by Fleischer and Stevens<sup>9</sup> is beyond the scope of this article. The general order of magnitude agreement is considered very satisfactory and the instrument seems to be capable of approaching the accuracy of other methods. The linearity of the intensity-concentration relation noted over several orders of magnitude is encouraging.

Table 1. VALUES FOR TRACE ELEMENTS IN STANDARD GRANITE, G-1, AND STANDARD DIABASE, W-1

Element	Results given in parts per million		
	Mass No.	G-1	W-1
U	238	3.5	0.60
Th	232	40.0	1.6
Bi	209	0.10	0.25
Pb	208	50.0	5.4
Tl	203	1.3	0.14
W	184	0.40	0.30
Hf	178	5.2	1.5
Lu	175	0.13	0.29
Yb	172	0.75	1.6
Tm	169	0.18	0.21
Er	167	1.7	1.5
Ho	165	0.62	0.55
Dy	163	4.5	2.3
Tb	159	1.1	0.60
Gd	158	6.4	2.3
Eu	153	2.3	0.96
Sm	147	7.6	2.5
Nd	146	53.0	7.4
Pr	141	15.0	1.7
Ce	140	105.0	19.0
La	139	60.0	11.0
Ba	137	1,250.0	160.0
Cs	133	2.1	0.80
Sb	121	0.30	0.80
Sn	118	2.3	1.0
In	115	0.05	0.30
Ag	109	0.040	0.030
Mo	98	9.5	0.70
Nb	93	21.0	5.4
Zr	90	185.0	70.0
Y	89	7.8	17.0

A detailed discussion and comparison with data of other workers will be presented elsewhere.

The results for the odd-numbered rare earth elements are plotted against results obtained by neutron activation methods by Haskin and Gehl<sup>10</sup> in Fig. 2. There is general agreement between the two sets of data, although some differences in detail.

The precision of the method is being evaluated but it appears to be of the order of 10 per cent. The instrument shows rather uniform sensitivity, with variations up to a factor of three or four. Work is proceeding on the factors responsible and preliminary data indicate a dependence both on volatility and ionization potential. Readily volatile and easily ionized elements such as the

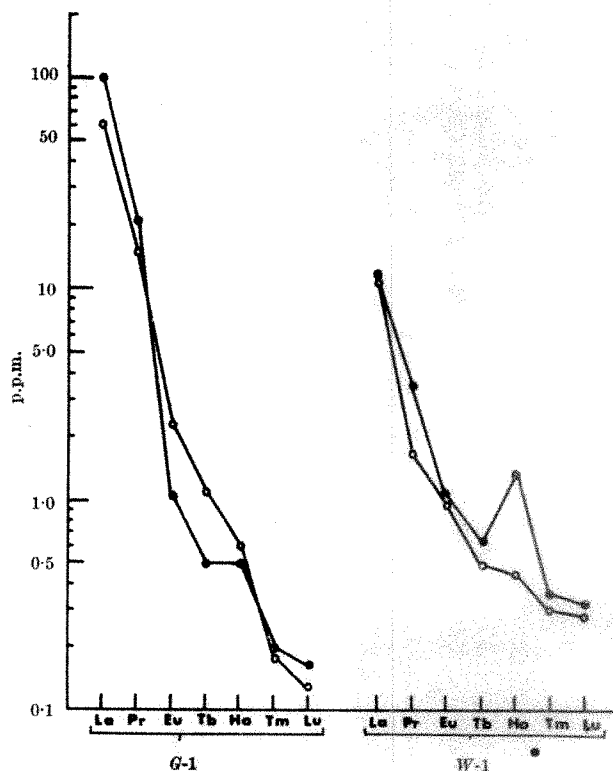


Fig. 2. Comparison of values for the odd-numbered rare earth elements by spark source mass spectrography (open circles—Taylor) and neutron activation analysis (filled circles—Haskin and Gehl, ref. 10)

alkalis show increased sensitivity. Other readily volatile elements, such as thallium, lead and antimony, which possess high ionization potentials, are less sensitive. The least sensitive appear to be the involatile elements such as titanium and zirconium.

Interferences in the mass spectra result from: (1) lines due to multiply-charged ions appearing, for example, at half the mass number for doubly-charged ions: the overall distribution pattern of the elements with decreasing abundances in the higher mass regions tends to minimize, but not remove, this effect: examples of interference are  $^{69}\text{Ga}^+$  by  $^{138}\text{Ba}^{2+}$  and  $^{45}\text{Sc}^+$  by  $^{90}\text{Zr}^{2+}$ ; (2) multiple ion formation. Under the sparking conditions used, there is a relatively low production rate of multiple ions, except for carbon, where lines appear at each multiple of twelve, up to the high mass numbers. It has so far proved possible to avoid the use of these mass numbers, and the resolution of the instrument is commonly sufficient to resolve ion lines of the same nominal mass.

The instrument is thus well suited to the determination of the rarer elements in geological materials, particularly those with isotopes of mass greater than 90. The lighter rarer elements, lithium and beryllium, suffer interference from multiply-charged silicon and aluminium, but boron can be determined using  $^{11}\text{B}$ .

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## SOME MEASUREMENTS OF THE TENSILE STRENGTH OF DRY POWDERS OF IRREGULAR PARTICLE SHAPE

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**D**URING the course of a general programme of research aimed at furthering the understanding of the flow of coal through bunkers we have observed that the irregularity of particles has a profound effect on the tensile strength of a powder packing. The measurement of tensile strength has been made using a split tilting-plate apparatus based on that described by Thouzeau and Taylor<sup>1</sup>: some control of the packing density of a powder has been achieved by a tamping procedure devised by J. C. Richards of these laboratories. The powders were of graded particle size and measurement was carried out in the open laboratory at relative humidities mostly in the range 30–55 per cent.

The form of variation in tensile strength as a function of packing density (fractional solids content of the packing), and the effect of particle size, are illustrated in Fig. 1 for a low-rank bituminous coal of National Coal Board Coal Rank Code No. (CRC) 900. In Fig. 2, results for 5–20 $\mu$  fractions of three coals are compared with those for similarly sized alumina and silica powders; a broadly similar pattern of curves, but at higher packing densities, was obtained with 20–33 $\mu$  sieved fractions. In contrast to the behaviour of these five materials of irregular particle shape, the cohesion of copper and glass spheres was found to be so small that they did not form packings sufficiently cohesive for readings to be taken.

The range of packing densities achieved with the tamping procedure varied both with the particle size and with the material, and the densities are all much lower than the value of 0.63 exhibited by 20–33 $\mu$  glass spheres and 5–20 $\mu$  copper spheres. While all the tensile strength curves are of similar shape, those for coals tend to fall to zero more quickly than those for silica and alumina. Tensile strength increases with the fineness of the powder but there is no simple relation, the relative cohesiveness of two powders varying widely with the packing density at which comparison is made. The 20–33 $\mu$  and 5–75 $\mu$  fractions of coal CRC 900 have specific surfaces that are almost equal in value and their tensile strength curves lie close together: thus size dispersion does not appear to affect tensile strength critically.

In view of the low cohesiveness of spherical particles, the results were examined for evidence of a dependence of tensile strength on particle shape. A simple measure of the shape of particles is afforded by comparing the esti-

mates of particle size yielded by different methods of sizing. For the present powders, the surface mean specific surface diameter  $d$  has been determined by the air permeability method and the surface mean projected diameter  $d_p$  of settled particles by the optical microscope method. The ratio  $d_p/d$  is a 'shape factor' that is expected to be unity for spheres and to increase with the degree of particle irregularity. Experimental values are shown in Table 1, that for copper spheres having been included as a check on the reliability of the procedures. Each value is a mean for at least three different size fractions in the overall range 5–75 $\mu$ , there being no evidence of any marked change of shape with particle size.

Table 1. PARTICLE SHAPE FACTOR  $d_p/d$

Material	Copper spheres	Silica	High-rank bituminous coal (CRC 301B)	Anthracite (CRC 101)	Alumina	Low-rank bituminous coal (CRC 900)
Ratio $d_p/d$	1.0	1.9	2.1	2.3	2.6	2.9

The results of Table 1 show the low-rank coal and alumina to have the most irregular particles and it is these powders that exhibit the highest tensile strengths at a given packing density. While these findings are in qualitative accord with a hypothesis of a shape dependence of tensile strength, too much should not be made of the precise numerical values of Table 1. A material has as many shape factors as there are pairs of ways of measuring particle size, so that a factor is in no way a unique characterization of shape. A single factor would not, for example, differentiate between needle-shaped particles and flaky ones, yet their tensile strength behaviour might well be very different. One might question also the choice of air permeability as one of the methods of size determination since it makes use of a powder packing. The present shape factors could conceivably, therefore, have been influenced by tensile strength—although the independence of particle size suggests that in fact they were not.

Visual examination of the low-rank coal confirms the presence of appreciable quantities of needle- and disk-shaped particles. At granular sizes these have been separated from the more rounded particles by sieving on a combination of square-aperture and slot-aperture meshes. If slot-aperture meshes become available at finer sizes it should be possible to test for the effects of

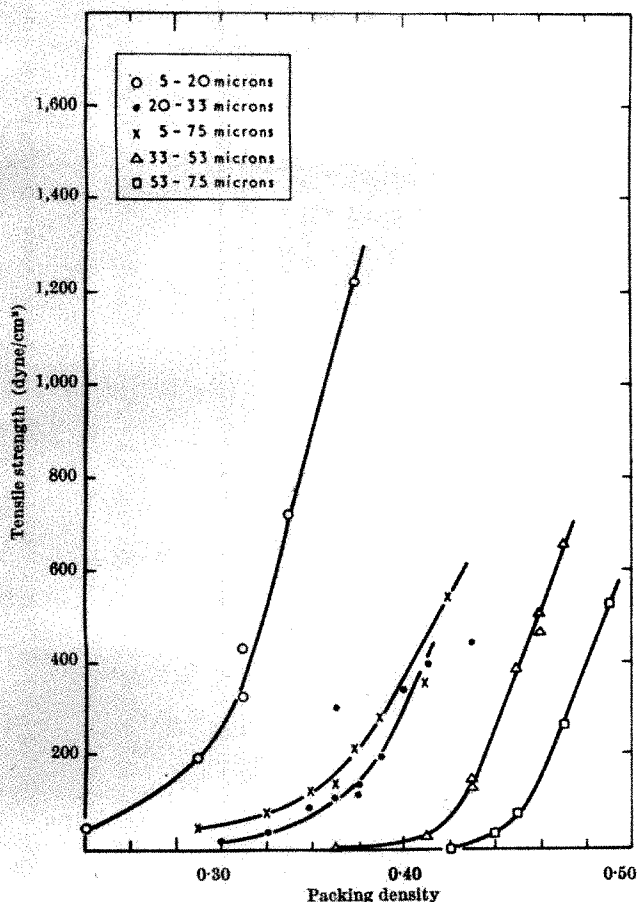
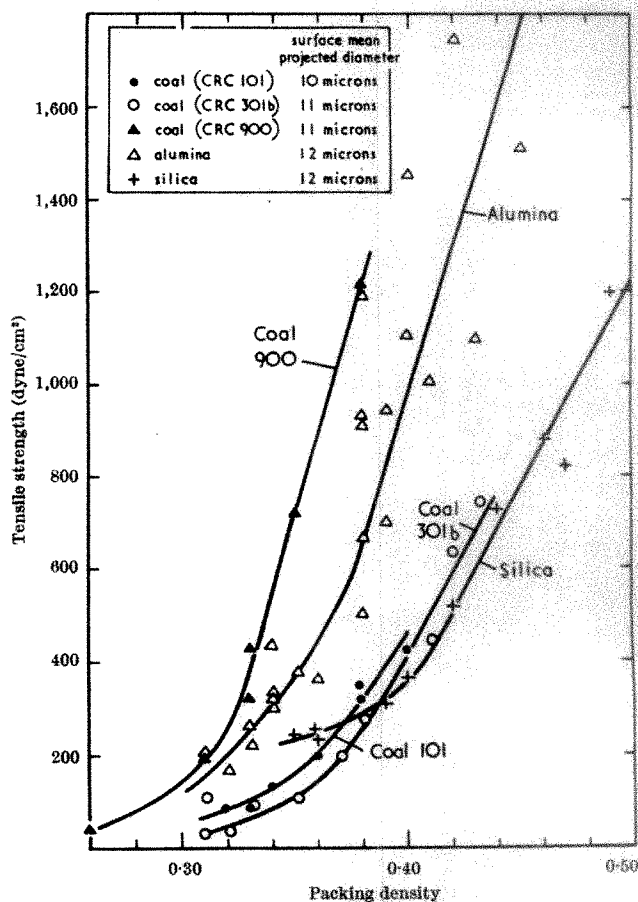


Fig. 1. Tensile strength of coal powder (CRC 900)

particle shape on powder properties more decisively than at present.

Theoretical expressions for the tensile strength of packings of monosize spheres have been given by Rumpf<sup>2</sup> and by Smalley and Smalley<sup>3</sup>, but the relationships they predict bear little resemblance to those we have found for irregular particles. Our results suggest that the challenge to theoretical analysis is considerable. An adequate treatment would have to explain why the relative cohesiveness of two powders of different particle size, but of the same shape, changes so markedly with packing density

Fig. 2. Tensile strength of 5-20 $\mu$  powders

and also the mechanism by which shape affects behaviour. At the present time it is difficult to see how a simple pattern can be imposed on the results unless the practice of making comparisons at equal packing densities is abandoned.

The measurements of tensile strength were made by Mr. A. C. Chilton.

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## INHALATION AND RETENTION OF FALL-OUT ZIRCONIUM-95 BY HUMAN BEINGS

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IN 1962 we reported<sup>1</sup> the detection of  $\gamma$ -rays at about 0.76 MeV from the chest region of all human subjects. We attributed this radiation to the presence of the fission product zirconium-95 (half-life 65 days) accompanied by its daughter-product niobium-95 (half-life 35 days), both of which emit  $\gamma$ -rays having energies between 0.72 MeV and 0.77 MeV. These radio-nuclides and other fission products had been found in human lungs examined *post mortem*, notably by Schönfeld *et al.*<sup>2</sup>

In this article we wish to report the results of our measurements of zirconium-95 in human lungs *in vivo* up to October 1963 and of our attempts to correlate the levels observed with the amounts in the air breathed. We may recall that the method of measurement involved the determination of the  $\gamma$ -ray spectrum with a 23-cm

diam. crystal of sodium iodide (TI) placed 2 cm below the thorax of the supine subject. The  $\gamma$ -ray spectra were analysed by a computer method of least squares using standard spectra from potassium-40 and caesium-137 and from zirconium-95/niobium-95 in a low-density lung phantom<sup>1</sup>.

Since these measurements began the amounts of both zirconium-95/niobium-95 and caesium-137 *in vivo* have shown substantial changes, as illustrated by the results in Figs. 1 and 2. In Fig. 1 are shown parts of the  $\gamma$ -ray spectra (0.5-0.9 MeV) from the thorax of Mrs. M. N. on four occasions at intervals of six months. In each case the spectrum derived by the computer has been drawn as a smooth curve; the agreement between the fitted curves and the experimental points is good even in the measure-

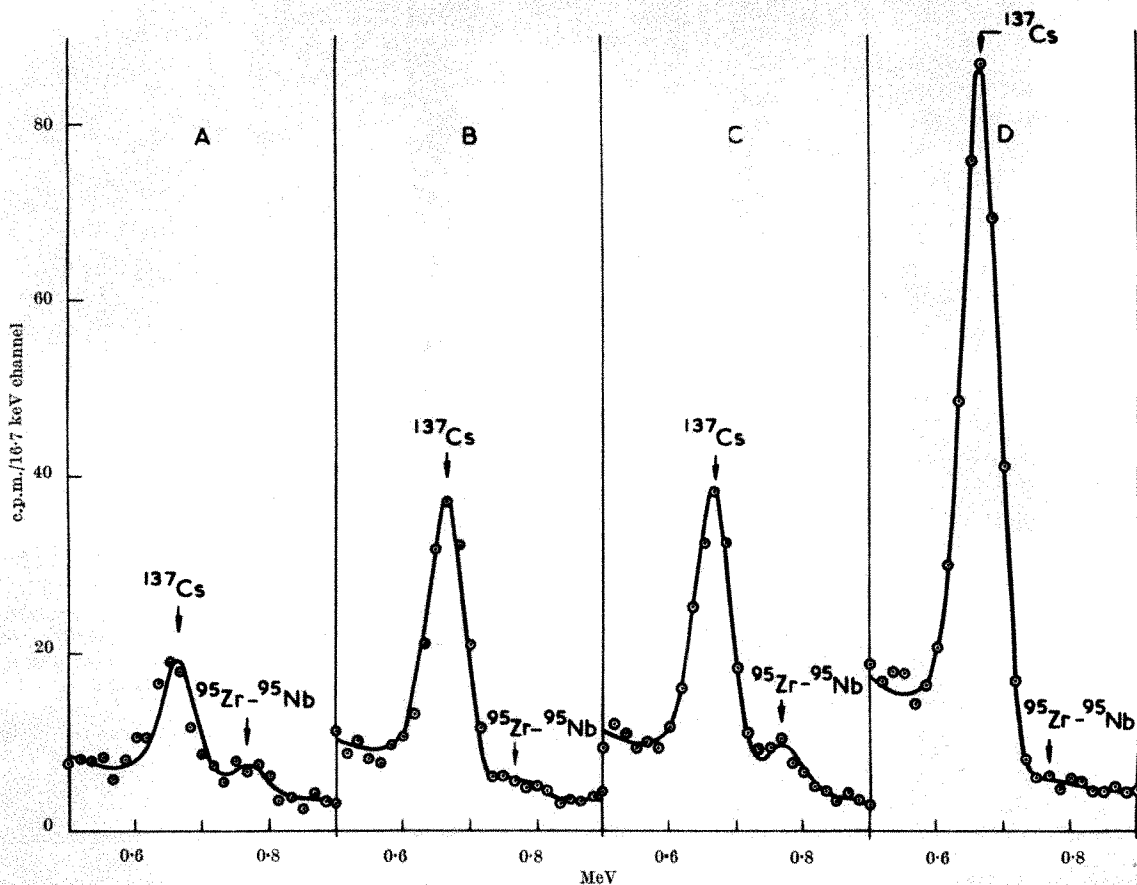


Fig. 1. Parts of the  $\gamma$ -ray spectra (0.5-0.9 MeV) from the thorax of Mrs. M. N. on four occasions at intervals of six months. A, April 21, 1962; B, October 20, 1962; C, April 24, 1963; D, October 6, 1963

ment of April 21, 1962, when the gain of the system was slightly different from normal. The values for the zirconium-95/niobium-95 content of Mrs. M. N., together with those for three other subjects, are plotted as a function of time in Fig. 2. The activities in all four subjects show the same trend, namely, a minimum in the autumn of 1962 and a maximum in the spring-summer of 1963. There seems to be a correlation between lung content and body-weight, as well as a possible sex difference. After October 1963 the effects of increasing whole-body contents of caesium-137 and decreasing lung contents of zirconium-95 combined to make the estimates of the latter nuclide quite unreliable.

A qualitative explanation for the fluctuations in the caesium-137 and zirconium-95/niobium-95 contents of the body, and for the differences between the two, is provided by the data plotted in Fig. 3. They are: (a) the quarterly mean concentrations of caesium-137 (from world-wide fall-out from nuclear test explosions) in milk produced on farms close to this Establishment<sup>3</sup>; (b) the monthly mean concentrations of zirconium-95 in air at ground-level at Chilton, Berks<sup>4</sup>. Because of the relatively short half-life of zirconium-95 (65 days) the levels *in vivo* followed quite closely the levels in the air. (It must be pointed out here that the measurements *in vivo* were of zirconium-95 with its daughter niobium-95 not necessarily in radioactive equilibrium, whereas the levels in air were determined when the niobium-95 had almost reached equilibrium with the parent

zirconium-95.) In the case of caesium-137 with its long half-life for radioactive decay, and an average biological half-life in adult man of about 100 days<sup>5</sup>, the levels *in vivo* at any time reflect the average levels in the diet over the preceding few months. We shall not consider further the caesium-137 levels *in vivo* as our results have been published<sup>6</sup>.

*Estimation of zirconium-95 present.* As already pointed out here, the measurements *in vivo* were of the total  $\gamma$ -ray activity of a mixture of zirconium-95 and its daughter niobium-95. In order to convert the measured

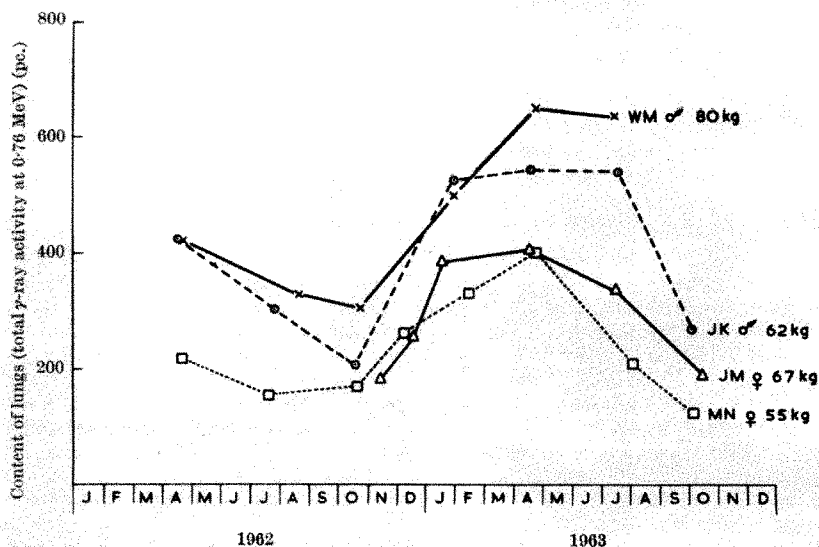


Fig. 2. The total  $\gamma$ -ray activity due to zirconium-95 and niobium-95 in the lungs of two men and two women as a function of time (April 1962-October 1963)



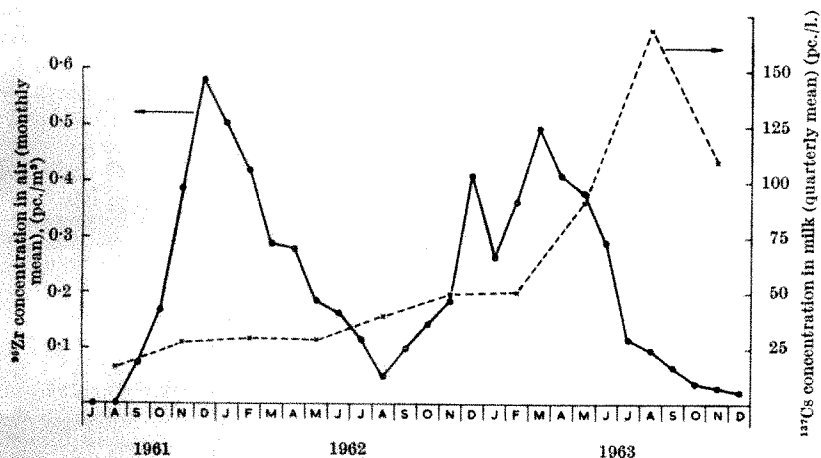


Fig. 3. Quarterly mean concentration of caesium-137 in milk produced on farms near Harwell (dashed line) and the monthly mean concentration of zirconium-95 in air at ground-level at Chilton, Berks (solid line)

activities into zirconium-95 activities, it was necessary to know the values of the ratio:

$$R_{\gamma} = \frac{\text{number of } \gamma\text{-rays at 0.76 MeV}}{\text{number of } \beta\text{-particles from Zr-95}}$$

at the various times the measurements were made. Provided that these were not too soon after a series of atmospheric nuclear weapon tests, a mean effective date of production could be assumed, and values of  $R_{\gamma}$  could be calculated easily. This was the situation for measurements made between January and July 1962 of zirconium-95 produced in September and October 1961, and for measurements made between January and July 1963 of zirconium-95 produced in the autumn of 1962. However, when the measurements were made shortly after the commencement of a test series the determination of  $R_{\gamma}$  for the  $\gamma$ -ray activity actually in the lung was more difficult. This was the case for our measurements in October 1962; it can be shown that in that month only about 9 per cent of the airborne zirconium-95 originated from the 1961 test series whereas it seemed unlikely that less than about 65 per cent of the lung content in October 1962 originated from the 1961 series (see following). An accurate value for  $R_{\gamma}$  could not therefore be determined; for the zirconium-95 inhaled during the month, a value of 1.73 would be deduced, whereas for the zirconium-95 in the lung a value somewhat below 3.0 would be reasonable. It is worth noting that immediately after fission,  $R_{\gamma} = 1$ , whereas when niobium-95 is in equilibrium with the parent,  $R_{\gamma} = 3.14$ .

In Table 1 are set out the average values for the total  $\gamma$ -ray activity and the zirconium-95 activity in subjects measured at intervals between January 1962 and October 1963, together with the values assumed for  $R_{\gamma}$ . No errors are shown; the statistical standard error on a single observation was in the range  $\pm 15$  to  $\pm 20$  per cent, while biological variation can cause a substantial spread in the results as indicated in Fig. 2. As we suggested in our previous report<sup>1</sup>, there may be a systematic error due to the method of calibration. The only possible way of assessing this is by comparison with the results of others

Table 1. AVERAGE VALUES FOR THE TOTAL ACTIVITY AT 0.76 MEV AND THE ZIRCONIUM-95 ACTIVITY IN THE LUNGS OF CONTROL SUBJECTS, JANUARY 1962-OCTOBER 1963

Date	No. of subjects	Total activity at 0.76 MeV (pc.)	$R_{\gamma}$ (in vivo)	Zirconium-95 content of lung (pc.)
January 1962	10	~300	2.3	~130
April 1962	14	355	2.75	129
July 1962	13	238	3.0	79
October 1962	16	205	<1.7	>120
Nov./Dec. 1962	7	280	~2.3	<68
Jan./Feb. 1963	9	376	2.6	~120
April 1963	12	450	2.8	145
July/Aug. 1963	12	447	3.0	161
October 1963	11	226	3.1	149

who have analysed lungs obtained *post mortem*. The data are very sparse, but in general our results agree well with those of Schönfeld *et al.*<sup>2</sup>, Osborne<sup>7</sup>, Wegst *et al.*<sup>8</sup> and Passant *et al.*<sup>9</sup>, and it seems clear that any systematic errors in our method of calibration must be quite small (< 20 per cent).

**Calculated lung contents.** From the monthly mean concentrations of zirconium-95 in air at ground-level<sup>3</sup> it should be possible to calculate values for the lung contents, with the aid of suitable models. Comparison with the observed contents may indicate which model best fits the facts. As a starting-point we used the single exponential model of the International Commission on Radiological Protection (I.C.R.P.)<sup>10</sup>; the lung content  $q$  pc. is given by the equation:

$$q = \frac{fCVT}{0.693} \left[ 1 - e^{-\frac{0.693t}{T}} \right] \quad (1)$$

after inhalation for  $t$  days of  $C$  pc./m<sup>3</sup> of air, where  $V$  is the breathing-rate in m<sup>3</sup>/day,  $f$  is the fraction of the inhaled material which is not exhaled or removed rapidly by ciliary action from the upper respiratory tract and  $T$  is the effective half-life in days. Inserting the values recommended by the I.C.R.P. of  $f = 0.125$ ,  $V = 20$  m<sup>3</sup>/day and  $T = \frac{T_b T_r}{T_b + T_r} = \frac{120 \times 65}{185} = 42$  days, and using the

values of  $C$  plotted in Fig. 3, the content of the lung at the end of each month due to zirconium-95 inhaled during that month was calculated. The fractional decrease per month caused by an effective half-life of 42 days was then applied repeatedly, in order to calculate the amounts remaining in the lung at the end of successive months. Finally, the amounts for individual months were summed to give the totals for those months; the results are plotted in Fig. 4 (lower curve, labelled 'I.C.R.P. model'), where the experimental results of column 5 of Table 1 are also plotted. This model underestimates the lung content by

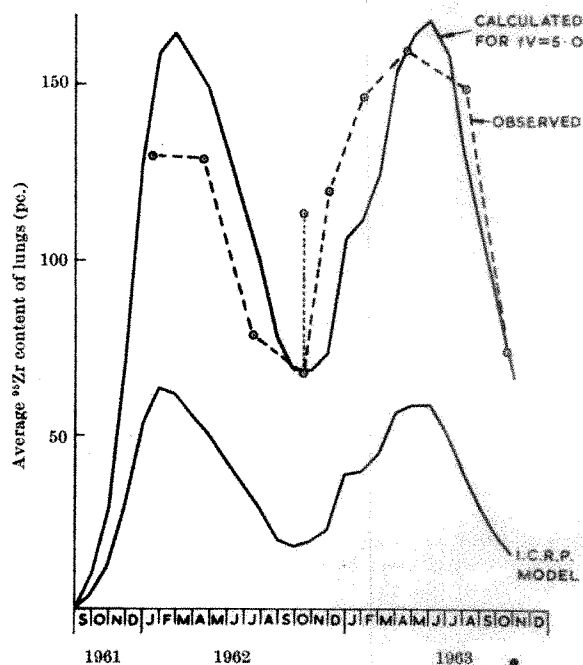


Fig. 4. Comparison of observed lung contents of zirconium-95 with contents calculated from the data in Fig. 3 on the basis of two different models for the retention in the lung

a factor of between two and three; also, it will be seen that the values of the calculated maxima in January/February 1962 and in April/May 1963 were both close to three times the minimum value in September/October 1962 whereas the observed maximum values were not more than twice the minimum. This suggests that the value for the effective half-life was too low.

The calculation of lung contents was next repeated with extreme values for the variables in equation (1). Thus  $f$  was assumed to be 1.0, and  $T$  was taken as 65 days (that is, no biological elimination from the lung);  $V$  was still taken as 20 m<sup>3</sup>/day. The observed average lung content at any time was expressed as a fraction of the maximum possible calculated value to give a series of estimates of  $f$ . The average of these (excluding that for October 1962, because of the uncertainty already discussed) was 0.25 and this value was then applied to the maximum possible lung contents (that is, those calculated with the assumption of  $f = 1.0$ ). The values obtained in this way are also plotted in Fig. 4; since the breathing rate is not known precisely the model is more correctly described as one in which  $fV = 5.0$  m<sup>3</sup>/day, rather than one in which  $f = 0.25$ . With the exception of the results for November/December 1962 and January/February 1963 the observed average lung contents agree with the calculated to within about  $\pm 20$  per cent. The discrepancies for the measurements during the winter of 1962-63 cannot be explained; possible causes might include such factors as variations in particle size and density, relative humidity and temperature of the air and breathing rates.

The conclusion which we may draw from these results is that use of the I.C.R.P. model for the retention in the lung of small ( $< 1\mu$ ) particles underestimates the actual lung content by a factor of 2-3, and the biological half-life of 120 days seems to be much too short. Unfortunately our results do not permit us to deduce a reliable value for the biological half-life. One way in which this might be estimated is by the determination of the lung content of one of the longer-lived fission products such as ruthenium-106 and cerium-144, and comparison with the calculated contents from the air-levels. These radio-nuclides could not be detected *in vivo* by external counting<sup>1</sup> but were determined in excised lungs examined *post mortem*<sup>2,3</sup>.

Finally, it is worth noting that our conclusion that the biological half-life is much longer than 120 days is not incompatible with our observation<sup>1</sup> that the average lung content of zirconium-95/niobium-95 of three men in April 1962 was 28 times their daily faecal excretion of these nuclides. Taken at its face value, this could imply that the biological half-life in the lung was only 19 days if: (a) a single exponential function describes completely the retention of all material deposited in the respiratory tract; (b) all the faecal activity were due to material which had been inhaled and then removed from the lung by ciliary action and swallowing, and none to ingested material. Even if condition (b) is valid it is easy to show that a value of 28 for the ratio mentioned here is obtained with a two-component exponential function to describe the lung retention. For example, with biological half-lives of 1 day and 250 days the fractions moving at these rates would be 0.05 and 0.95 respectively. A half-life of about 1 day is not unreasonable for the phase of rapid clearance by ciliary action, while we obtained a value of 250 days for the long-term clearance of zirconium-95 in one case, following inhalation of irradiated uranium<sup>11</sup>. It should not be assumed that 250 days is necessarily the biological half-life for inhaled fall-out zirconium-95; we merely use this to show that if a few per cent of the inhaled material had a very short half-life and the remainder a long half-life, then the excretion rate would be such as to suggest a short half-life (that is, 19 days) with assumptions (a) and (b) above.

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## ABSCOPAL STIMULATION OF THE THYMUS OF RATS BY EXPOSURE OF THE HEAD TO X-RAYS

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THE appearance of radiation changes in organs remote from those that have been exposed to ionizing radiations has frequently been claimed, and the phenomenon has been called 'abscopal'<sup>1</sup>. Loss of weight of spleen and thymus has been observed<sup>2</sup> after part-body irradiation, but was attributed to general malaise<sup>3</sup>. Recently such changes were seen four days after exposure of small parts of the abdomen of mice<sup>4</sup> when the animals did not appear to be sick. Benjamin and Yost<sup>5</sup> found a decrease in oxidative phosphorylation of the spleen after irradiation of the head of animals. We<sup>6</sup> observed marked increases in the content of alkaline ribonuclease (RNase) in the spleen and thymus of rats the heads of which had been exposed to 1,000 r. of X-rays. The measurements made are believed to represent the total RNase activity of the organs, since the cells were completely disrupted by repeated freezing

and thawing before enzyme assay. The increased RNase activity was seen in the spleen within a few hours after head irradiation, but was not pronounced in the thymus until more than 24 h had elapsed. Elevation of the RNase activity of the thymus following whole-body irradiation of mice was first reported by Weymouth<sup>7</sup>, and our experiments indicated that part of this effect was abscopal and could be attributed to exposure of the head.

We have now found that the rise in RNase due to head irradiation is preceded by an increase in the rate of incorporation of radioactive thymidine into the DNA of the thymus, though not of the spleen.

**Experimental procedures.** 24-28-day-old rats, ranging in weight from 42 to 50 g, were placed in a coffin-shaped lead box from which only the head protruded. To determine the effectiveness of the shielding a 'Victoreen' dose meter

Table 1. EFFECT OF HEAD IRRADIATION AND STRESS ON SPLEEN AND THYMUS OF RATS (AVERAGE OF AT LEAST FIVE RATS PER GROUP)

Treatment	Age (days)	Weight (g)			DNA content (mg)		RNase $\mu$ g of organ		Thymidine uptake $\dagger$	
		Animal	Thymus	Spleen	Thymus	Spleen	Thymus	Spleen	Thymus	Spleen
None	26	42	0.08	0.14	2.1	1.7	0.38	2.1	2.8	85
24 h after 1,000 r. to head only	26	39	0.06	0.11	1.4	1.2	0.80	27.0	19.0	95
None	27	50	0.13	0.20	3.9	4.6	0.33	2.0	4.0	81
24 h after sham irradiation	27	49	0.13	0.22	3.2	2.5	0.30	2.0	3.6	78
24 h after 1,000 r. to hind legs only	27	48	0.12	0.23	3.2	2.3	0.42	2.7	—	—
None	24	37	0.07	0.11	2.2	1.5	0.38	2.3	—	—
48 h complete starvation	24	28	0.04	0.08	1.1	1.3	0.32	2.4	—	—
20 days after thymectomy	46	105	—	0.33	—	1.2	—	6.0	—	140
20 days after thymectomy followed by 1,000 r. to head; killed 24 h later	46	90	—	0.29	—	1.1	—	3.0	—	109
25 I.U. ACTH on days 1 and 2 killed 3 h after last injection	52	110	0.26	0.41	8.7	4.9	0.25	0.9	—	—

\* Expressed as activity of crystalline pancreatic RNase in  $\mu$ g.  $\dagger$  Counts per min  $\times 10^3$  in the DNA per g of organ wet weight.

Table 2. CHANGES PRODUCED 24 AND 48 H AFTER HEAD IRRADIATION, EXPRESSED AS PERCENTAGE OF CONTROL (AVERAGE OF SIX RATS PER GROUP)

Dose	Thymidine uptake in thymus 24 h      48 h	RNase per mg DNA				DNA content of organ, 24 h	
		Thymus		Spleen		Thymus	Spleen
		24 h	48 h	24 h	48 h		
100 r.	168%	109%	122%	220%	492%	98%	76%
250 r.	448%	122%	182%	492%	475%	85%	74%
500 r.	510%	166%	2,900%	770%	1,500%	76%	64%
1,000 r.	450%	190%	—	1,400%	—	59%	71%

was placed in the thymus and spleen in the carcass of a rat. 280-kV X-rays (no filter) were used at a dose rate of 250 r./min. The dose received by the spleen was less than 0.5 per cent, and that by the thymus was 1.2 per cent, of that received by the head. The experimental procedures for determining RNase were as described earlier<sup>6</sup>, but the present series differs in that four-week-old rats were used instead of eight-week-old rats weighing 110 g, so as to conserve radioactive thymidine. The rate of thymidine incorporation was measured by intraperitoneal injection of 1  $\mu$ c./g body-weight of tritiated thymidine with an activity of 8.8 curie/m.mole 15 min before killing the animals. The DNA from the thymus and spleen was then separated by extracting the tissues with cold 15 per cent trichloroacetic acid and then hydrolysing the precipitate. The radioactivity of the hydrolysate was measured in a scintillation counter.

**Increase of DNA synthesis in the thymus.** Table 1 shows that 24 h after irradiating the head of rats with 1,000 r. there is a pronounced increase in the rate of incorporation of thymidine into the DNA of the thymus and in the RNase content of the spleen. The RNase activity of the thymus is also substantially higher at 24 h, but the dramatic increase is only seen later in this organ (see Fig. 1). Table 1 shows that these effects of head irradiation are not produced by generalized stress such as loading the animals into the irradiation container, starvation, or injection of ACTH. Irradiation of the hind legs does not produce the abscopal effects seen in spleen and thymus after head irradiation. No change whatsoever was observed in thymidine incorporation or RNase activity in the liver at 24 and 48 h following 1,000 r. to the head.

Fig. 1 shows the development of these changes with time after 250 r. to the head. Increase in thymidine uptake in the thymus is already apparent at 2 h, and at 19 h the rate is close to its maximum value. The effect begins to disappear on the fourth day after irradiation. It is noteworthy that no significant change in the thymidine uptake of the spleen could be observed at any time although in this organ the RNase content was greatly increased by head irradiation. Changes in splenic RNase seem to occur a few

hours later than the increase in thymidine incorporation in the thymus, but are close to their maximum at 24 h. The increase in thymic RNase occurs later, and with a dose of 250 r. is not significant 24 h post-irradiation. In Table 2 the effects of different doses of head irradiation are compared; the data suggest that the enhancement of thymidine incorporation becomes independent of dose after 250 r., while the effect on the RNase content shows no indication of levelling off below 1,000 r., the highest dose examined.

The increased rate of thymidine incorporation is unlikely to be a pool-dilution phenomenon, since it is independent of the amount of radioactive thymidine injected, and it probably represents a true increase in the rate of DNA synthesis of certain thymus cells after head irradiation. Histological examination showed an increase in the number of dividing cells in the thymus after irradiation, and it seems probable, therefore, that the thymidine incorporation measurements reflect a true stimulation of cell division in the thymus by exposure of the head of young rats to X-rays. The rise of RNase which occurs much later in the thymus would appear to be a result of the stimulation and may be related to the return of the rate of cell division to normal.

The effect of head irradiation in the RNase content of the spleen appears to be mediated by the thymus since it

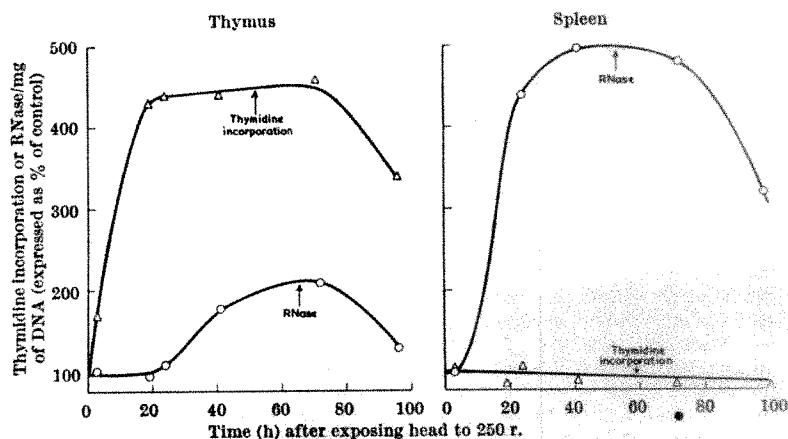


Fig. 1. Changes in the rate of thymidine incorporation ( $\Delta$ ) and in the content of RNase ( $\circ$ ) of spleen and thymus at different times after exposure of the head (only) to 250 r. of X-rays (each point is the average of measurements on five rats)

is largely abolished in animals that have been thymectomized (see Table 1). The thymus may exert an action in the spleen by excretion of humoral factors, and this form of control may be altered by head irradiation. Another interpretation is that head irradiation induces a discharge of cells from the thymus to the spleen and that on reaching the spleen these cells undergo changes which are accompanied by an increase in RNase. This hypothesis would explain why the number of cells in the thymus (that is, the DNA content of the organ) is not increased after head irradiation (see Table 1) in spite of the fact that the thymidine uptake data indicate a very large increase in the rate of cell division in this organ. Indeed, our data (see Table 2) consistently show some fall in the total content of thymus DNA after head irradiation though the magnitude of this decrease is too small to be reliably interpreted since changes due to anorexia are to be expected.

The mechanism by which exposure of the head stimulates cell division of the thymus is obscure. The experiments shown in Table 1 establish that it is not a normal

stress phenomenon following on an increase in the secretion of ACTH. In this connexion the finding of Hameed and Haley<sup>8</sup> may be relevant—that early elevation of plasma corticosterone is obtained only if the body is exposed to X-rays, head irradiation being without effect. We suggest that head irradiation, by stimulation of hypothalamic centres<sup>9</sup> or repression of some hypophyseal function, interferes temporarily with neuro-endocrine factors responsible for controlling the rate of cell turnover in the thymus. Experiments to test this hypothesis are being carried out.

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## DISTRIBUTION OF METALS AND CATECHOLAMINES IN BOVINE ADRENAL MEDULLA SUB-CELLULAR FRACTIONS

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THE ability of adenosine triphosphate (ATP) to chelate many divalent cations is well known. ATP is also able to interact with catecholamines in an ionic linkage reinforced by hydrogen bonding<sup>1</sup>. Since ATP occurs in high concentration in adrenal granules, it appeared possible that a divalent metal might be associated with it in the granules, and that this metal might be involved in the binding or release of catecholamines. For this reason, sub-cellular fractions of adrenal medulla were analysed for a series of multivalent metals.

Fresh adrenal medullae, obtained from cattle at a local abattoir, were homogenized in 0.3 M sucrose, and separated into various sub-cellular fractions using differential and gradient density centrifugation. Aliquots from these fractions were either ashed at 500° C for 2 days, or digested with acid and peroxide. The samples were then analysed for their metal content. In three experiments, multivalent metals were determined using emission spectroscopy<sup>2</sup>. In one experiment, calcium was estimated using absorption spectroscopy, and magnesium was measured by the thiazole yellow method<sup>3</sup>. Results obtained using these methods were in good agreement. The glass distilled water and sucrose used in these experiments were found to contain negligible amounts of metals. Additional aliquots of adrenal medulla fractions were taken for the determinations of protein by the biuret method<sup>4</sup>, catecholamines by the method of von Euler and Hamberg<sup>5</sup>, and succinic oxidase by the manometric method<sup>6</sup>.

Fig. 1 shows the distribution of the three major multivalent metals and catecholamines in sub-cellular fractions of bovine adrenal medulla. The metal content of chromaffin granules is small compared with the concentration of catecholamines present. For example, the ratio of catecholamine molecules to calcium atoms present in chromaffin tissue is about 27. Calcium concentration generally parallels catecholamine concentration in all fractions except mitochondria, where the calcium concentration is relatively high. This is in agreement with the observation that the calcium content of rat liver mitochondria is high<sup>7</sup>. In granules, the calcium concentration is approximately 60  $\mu$ moles/g protein, nearly 2.5 times

that in the total homogenate (25  $\mu$ moles/g protein). In contrast, magnesium, which is the most predominant divalent metal present in adrenal medulla homogenate (41.5  $\mu$ moles/g protein), is found in relatively low concentrations in granules (23.6  $\mu$ moles/g protein).

The concentration of iron in granules averages 3.9  $\mu$ moles/g protein. In the other sub-cellular fractions of adrenal medulla, the distribution of iron parallels that in analogous rat liver sub-cellular fractions<sup>7</sup>. The highest concentrations of iron are found in microsomes and mitochondria in both cow adrenal medulla and rat liver.

A number of other metals were also determined by atomic emission spectroscopy. Zinc was fairly constant at 2  $\mu$ moles/g protein in all sub-cellular fractions of medulla. Chromium, strontium, barium and manganese were found in trace quantities, and were not concentrated in any fraction.

The distributions of catecholamines and succinic oxidase activity in the several fractions of the gradient density tubes show that an almost complete separation of mitochondria from granules was achieved. In each of the four separate experiments, the calcium concentration was greater in granules than in mitochondria. Magnesium, on the other hand, was found in higher concentrations in mitochondria than in granules. In fraction III, containing both mitochondria and granules, the calcium and magnesium concentrations were intermediate between those in mitochondria and in granules (Fig. 2).

The content of calcium in chromaffin granules is especially notable when compared with that of other soft tissue fractions. For example, granules from cow adrenal medulla contain six times as much calcium/g protein as rat liver mitochondria, which, in turn, contain more calcium than any other sub-cellular fraction of rat liver<sup>7</sup>.

It was thought that this large concentration of calcium in granules might be due to chelation by ATP. Calcium, however, forms a chelate with ATP which has a slightly lower stability constant than the Mg-ATP chelate<sup>8</sup>. Since magnesium occurs in high concentration in the supernatant of adrenal medulla, one might expect mag-



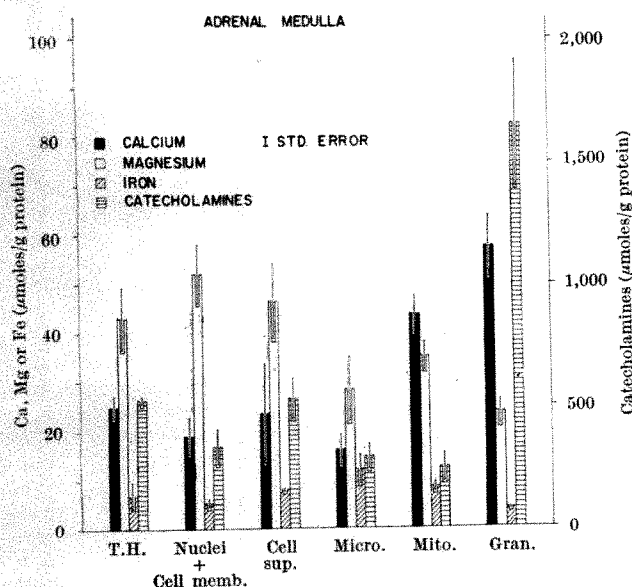


Fig. 1. Distribution of metals and catecholamines in total homogenate (TH), nuclei and cell membranes, cell supernatant, microsomes, mitochondria, and chromaffin granules of bovine adrenal medulla

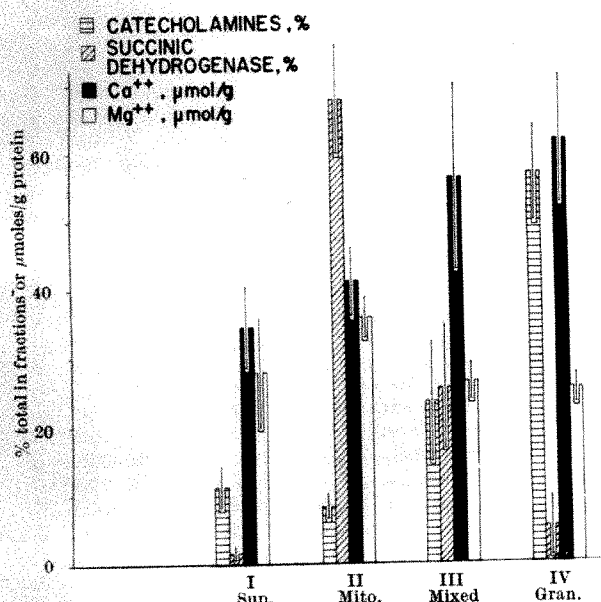


Fig. 2. Distribution of catecholamines, succinic dehydrogenase, calcium, and magnesium in four sucrose gradient density fractions of bovine adrenal medulla. The fractions are: I, supernatant or top; II, mitochondria; III, mixed, containing both mitochondria and granules; IV, granules

nesium to be more concentrated than calcium in granules, if simple sequestration by ATP were the only factor involved.

The ability of granules to take up calcium *in vitro* was tested at various temperatures using radioactive calcium. Approximately 0.2  $\mu$ c. of  $^{45}\text{CaCl}_2$  (0.375 mc./g) was added to 40 ml. of a 10 per cent homogenate (in 0.3 M sucrose) of cow adrenal medulla and a portion of the homogenate was then directly carried through the fractionation procedure at 0° C. Other aliquots were incubated for 15 min at either 4° C or 37° C followed by fractionation at 0° C. Protein was precipitated from aliquots of each fraction with 10 per cent trichloroacetic acid (TCA). The precipitates were washed twice with 5 per cent TCA and the supernatants were combined. Carrier  $^{45}\text{CaCl}_2$  (0.2 mg) was added to the acid extracts and the calcium was precipitated as the oxalate from basic solution. The calcium precipitate was uniformly deposited on a planchet and counted in a gas flow counter (efficiency = 18 per

Table 1. DISTRIBUTION OF CALCIUM-45 IN FRACTIONS OF BOVINE ADRENAL MEDULLA\*

Fraction	0° C†	4° C†	37° C†
Supernatant	0.30 ± 0.06	0.49 ± 0.04	0.96 ± 0.18
Mitochondria	2.37 ± 0.25	2.17 ± 0.27	1.10 ± 0.15
Granules	1.42 ± 0.16	1.85 ± 0.23	1.96 ± 0.24

\* Results expressed as the ratio ( $\pm$  S.E.) of c.p.m./mg protein in the fraction/c.p.m./mg protein in the total homogenate.

† No incubation prior to fractionation.

‡ Incubated for 15 min at the temperature noted prior to fractionation.

cent). Results are expressed as the ratio: c.p.m./mg protein sub-cellular fraction : c.p.m./mg protein in the whole homogenate (Table 1).

Calcium-45 is taken up by both mitochondria and chromaffin granules of bovine adrenal medulla at 0° C and at 4° C. At 37° C the mitochondria contain about the same amount of  $^{45}\text{Ca}$ /mg protein as the total homogenate.

The uptake of calcium-45 by chromaffin granules, on the other hand, is relatively independent of temperature. There is no significant difference in the uptake of calcium-45 by granules at any of the three temperatures examined (Table 1). A similar temperature-independent uptake of  $\text{Ca}^{++}$  by polymorphonuclear leucocytes was observed by Woodin and Wieneke<sup>9</sup>.

In another series of experiments EDTA (final concentration,  $10^{-3}$  M) was added to bovine adrenal medulla homogenate and the homogenate was shaken for 15 min in air at 4° C. The subcellular fractions were separated as before and analysed for protein and catecholamines in the usual manner. Calcium was measured in each fraction by atomic absorption spectroscopy. The presence of EDTA resulted in a decrease in the calcium concentration of all particulate fractions and an increase in the calcium concentration in the soluble portion of the cell. The calcium concentration of mitochondria was significantly decreased from  $43.2 \pm 2.7$  S.E.  $\mu$ moles/g protein to  $27.3 \pm 1.5$   $\mu$ moles/g protein. The calcium concentration of chromaffin granules was also decreased from  $67.8 \pm 3.7$  to  $53.4 \pm 3.3$   $\mu$ moles/g protein although this decrease was not statistically significant. It thus appears that most of the calcium of chromaffin granules is firmly bound, and is not affected by the presence of  $10^{-3}$  M EDTA.

Douglas and Poisner<sup>10</sup> showed that the uptake of calcium-45 by isolated perfused cat adrenal medulla is markedly enhanced during stimulation of the gland. The site of intracellular uptake of calcium-45 was not determined in their investigations. It is possible that calcium is taken up by the granules during adrenal stimulation and that this process may initiate the release of catecholamines from their intragranular storage complex. The existence of a high concentration of calcium in the granules is compatible with the theory that calcium is directly involved in intragranular catecholamine release. Woodin and Wieneke have shown that the leucocidin-induced release of leucocyte granule enzymes is markedly increased in the presence of calcium and ATP<sup>11</sup>. The vesicles remaining in the depleted leucocytes contain several times more calcium than granules of unstimulated leucocytes<sup>9</sup>. These investigations suggest that calcium plays a part in the initiation of the release of enzymes from granules of polymorphonuclear leucocytes. Calcium may play a similar part in the release of catecholamines from granules of adrenal medulla.

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# EFFECTS OF METABOLIC INHIBITORS ON ENERGY METABOLISM OF EHRLICH ASCITES CARCINOMA CELLS

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**I**N the course of work on the action of anti-cancer agents on metabolism of tumours, *in vitro* routine observations and evaluations have been made of the effects of several well-known metabolic inhibitors on energy-yielding processes and on operations such as protein and nucleic acid biosyntheses in tumours.

It is our objective to present one or two broad generalizations which have come to light as a result of this work.

We have worked largely with ascites tumour cells, which under appropriate physiological conditions are very suitable for such investigations. They provide uniform, highly malignant, cancer cells in which the observed effects of the agents are not obscured by factors such as necrosis and stroma which are commonly encountered in solid tumours.

Animals with 6-8 days old ascites were killed by cervical dislocation and the ascites fluid from the peritoneum was collected with a Pasteur pipette in a graduated centrifuge tube. The cells were washed several times by suspending in ice-cold Krebs-Ringer medium (without buffer or glucose) and centrifuging in the six-place head of the international clinical centrifuge at maximum (800g) speed: once for 1 min and twice for 20 sec. This procedure removed the ascites fluid and blood elements. The re-suspended cells were then centrifuged for 2 min, the supernatant was removed, and the packed cells were re-suspended in nine parts of the same Krebs-Ringer medium. Portions (0.5 ml.) of the final suspension were added to each incubation vessel. Under these conditions 0.5 ml. cell suspension corresponded to approximately 50 mg wet- or 5 mg dry-weight of tumour cells.

It is important that the cell density per vessel in different experiments should be kept constant if comparable results are required. Our observations indicate that the specific activities (that is, activities per unit weight) of measured metabolic processes (respiration, glycolysis, incorporation of radioactivity into proteins) tend to diminish with increasing cell density and that the relative effects of drugs (that is, per cent inhibitions) also decrease. The fact, for example, that the effects of actinomycin D diminish with increased density of cell population of bacteria, or of L cells, has already been reported<sup>1,2</sup>.

The conventional Warburg manometric technique was used for the measurement of respiration and glycolysis. This technique was also used during the estimation of the incorporation of radioactivity of labelled glycine into cell proteins. The Ringer media used were those described by Umbreit *et al.*<sup>3</sup>, except that calcium chloride was present at a concentration of 1.5 mM. Glucose when added was 10 mM. For measuring respiratory rates, the gas phase was air, but aerobic and anaerobic glycolytic rates were measured after appropriate gassing with mixtures of 95 per cent oxygen and 5 per cent carbon dioxide or 95 per cent nitrogen and 5 per cent carbon dioxide respectively. To secure complete anaerobiosis, in some experiments, a stick of yellow phosphorus was placed in the centre wells of the manometric vessels. The drug and any radioactive tracer were placed in the side-arm of the manometric vessel and tipped into the main compartment after 10 min thermal equilibration. The incubation time was normally 1 h at 37° C. The total volume of fluid in the vessels was 3 ml.

Radioactive glycine (glycine-1-<sup>14</sup>C) was used in the experiments described below. Its final concentration in

the manometric vessel was 2 mM. Estimation of its incorporation into the proteins of Ehrlich ascites cell was carried out as described in an earlier paper<sup>4</sup> using a thin window gas flow Geiger-Müller tube and automatic scalar. Weights of proteins from different vessels of the same experiment were approximately the same (3-4 mg) and no correction for self-absorption was necessary. The incorporation of radioactivity into proteins has been calculated in c.p.m./mg protein.

Results of individual experiments are given in Tables 1-3, where for each experiment (consisting of a control and control + inhibitor) a different preparation of Ehrlich ascites cells was used. Each experiment was carried out at least three times, and with any one preparation of ascites cells the deviation from the mean was not greater than  $\pm 4$  per cent.

*Constancy of rates of ATP formation in tumour cells in presence of certain metabolic inhibitors.* We have shown<sup>4</sup> that, in ascites tumour cells, the fall in rate of formation of adenosine triphosphate (ATP) by operation of the Crabtree effect is balanced by an increased rate of formation of ATP by enhanced aerobic glycolysis, that is, the fall in respiratory ATP is compensated by increased glycolytic ATP. Typical results given in Table 1 show that the combined rates of aerobic formation of ATP in the cell (that is, the sum of respiratory and aerobic glycolytic ATP) is a constant, in the presence or absence of a variety of metabolic inhibitors. That is:

$$6Q_{O_2} + Q_{CO_2(O_2)} = K$$

= respiratory ATP,      = aerobic      (constant)  
assuming P/O = 3      glycolytic  
ATP

The results demonstrate that in the presence of a variety of metabolic inhibitors, which affect the energetics of the cell, the generalization holds that, in the tumour in the presence of glucose, loss of ATP by diminished respiration is balanced by an approximately equal gain of ATP by increased aerobic glycolysis and that loss of ATP by inhibited glycolysis is compensated by a gain of ATP by an increased rate of respiration. For example, malonate, diazouracil, chlorpromazine or potassium cyanide, each of which decreases the respiratory rate, increases the rate of aerobic glycolysis, while DL-glyceraldehyde or carzinophilin, each of which decreases the glycolytic rate, increases the respiratory rate (Table 1).

*Effects of 'uncoupling' metabolic inhibitors.* Experiment shows that the total energy made available by respiration and glycolysis in Ehrlich ascites cells respiring in a glucose-Ringer medium is about equal to, though often

Table 1. CONSTANCY OF THE EXPRESSION  $6Q_{O_2} + Q_{CO_2(O_2)}$  OBTAINED WITH EHRLICH ASCITES CELLS IN PRESENCE OF VARIOUS METABOLIC INHIBITORS

Exp.	Glucose-Ringer media			
	$Q_{O_2}$	$6Q_{O_2}$	$Q_{CO_2(O_2)}$	$6Q_{O_2} + Q_{CO_2(O_2)}$
1 Control	7.4	44.4	24.4	68.8
+ sodium malonate 20 mM	5.8	34.8	31.0	65.8
2 Control	7.4	44.4	28.4	72.8
+ phloretin 0.1 mM	2.4	14.4	60.4	74.8
3 Control	5.8	34.8	21.2	56.0
+ glyceraldehyde 2 mM	9.8	58.8	3.7	62.5
4 Control	7.0	42.0	25.7	67.7
+ diazouracil 1 mM	4.0	24.0	52.6	76.6
5 Control	6.2	37.2	23.6	60.8
+ carzinophilin 2 µg/ml.	8.2	49.2	13.2	62.4
6 Control	6.8	40.8	24.4	65.2
+ chlorpromazine 0.1 mM	4.8	28.8	39.4	68.2
7 Control	7.0	42.0	22.7	64.7
+ potassium cyanide 1 mM	0.3	1.8	65.3	67.1

Table 2. EFFECTS OF DIFFERENT METABOLIC INHIBITORS ON ATP METABOLISM IN EHRlich ASCITES CARCINOMA CELLS  
Calculated relative amounts of ATP formed during incubation for 1 h

Exp.	Additions	n = P/O	Aerobically				Anaerobically	Action of inhibitor
			Without glucose	With 10 mM glucose			With 10 mM glucose	
			$Q_{ATP} = \frac{2nQ_{O_2}}{2nQ_{O_2} + Q_{CO_2(O_2)}}$	$2nQ_{O_2}$	$Q_{CO_2(O_2)}$	$2nQ_{O_2} + Q_{CO_2(O_2)}$	$Q_{ATP} = \frac{Q_{CO_2(N_2)}}{Q_{CO_2(N_2)}}$	
1	Nil	3	79	38	27	65	62	Uncoupler
	+ 2:4-dinitrophenol 0.1 mM	3	88	125	63	188	74	
2	Nil	0.26	7.6	11	63	74	74	Uncoupler
	+ dicumarol, 0.01 mM	3	84	46	28	74	64	
3	Nil	0.26	7.4	10	61	71	71	Respiratory inhibitor
	+ KCN, 1 mM	3	72	42	23	65	59	
4	Nil	3	3	2	55	57	55	Respiratory inhibitor
	+ sodium malonate, 20 mM	3	77	44	24	68	65	
5	Nil	3	54	35	31	66	60	Glycolytic inhibitor
	+ sodium iodoacetate 0.1 mM	3	76	42	24	66	58	
6	Nil	3	67	66	3	69	5	Glycolytic inhibitor
	+ carzinophilin, 2 µg/ml.	3	70	37	24	61	58	
7	Nil	3	64	49	13	62	23	Respiratory inhibitor
	+ diazouracil, 1 mM	3	78	42	26	68	62	
8	Nil	3	44	24	51	75	62	Inhibitor of both respiration and glycolysis
	+ phaloretin 0.2 mM	3	68	38	21	59	53	
		3	7	2	43	45	44	

Table 3. EFFECTS OF UNCOUPLERS OF ENERGY METABOLISM IN EHRlich ASCITES CARCINOMA CELLS  
1 h incubation. Glycine-1-<sup>14</sup>C = 2 mM, 10<sup>6</sup> c.p.m./vessel

No.	Additions		Ringer-phosphate medium-O <sub>2</sub>		Ringer-bicarbonate glucose medium-O <sub>2</sub> /CO <sub>2</sub>		Ringer-bicarbonate glucose medium-N <sub>2</sub> /CO <sub>2</sub>	
			No glucose		10 mM glucose			
			Q <sub>O<sub>2</sub></sub>	Inc.	Q <sub>O<sub>2</sub></sub>	Q <sub>CO<sub>2</sub>(O<sub>2</sub>)</sub>	Inc.	Q <sub>CO<sub>2</sub>(N<sub>2</sub>)</sub> corr. Inc.
1	Nil		13.2	146	6.4	27.6	131	61.8 131
	+ 2:4-dinitrophenol 0.05 mM		16.8	73	17.0	41.2	130	75.2 144
	+ 2:4-dinitrophenol 0.1 mM		14.6	36	20.8	63.2	124	73.8 142
2	Nil		14.0	148	7.6	28.0	164	64.2 142
	+ Dicumarol 0.005 mM		16.2	81	16.6	50.2	159	71.4 131
	+ Dicumarol 0.01 mM		14.2	21	19.2	61.0	158	71.8 130
3	Nil		10.6	68	6.0	30.8	106	51.2 85
	+ p-Nitrobenzene-azo-resorcinol 0.005 mM		11.8	36	12.0	40.4	110	51.8 77
	+ p-Nitrobenzene-azo-resorcinol 0.01 mM		11.0	18	14.6	48.8	108	52.4 67

Inc. = incorporation of glycine-<sup>14</sup>C into proteins expressed as c/min/mg protein

somewhat higher than, that obtained by glycolysis under anaerobic conditions in a glucose-Ringer medium. This is seen in the various typical values shown in Table 2 and Table 3, where it is clear that the aerobic value  $Q_{ATP}$  calculated as  $2nQ_{O_2} + Q_{CO_2(O_2)}$  (where  $n = P/O$  ratio = 3) is approximately equal to, but usually a little higher than, the anaerobic value of  $Q_{ATP}$  calculated as  $Q_{CO_2(N_2)}$ . This result is consistent with the fact that the incorporation of glycine-1-<sup>14</sup>C into the tissue proteins is approximately the same under aerobic and anaerobic conditions<sup>4</sup> (see also Table 3).

It is possible to arrive at a measure of the amount of uncoupling brought about by a drug that is neither a respiratory nor a glycolytic inhibitor. In the presence of a substance that uncouples phosphorylations from respiration, but is not a respiratory or glycolytic inhibitor, there is an increased rate of aerobic glycolysis. In such circumstances the value of the sum of  $6Q_{O_2} + Q_{CO_2(O_2)}$  may greatly exceed that of  $Q_{CO_2(N_2)}$  (these values being obtained in a glucose-Ringer medium in presence of the drug). For example, the well-known uncoupler 2:4-dinitrophenol at 0.1 mM gives a value of  $6Q_{O_2} + Q_{CO_2(O_2)} = 188$  which is far in excess of the value for  $Q_{CO_2(N_2)} = 74$  (Table 2). This is due to the fact that not only is the aerobic glycolysis increased but the rate of respiration is also increased. The respiratory increase, however, is not coupled with ATP formation and hence the value of  $6Q_{O_2}$  gives a wholly inaccurate measure of the ATP produced in the respiratory process in presence of the dinitrophenol. The coupled  $Q_{O_2}$  would be given approximately by the value  $\frac{Q_{CO_2(N_2)} - Q_{CO_2(O_2)}}{6}$ , taking the P/O ratio for Ehrlich ascites cells as equal to 3 (ref. 4).

This value in the case of 2:4-dinitrophenol (Table 3) at 0.1 mM is  $\frac{73.8 - 63.2}{6} = 1.8$ . This may be contrasted with the value of  $Q_{O_2} = 20.8$  obtained aerobically in a glucose-phosphate medium (Table 3). The difference between the two values, that is,  $20.8 - 1.8 = 19.0$ , may be regarded as a measure of the uncoupled rate of respiration, that is, a measure of the amount of oxygen consumption that is ineffective as a source of energy to the cell.

The value of  $n$  (that is, the P/O ratio) of the Ehrlich ascites cells in presence of glucose and an uncoupling agent may be estimated using the expression:

$$2n = \frac{Q_{CO_2(N_2)} - Q_{CO_2(O_2)}}{Q_{O_2}}$$

From the values given in Table 2 it may be calculated that in presence of either 2:4-dinitrophenol (0.1 mM) or dicumarol (0.01 mM), the P/O ratio = 0.26.

Another approach towards this measurement of ineffective or uncoupled respiration is to compare the rates of incorporation of glycine-1-<sup>14</sup>C into the tissue proteins (for such a reaction is well known to be ATP-dependent) in the presence and absence of the uncoupler. It will be seen in the results given in Table 3 that, in the absence of glucose, when glycolytic ATP is not available, the ratio of the rates of incorporation of glycine-1-<sup>14</sup>C, into Ehrlich ascites proteins, in the presence and absence of 0.1 mM dinitrophenol =  $36/146 = 0.25$ . Assuming that this ratio represents the ratio of the rates of formation of ATP in the cell, in the presence and absence of the drug, this value will also express the ratios of the coupled rates of respiration in the cell under the two conditions. As the

$Q_{O_2}$  (in absence of glucose) in absence of the drug = 13.2 (Table 3), the effective or coupled  $Q_{O_2}$  (in absence of glucose) will be  $13.2 \times 0.25 = 3.3$  in presence of the drug. This may be compared with the value of 1.8 already given here, which, however, is the value calculated from experimental results obtained in the presence of glucose.

Calculations of the extent of uncoupling may be made, therefore, either from the ratios of the rates of incorporation of glycine-1- $^{14}C$  into tissue proteins, or from the aerobic and anaerobic glycolytic rates, or on the basis of the conclusion that the sum of the rates of formation of respiratory ATP and glycolytic ATP (in the presence of glucose) is constant. Such calculations show that, in the presence of 0.05 mM and 0.1 mM 2:4-dinitrophenol, the coupled respiratory rates, calculated from the respiratory and aerobic glycolytic rates, are far lower than the observed respiratory rates in presence of glucose, but approximate to the value of the observed respiratory rates calculated from the ratios of the rates of incorporation of glycine-1- $^{14}C$  into the tissue proteins in absence of glucose. For example, from Table 3, the coupled  $Q_{O_2}$  in presence of 0.05 mM dinitrophenol and in absence of

glucose is equal to  $\frac{73 \times 13.2}{146} = 6.6$  when calculated

from glycine incorporation rates and equal to  $\frac{75.2 - 41.2}{6}$

= 5.7 when calculated from the anaerobic and aerobic glycolytic rates. The observed respiratory rate,  $Q_{O_2}$ , is equal to 16.8. Concentrations of 2:4-dinitrophenol higher than 0.1 mM lead to diminutions of respiratory rates. It is evident that other effects of dinitrophenol are operating at relatively high concentrations<sup>4</sup>, the reasons for which are not yet clear and allowance for them cannot be made at present.

Dicumarol acts as a typical uncoupler at the concentrations, 0.005 mM and 0.01 mM, investigated. Again, it is evident from results quoted in Table 3 that the coupled  $Q_{O_2}$  calculated from the aerobic and anaerobic glycolytic rates is far less than the observed respiratory rate in presence of glucose, and approaches the value of the coupled  $Q_{O_2}$  calculated from the values of the glycine incorporation rates into proteins found in the absence of glucose. A similar observation applies to the metal chelator, *p*-nitrobenzene-azo-resorcinol.

Values of incorporation of glycine into tissue proteins in the presence of glucose, aerobically or anaerobically,

show that 2:4-dinitrophenol and dicumarol have negligible inhibitory effects on protein biosynthesis at the concentrations tested (Table 3). *p*-Nitrobenzene-azo-resorcinol acts as an uncoupler without significant effect on total energy metabolism. It seems, however, to exercise an inhibitory effect, that gradually increases with increase of concentration, on anaerobic incorporation of glycine-1- $^{14}C$  into the proteins of Ehrlich ascites tumour cells.

Measurements of respiratory rates, and aerobic and anaerobic glycolytic rates in presence of an inhibitor are obviously helpful in indicating whether the inhibitor is acting mainly as a respiratory uncoupler, respiratory inhibitor or glycolytic inhibitor. Thus it will be seen from the results given in Table 2 that malonate, iodoacetate, carzinophilin, diazouracil and phloretin exercise respiratory or glycolytic inhibitions without any demonstrable uncoupling effects at the concentrations tested.

In summary, the present work shows that, in presence of a variety of metabolic inhibitors which affect the energetics of the Ehrlich ascites cell, the generalization holds that the fall in the rate of formation of ATP by diminished effective respiration, due to the inhibitor, is balanced by an approximately equal rise in the rate of formation of ATP by increased glycolysis. Moreover, the fall in the rate of formation of ATP by a decreased glycolytic rate, due to an appropriate inhibitor, is balanced by a rise in the rate of formation of ATP by increased respiration in the presence of glucose. From a knowledge of the different rates of incorporation of radioactivity from glycine-1- $^{14}C$  into tissue proteins, in the presence or absence of the inhibitor, or from the aerobic and anaerobic glycolytic rates under the same conditions, it is possible to estimate approximately the amount of uncoupling of phosphorylation from oxidation that occurs with inhibitors which act solely as uncouplers and thereby that amount of tumour respiration which is coupled to biosynthetic processes.

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## SUBSTRATE EFFECT ON HEAT INACTIVATION OF TAKA-AMYLASE A

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IT is known that some enzymes, for example, D-amino-acid oxidase<sup>1</sup>, glutamic dehydrogenase<sup>2</sup>, lactic dehydrogenase<sup>3</sup>, alcohol dehydrogenase<sup>3</sup>, bacterial  $\alpha$ -amylase<sup>4</sup> and taka- $\alpha$ -amylase<sup>5</sup>, are protected from the inactivation and denaturation by coenzymes or substrates or related compounds. Okunuki and others<sup>4,5</sup> concluded that the protective effect of starch on the inactivation and denaturation of taka- and bacterial  $\alpha$ -amylase by heat, acid and urea must be derived from the hydrolysis products of starch, for the reason that starch was completely hydrolysed within 1 min under experimental conditions. Furthermore, they concluded that the protective effect of digestion products of starch was attributed to protection of the secondary structure of enzyme proteins from denaturation.

This article deals with more detailed protective action of substrate, starch, from heat inactivation in the case of taka-amylase A, and the separation of protective effect

of starch from that of decomposition products from starch was performed under the suitable experimental conditions.

Taka-amylase A is highly protected from heat inactivation by the presence of substrate, starch. This is shown in Fig. 1. Curve (1) shows activity-incubation temperature relation (incubated for 10 min). In the absence of substrate, activity decreases suddenly over 50° C and is completely lost at about 65° C. On the other hand, the presence of substrate (0.6 per cent starch) gives rise to remarkable resistance against heat inactivation, and the activity-incubation temperature curve shifts towards higher incubation temperature than curve (1) as shown in Fig. 1 (2).

The amount of this shift is about 3.5° C under the present experimental conditions, and this is thought to be the 'effective temperature' lowered as substrate effect. Curve (3) is the difference between curve (2) and (1) and shows a sharp peak at about 60° C. From curve (3), it

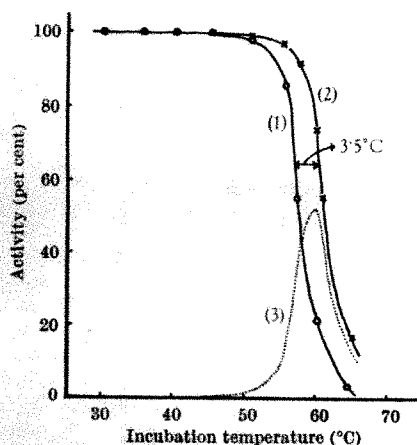


Fig. 1. Change of activity of taka-amylase A with incubation temperature. Incubation time, 10 min; concentration of taka-amylase A, 0.005 per cent at pH 5.6 (0.2 M acetate buffer). Curve (1), taka-amylase A alone; curve (2), taka-amylase A + starch (0.6 per cent); curve (3), difference of activity between (2) and (1).

is understood that the protective action is most remarkable at the 'transformation temperature' from native to denaturation. Heat inactivation was carried out by the following procedure. Aqueous solutions of taka-amylase A (0.005 per cent), in the absence and presence of 0.6 per cent soluble starch, are incubated for 10 min at various temperatures in the thermostat and rapidly cooled at 0°C after incubation. The activity of taka-amylase A was measured at 40°C by the blue value method<sup>6</sup>. All the experiments were made in 0.2 M acetate buffered solutions at pH 5.6. Taka-amylase A was prepared from 'takadiastase Sankyo' and recrystallized three times from aqueous acetone solution containing 0.01 M calcium acetate by Akabori's method<sup>7</sup>.

Fig. 2 shows the dependence of activity on incubation time at an incubation temperature of 60°C, which is the temperature with the most effective protective action. Curves (1) and (2) show activity-incubation time relation in the absence and presence of substrate respectively. It takes a much longer incubation time in the latter case than in the former to obtain an equal degree of inactivation.

This is shown in curve (4) obtained from the difference between the incubation time in curve (2) and (1) at the same inactivation. From the extrapolation of curve (4) to zero activity, it is known that the longer incubation time in the coexistence system of substrate by about 9 min over that of the substrate-free system is necessary for complete inactivation. Curve (3) shows the difference between the activity in both systems at the same incubation time. The optimum activity is observed after about 9 min

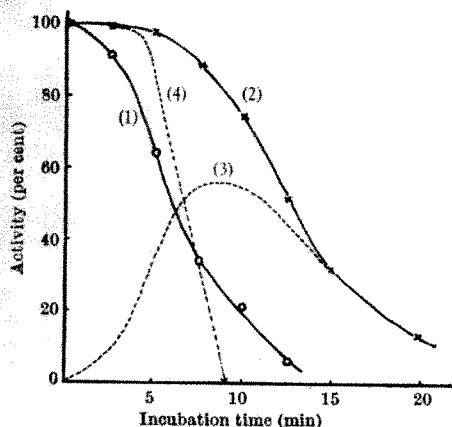


Fig. 2. Change of activity of taka-amylase A with incubation time. Incubation temperature, 60°C; concentration of taka-amylase A, 0.005 per cent, at pH 5.6 (0.2 M acetate buffer). Curve (1), taka-amylase A alone; curve (2), taka-amylase A + starch (0.6 per cent); curve (3), difference of activity between (2) and (1); curve (4), difference of incubation time between (2) and (1).

of incubation. The protective action observed in the presence of substrate is thought to be derived from the superimposed effects of starch and its hydrolysis products. In order to separate the protective effect of starch from that of hydrolysis products, the incubation was made after various periods of digestion. The dependence of activity on digestion time is shown in Fig. 3 (1).

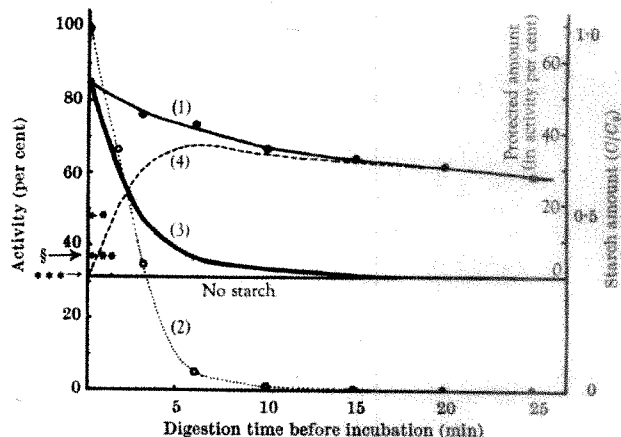


Fig. 3. Changes of activity of taka-amylase A with digestion time before heat treatment and of starch concentration with digestion time, and contributions to protective action from starch and hydrolysis products. Incubation temperature, 59°C; incubation time, 10 min; concentration of taka-amylase A, 0.005 per cent at pH 5.6 (0.2 M acetate buffer). Curve (1), taka-amylase A + starch (0.6 per cent); curve (2), starch concentration at various digestion times; curve (3), protection by starch (degree of polymerization  $> \sim 6$ ); curve (4), protection by hydrolysis products (degree of polymerization  $< \sim 6$ ); \*, \*\*, \*\*\* and § are, respectively, the activity after heat treatment in the presence of dextrin, maltose, glucose and limit dextrin.

The incubation temperature and time are, respectively, 59°C and 10 min, at which the protective action is most remarkable, as shown in Figs. 1 and 2. In the absence of starch, activity decreases to 32 per cent after heat treatment. The amount of substrate (degree of polymerization  $n > \sim 6$ ) in the course of digestion, which was measured by iodostarch reaction, is shown in Fig. 3, curve (2).

As shown in Fig. 4, the decrease in activity by heat treatment is proportional to the concentration of substrate in the region of low substrate concentration, where the incubation temperature and time are, respectively, 60°C and 10 min and incubation was started immediately after mixing the substrate with amylase solution.

This proportionality makes it possible roughly to separate the total protected amount (expressed in activity difference between curve (1) and the activity after heat treatment in the absence of substrate) into two parts: protected amount by substrate ( $n > \sim 6$ ) and that by decomposed compounds ( $n < \sim 6$ ). These are shown in Fig. 3 (3) and (4) respectively. In the short period of digestion, the protection is mainly due to the effect of substrate ( $n > \sim 6$ ). This protective effect is thought to be attributable to the stabilization of the secondary structure of enzyme protein by the formation of enzyme-substrate complex. The formation of an intermediate enzyme-substrate complex in enzyme-catalysed reaction was first postulated by Michaelis and Menten<sup>8</sup>. This concept has been experimentally proved from kinetics of enzyme reactions by various workers, in various systems. Keilin and Mann, and others<sup>9</sup>, have shown spectroscopically the existence of complexes of peroxides with catalase and peroxidase and calculated the thermodynamic quantities of enzyme-substrate complex such as free energy, entropy and enthalpy. Recently, the change of higher order structure of enzyme on the formation of enzyme-substrate complex has been investigated by means of optical rotation<sup>10</sup> and difference spectra<sup>11</sup>.

\* The minimum degree of polymerization of amylose to colour on treatment with iodine. cf. Swanson, M. A., *J. Biol. Chem.*, 172, 825 (1948).



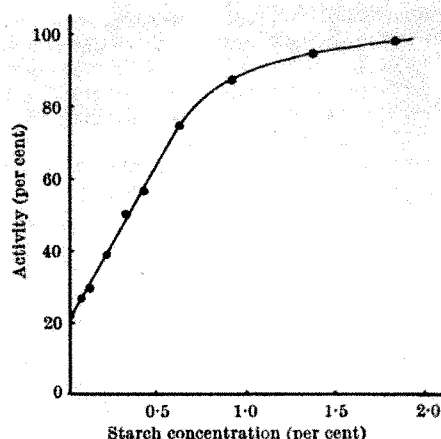


Fig. 4. Dependence of activity after heat treatment on starch concentration. Incubation temperature, 60° C; incubation time, 10 min; concentration of taka-amylase A, 0.005 per cent at pH 5.6 (0.2 M acetate buffer)

These experimental results show that the structure of enzyme protein changes from disorder to order on the formation of enzyme-substrate complex. Such a structural change may lead to stabilization of the enzyme and to protection from external disturbances, for example by heat treatment. On heat inactivation of taka-amylase A, the presence of substrate seems greatly to stabilize the secondary structure of enzyme protein by the formation of enzyme-substrate complex and to give rise to a profound resistance against inactivation and denaturation. A difference spectrum of taka-amylase on the formation of complex with substrate has not been observed, but in the cases of lysozyme and  $\alpha$ -chymotrypsin<sup>11</sup>, difference spectra on the formation of enzyme-substrate complex are well established. On the formation of enzyme-substrate complex, ultra-violet absorption spectra of enzymes usually show 'red shift' and this means the conformation change of enzyme protein from disorder to

order. This conformation change may conjugate with the appearance of enzyme activity. The ordered and rigid structure of enzyme protein may be essentially responsible for the appearance of enzyme activity. Furthermore, this structure may be highly stabilized and protected from the external disturbance such as thermal agitation. This seems to be the main reason why taka-amylase can be protected from heat inactivation by the presence of substrate. The decomposition products from starch,  $\alpha$ -limit dextrin and maltose are also effective for protection of amylase from heat inactivation, but glucose has no protective effect. In the presence of these substances (0.6 per cent in each), the activity decreases by heat treatment (at 59° C and for 10 min) to 38, 37 and 32 per cent respectively, as shown in Fig. 3. Limit dextrin and maltose seem to stabilize the secondary structure by combination with amylase, and to protect it from heat inactivation and denaturation, though the effect is not so great. The site with which these compounds are combined, unlike substrates, is not necessarily the active point of amylase. So, the induced stabilization is considered to be probably similar to that by calcium ions<sup>12</sup>.

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## REPRESSION OF GROWTH, AND SUBSEQUENT ADAPTATION, OF A PARENTAL STRAIN TUMOUR IN GENETICALLY COMPATIBLE $F_1$ HYBRID MICE

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AS is known from the work of many investigators<sup>1</sup>, starting with C. C. Little, the successful transplantation of neoplasms, as of normal tissues, depends on their not possessing histocompatibility antigens different from those of the host. Thus, transplantation generally succeeds within inbred lines of animals or from animals of an inbred line to  $F_1$  hybrids between that inbred line and some other (not necessarily inbred) line.

However, recently a number of investigators<sup>2-5</sup> have reported experiments in which a tumour growing in a particular inbred line of mice grows less readily, or not at all, in  $F_1$  hybrid hosts. A comparable phenomenon of deficient growth of normal parental bone marrow cells in the  $F_1$  has also been reported<sup>6,7</sup>. The purpose of this article is to report a similar  $F_1$  hybrid effect with a transplantable tumour and, in addition, an adaptive change in the tumour.

Female mice of the A/J and C57BL/6J strains, and their  $F_1$  hybrid ( $B_6A.F_1$ ) (designated as A, B and  $F_1$ , respectively), were used. A suspension of lymphoma, minced with not more than 0.25 volume of physiological saline, was injected through a 16-gauge needle into the sub-

cutaneous tissues of the neck-and-shoulders region. The lymphoma had originated in a B mouse near the site at which syngeneic spleen fragments had been injected 97 days previously in connexion with other experiments<sup>8</sup>.

The primary tumour was transplanted after 28 days into 4 A, 4 B and 4  $F_1$  mice, and grew progressively only in 2 of the B hosts. In one (designated 21) the tumour developed more slowly than in the other (21a). Tumour 21a was transplanted (0.4-0.8 ml. of suspension) after 71 days into 5  $F_1$  and 3 B hosts, two of the latter having previously rejected the original tumour. It failed to grow progressively in the rejectors and  $F_1$  hybrids, but grew to large size in one B host. On days 198 and 127, respectively, after the previous transfers, a second transplantation with a single tumour of line 21 and a third with a single tumour of line 21a were made, 0.2 ml. of suspension of each tumour having been injected into six animals of each of the three genotypes. The results are shown in Table 1.

Histological examinations of specimens removed from all 18 tumour-bearing mice failed to reveal any obvious



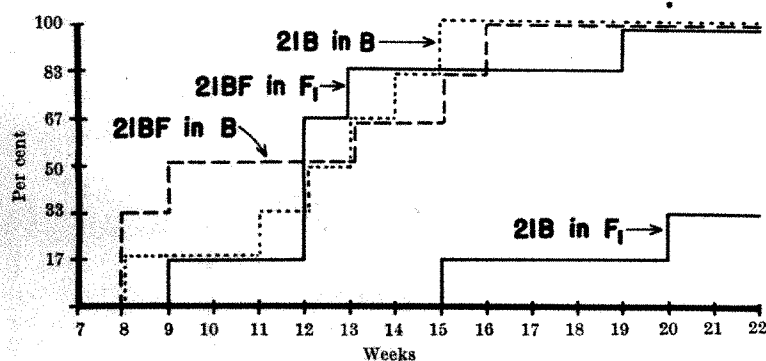


Fig. 1. Cumulative mortality (percentage of tumour-bearing mice having died with tumours) in  $B$  and  $F_1$  groups, exclusive of resistant  $F_1$ s.

differences in appearance between lines 21 and 21a, and between tumours grown in  $B$  or  $F_1$  hosts.

One  $B$  and one  $F_1$  recipient of tumour 21 were killed, as indicated in Table 1, in order to transplant their tumours which weighed, respectively, 6.96 g (21B tumour) and 11.18 g (21BF tumour) exclusive of metastases. The 21B tumour was transplanted (0.2 ml. of suspension) to 7 stock  $B$ , 7 stock  $F_1$ , and 7 'resistant'  $F_1$  that had rejected previous grafts of the original tumour or its sublines 21 and 21a. Likewise, the 21BF tumour was transferred to 6 stock  $B$ , 7 stock  $F_1$  and 7 'resistant'  $F_1$ . The results are shown in Table 2 and Fig. 1. In Table 2, persisting tumours are those which were present until the death of the animal or until the termination of the 25-week observation period; regressing tumours were present at some time after the second week but were not present at the end of 25 weeks or when the animal died.

Table 1. BEHAVIOUR OF TWO TRANSPLANTABLE LYMPHOMAS OF COMMON ORIGIN IN HOMOZYGOUS AND  $F_1$  HYBRID HOSTS

Tumour	Hosts	No. in group	No. with tumours	Days post-grafting on which deaths occurred
21	$B$	6	6	56, 64, 75, 78, 81
	$F_1$	6	1	(one killed on day 82)
	$A$	6	0	(one killed on day 87)
21a	$B$	6	6	56, 60, 60, 62, 71, 73
	$F_1$	6	5	(All 5 killed on day 85)
	$A$	6	0	—

Table 2. RESULTS OF TRANSPLANTATION EXPERIMENTS WITH  $F_1$ -PASSED (21BF) AND UNADAPTED (21B) TUMOURS

Tumour	Type of host	No. of mice in group	No. with persisting tumours	No. with regressing tumours	No. dying with tumours	Survivors through 25th week
21BF	$B$	6	6	0	6	0
	$F_1$	7	6	0	6	0
	Resistant $F_1$	7	3	3	3	4
21B	$B$	7	6	0	6	1
	$F_1$	7	6	0	2	5*
	Resistant $F_1$	7	1	5	1	5

\* Four bearing tumours. These died during the 28th, 35th, 37th and 48th weeks.

The 21B tumour, which had never been exposed to the  $F_1$  environment, exhibited deficient growth in the  $F_1$  hosts, whereas the 21BF tumour, which had been passed through an  $F_1$  host, behaved toward ordinary  $F_1$  hosts as it did toward syngeneic  $B$ -hosts. Of the resistant  $F_1$  hosts, a greater proportion survived challenge with the 21B tumour than with the 21BF tumour, and there were more cases of tumour regression among resistant  $F_1$  recipients of  $B$  tumour.

(The sixth transplant-generation of the 21B tumour was grown in 9  $B$  and 9  $B_6D_3F_1$  hosts. Deficient growth of the tumour in  $F_1$  hosts was again observed.)

It is known that tumours occasionally undergo alterations that permit them to grow in a greater percentage of  $F_1$  hybrid hosts or hosts produced by back-crossing  $F_1$  hybrids to the resistant parental strain<sup>9</sup>, and these changes are generally regarded as being due to loss or inactivation of isoantigenic determinants within the tumour cells, permitting the cells to avoid provoking the homograft-rejection reaction in foreign hosts<sup>10-14</sup>. The methods by which tumours may be adapted to foreign hosts include

passage through adult<sup>11</sup> and embryonic<sup>13</sup> foreign genotypes, through radiation chimaeras<sup>14</sup> and through half-foreign  $F_1$  hybrids<sup>15,16</sup>. In the latter experiments<sup>15,16</sup> the  $F_1$ -passed tumours grew in a greater proportion of resistant back-cross mice and thus behaved as though exposure to the  $F_1$  environment had caused them to lose certain isoantigens.

Snell and Stevens<sup>9</sup> suggested that the deficient growth of parental tumours in  $F_1$  hybrids might be due to an abortive graft-versus-host reaction. Presumably the mechanism for this might be allergic death of the grafted cells<sup>17</sup>. Hellström<sup>8</sup> argues against abortive graft-versus-host reaction inasmuch as the effect is obtained with grafts of

tissues that are presumably not immunologically competent. This argument presupposes that the relevant tumours or their normal counterparts do not possess cells that are immunologically competent or capable of becoming so<sup>8,18</sup>. However, without referring specifically to allergic death, Hellström<sup>8</sup> suggested that exposure to foreign antigens of the host may inhibit the growth of transplanted cells. He<sup>4</sup> also considered the possibility of recessive histocompatibility ( $H$ ) genes, but argued against this because: (1) recessiveness of  $H$ -genes has not been unequivocally demonstrated in various experiments; (2) irradiation of the  $F_1$  hybrid prior to grafting does not abrogate its relative resistance.

For the similar phenomenon with normal bone marrow, Cudkovic and Stimpfling<sup>6</sup> offer modifications of the recessive  $H$ -gene and allergic-death hypotheses, and favour the view that, in heterozygotes, interallelic interaction prevents or modifies the expression of a genetic determinant in the  $H$ -2 locus "whose expression is required for the growth of transplanted marrow cells". The unimpaired growth of transplanted marrow cells in allogeneic homozygous hosts is considered<sup>6</sup> to be opposed to the allergic-death interpretation.

Since the present findings of preferential growth of the tumour in the parental strain of origin are similar to those of the authors previously cited<sup>2-7</sup>, they are subject to similar interpretations; but the additional phenomenon of adaptive change would tend to favour explanations based on selection of genetic variant cells. If one or more parental isoantigens were not expressed on cell-surface sites in the  $F_1$  hybrid, then the deficient growth of the 21B tumour in the  $F_1$  hybrids initially could be due to an  $F_1$ -antiparent immunological reaction, conditioned by the  $H$ -genes of that parent being recessive in the  $F_1$ . The unimpaired growth in the  $F_1$  of the 21BF tumour in later transplant generations could be interpreted as due to an antigenic simplification of the tumour, the lost isoantigens being those that are non-codominant. If it is assumed that the proliferative stimulus to the tumour is greater the greater the number of host antigens that it lacks<sup>6,18</sup>, then selection would operate in the direction of such antigenic simplification. This would also permit growth in a greater percentage of resistant back-cross animals, as has been described<sup>15,16</sup>.  $F_1$  serum factors would in this case be effective as additional selective agents in conditioning—or enhancing—the parental tumour<sup>19</sup>, as antisera are known to be<sup>9</sup>. The results presented here are also consistent with the allergic-death explanation on the assumption that exposure to the  $F_1$  hybrid environment has selected a population of parental-strain tumour cells which are resistant to such destruction. A third possibility, consistent with the present results and that of the other authors, is that a naturally occurring autoantibody, perhaps possessing normally a growth-regulating function, is expressed more completely in heterozygotes and is capable of inhibiting the growth of homozygous cells<sup>20</sup>. The homozygous cells would adapt to its presence through suppressed production of isoantigens.

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## GLUCOSE METABOLISM VIA THE PENTOSE PHOSPHATE PATHWAY RELATIVE TO CELL REPLICATION AND IMMUNOLOGICAL RESPONSE

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**R**EGULATION of glucose metabolism via the pentose phosphate pathway by pharmacological agents may provide a means of influencing cell replication and protein production.

The metabolism of glucose via the pentose route produces pentose sugars, the necessary building blocks for the biosynthesis of RNA and DNA, and, in turn, for protein formation. Thus inhibition—whether physiological or biochemical—of glucose utilization via the pentose cycle will result in a reduced production of D-ribosyl units and consequently diminish certain cellular function such as that of replication and the formation of proteins.

*In vitro* experiments have demonstrated a parallelism between the rate of pentose shunt activity and that of RNA synthesis in normal tissue<sup>1</sup>, and the rate of pentose shunt activity and that of RNA and DNA synthesis in malignant tissue<sup>2</sup>. It has also been established that a decrease in glucose metabolism via the pentose route, whether due to normal physiological response<sup>3</sup> or to the effects of chemical compounds<sup>1,2</sup>, entrains simultaneous corresponding repercussions in cell processes requiring nucleic acid and protein synthesis.

Thus, while there is consistently clear evidence of a parallelism between the rate of pentose shunt activity, nucleic acid synthesis and protein production in both normal and malignant tissues, definite confirmation has been lacking as to whether alterations in the rate of pentose shunt activity directly induce corresponding fluctuations in the formation of pentose sugars.

Such evidence was sought by isolating and identifying the amount of pentose sugars in the RNA and estimating its turn-over rate relative to that of pentose shunt activity and total RNA synthesis.

Three types of tissue were examined: human placenta during the first trimester of pregnancy and at term, normal and regenerating rat liver and adenocarcinoma of the large bowel.

The tissue samples were cut with a Stadie-Riggs slicer into thin slices weighing about 100 mg which were transferred to Warburg flasks containing ice-chilled Krebs-Ringer bicarbonate buffer medium at pH 7.4 with a

glucose concentration of 0.15 per cent. The samples were incubated for 150 min at 37° C in 95 per cent oxygen and 5 per cent carbon dioxide. Four types of radioactive material were used in this investigation: U-<sup>14</sup>C glucose, glucose-1-<sup>14</sup>C, glucose-6-<sup>14</sup>C and U-<sup>14</sup>C glycine.

The specific activity of nucleic acid and ribose turnover was measured by their incorporation of U-<sup>14</sup>C glucose. The RNA was isolated from the homogenized tissue by Kirby's phenol method<sup>4</sup> and recovered from the two-phase partition system as a cetyltrimethyl ammonium salt<sup>5</sup>. Approximately a half of the RNA sample was used to determine the total radioactivity; the other aliquot was hydrolysed with 1 N hydrochloric acid for 1 h at 100° C, after which the hydrolysate was run on Whatman No. 3 paper in a system of *n*-butanol (50 vol.), pyridine (30 vol.) and water (20 vol.). The positions of the pyrimidine and purine bases were located by ultra-violet examination of the chromatogram, and the positions of the ribose and glucose were determined by spraying the chromatogram with aniline oxalate and developing it at 100° C dry heat for 10–20 min. Glucose appeared as a brown spot and ribose as a pink one. Originally, the radioactivity was measured by an automatic chromatographic scanner, but because of the relatively low counts obtained this was consequently done in a liquid scintillation counter.

To some flasks, for each 3 ml. of incubating medium, was added a TPNH generating system which depresses the activity of the shunt<sup>1</sup>. In our experiments, this system comprised 0.1 ml. of 10 per cent ethanol containing 150 µg of crystalline alcohol dehydrogenase (ADH). To other flasks was added 0.2 ml. of 0.05 per cent methylene blue per 3 ml. of incubating medium, this being an electron accelerator which increases the metabolism of glucose via the pentose phosphate pathway.

To minimize the often varied effects of ethanol alone on tissue metabolism obtained in previous investigations<sup>1</sup> the concentration in the present series of experiments represents one-fifteenth of that previously used. In this concentration, ethanol alone produced practically no alteration in the rate of glucose metabolism via the pentose cycle; yet when the ADH was added the desired depressant effect was constant and always reproducible.

The relative utilization of the pentose phosphate pathway in glucose metabolism was determined on the basis

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Table 1. D-RIBOSE FORMATION, RIBONUCLEIC ACID AND PROTEIN SYNTHESIS

Tissue	Addition to medium	C.p.m./mg ribonucleic acid *				C.p.m./mg of dry protein†
		Unhydrolysed RNA	Hydrolysis products			
			Ultra-violet absorbing areas	D-ribose	Counts not recovered	
Placenta of 9 weeks gestation	None	16,580	11,845	2,900	2,335	5,210
Placenta of 8 weeks gestation	None	18,870	10,950	2,240	5,680	6,430
Placenta: vaginal delivery at term	None	6,340	4,700	810	830	2,412
Placenta: Caesarean section at term	None	5,730	4,220	648	862	2,740
Placenta of 11 weeks gestation	None	13,450	10,400	2,006	1,044	6,754
Placenta: vaginal delivery at term	ADH	9,440	7,824	1,290	326	5,100
	None	5,016	4,146	840	30	3,175
	M.B.	6,860	4,372	926	1,562	3,036
Normal liver	None	1,540	1,190	193	157	—
	ADH	938	760	64	110	—
Normal liver	None	1,800	1,316	268	216	4,008
	M.B.	1,920	1,450	290	180	3,810
	ADH	1,406	1,134	148	124	2,143
Regenerating liver	None	4,170	3,460	590	120	—
	ADH	3,054	2,191	405	458	—
Adenocarcinoma of large bowel	None	2,635	1,740	398	497	—
	ADH	2,084	1,396	240	448	—

\* Precursor U-<sup>14</sup>C glucose. † Precursor U-<sup>14</sup>C glycine.

of the fractional recoveries of carbon dioxide from glucose-1-<sup>14</sup>C and glucose-6-<sup>14</sup>C, and also by measuring the amount of radioactive material in the medium before and after incubation. Protein production was determined by the rate of incorporation of U-<sup>14</sup>C glycine into total protein.

All tissues examined showed that, in the presence of ADH, pentose shunt activity was decreased, the carbon dioxide yields from glucose-1-<sup>14</sup>C being reduced by at least 30 per cent, while those from glucose-6-<sup>14</sup>C remained virtually unchanged. This reduction was also borne out by calculating the amount of radioactive glucose utilized during incubation. Methylene blue, on the other hand, greatly increased the rate of glucose metabolism via the pentose phosphate route, the carbon dioxide yield from glucose-1-<sup>14</sup>C often being doubled. These findings are in accord with data already obtained in previous investigations<sup>1,2</sup>.

The incorporation of U-<sup>14</sup>C glucose into RNA and its ribose fraction are shown in Table 1. Term placenta reveals an RNA value about one-third of that present during the first trimester of pregnancy. This is in accord with the observation<sup>3</sup> that in the first trimester of pregnancy the rapidly growing placenta demonstrates a high rate of glucose metabolism via the pentose phosphate pathway; whereas, at term, the utilization of this pathway is so much reduced as to be almost immeasurable. After hydrolysis and subsequent chromatography, some 80 per cent of the total RNA count was found to be concentrated in ribose and pyrimidine spots. The former comprised 10–20 per cent of the total radioactivity measured and this proportion of ribose corresponded in all tissues examined with the RNA turnover and the rate of pentose shunt activity, whether the latter was influenced by physiological or pathological processes, or the addition of ADH to the medium.

Normal and regenerating liver revealed a similar picture to placenta inasmuch as the regenerating liver, being an actively growing tissue, has an increased pentose shunt activity with correspondingly increased D-ribose formation and nucleic acid synthesis.

In all the experiments using placenta, liver and adenocarcinoma of the bowel, the presence of ethanol ADH in the medium during incubation of the tissue reveals a decreased ribose formation and nucleic acid synthesis consequent to the decreased rate of pentose shunt activity.

In all instances, the decrease in pentose shunt activity—whether produced by physiological or pharmacological means—was followed by a reduction in ribose formation and nucleic acid synthesis. The addition of the electron accelerator to the medium, while always producing a marked elevation in glucose metabolism via the pentose route, did not entrain a parallel augmentation in ribose and RNA formation, these revealing only a slight increase.

A rise in pentose shunt activity will produce a corresponding simultaneous increase in ribose formation and RNA synthesis only in response to physiological or pathological demands *in vivo* where the other necessary intermediate products become available in the elevated quantities required. *In vitro*, this cannot be accomplished by increasing the pentose shunt alone.

Alternatively, a decrease in glucose metabolism via the pentose route will always entrain parallel reduction in the formation of ribose and nucleic acids, as a decreased availability of any one of the compounds essential for the synthesis of nucleic acids will limit their formation.

Timed experiments and others still in progress suggest that the reduction in pyrimidine and purine bases (Table 1) can be considered, in the main, as secondary to a decrease in ribose formation. Furthermore, the metabolic effects of ethanol-ADH may involve some action additional to that on the TPNH generating system.

In all types of tissue examined, reduction in glucose metabolism via the pentose phosphate pathway is also concurrent—as can be seen from Table 1—with a decrease in protein formation. This is probably mainly a repercussion of reduced RNA synthesis, since it is now believed that the rate of protein production is dependent on the availability of messenger RNA<sup>6</sup>. Thus, the effects on general protein biosynthesis of alterations in the rate of pentose shunt activity can be considered secondary to the primary effects on nucleic acid formation.

Antigenic stimulation producing antibodies is essentially a process involving protein (γ-globulin) formation. Investigations of antibody formation in rabbit spleen show that initiation of the immunological response is characterized by increased glucose metabolism via the pentose cycle with augmented ribose formation and, thus, nucleic acid synthesis.

The results of the investigation recorded here therefore illustrate that: (a) ribose formation runs in parallel with the rate of glucose metabolism via the pentose phosphate pathway; (b) if the rate of glucose metabolism via the pentose phosphate pathway is altered by physiological or pharmacological means simultaneous changes are produced in the rate of D-ribose formation and consequently in RNA synthesis and protein production.

It can thus be stated that a prolonged reduction in pentose shunt activity with the concomitant decrease in nucleic acid synthesis should incur repression of cell replication and interference with some aspects of immunological reaction.

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## ANALYSIS OF FOVEAL DENSITOMETRY

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THE identification and analysis of the visual pigments *in situ* are based on fundamental principles of spectrophotometry. Moreover, they involve those factors which are unique to the physico-chemical organization of the pigment molecules within the receptors and the spatial distribution and orientation of these elements within the retina. Spectral densitometry of a light-absorbing medium relates the wave-length of the incident radiation to the attenuation of said radiation by absorption on passage through the medium. The interpretation of densitometric data is greatly simplified in the usual circumstance of measurement where the pigment molecules are distributed uniformly throughout their container and the Beer-Lambert laws are directly applicable. If these conditions are satisfied, then the optical density of a mixture of solutes is given by the sum of the component densities. The situation becomes more complicated when the molecules are not distributed uniformly in space.

For purposes of this article one can distinguish two special cases: (a) the existence of concentration gradients or boundaries in planes perpendicular to the direction of the light beam utilized for transmissivity measurements (Fig. 1a); (b) occurrence of such gradients or boundaries in planes parallel to the measuring beam (Fig. 1b).

Both these conditions are represented in the organization of pigments within the receptor layer of the retina (Fig. 1c). It is the object of this article to compare densitometry for these two conditions, to illustrate how each obtains as regards the quantitative analysis of visual pigments, and to examine some predictions derived from this analysis in the light of experimental measurements on the human retina.

**Theory.** The laws of Beer and Lambert state that the optical density  $D_\lambda$  (defined as the negative common logarithm of the spectral transmission factor  $T_\lambda$ ) is proportional to the concentration  $c$  of the medium and the path-length  $x$  of the light therein:

$$D_\lambda = -\log_{10} T_\lambda = \alpha_\lambda c x \quad (1)$$

where  $\alpha_\lambda$ , the extinction coefficient, is determined by the molecular configuration of the medium and is a function of the wave-length of the measuring light.

It is readily seen that if there is more than one light-absorbing substance ordered in the form of layers perpendicular to the test beam (Fig. 1a), the total density is equal to the sum of the component densities. The fraction of light transmitted by layer 1 is  $T_1$ , that by layer 2 is  $T_2$ , and so on. The total fraction of the incident light transmitted  $T_{\text{total}}$  is given by:

$$T_{\text{total}} = T_1 \cdot T_2 \cdot T_3 \cdot \dots \cdot T_n \quad (2)$$

or, from equation (1):

$$D_{\text{total}} = D_1 + D_2 + D_3 + \dots + D_n \quad (3)$$

In this result the individual layers have lost their identity, and it follows that this is equivalent to the situation of uniformly mixed solutes cited previously. In particular, if a given single type of pigment is arranged in laminae, such an ordered distribution would not appear to affect the issue, provided one recognizes that the longitudinal dimension of the container does not represent the optical path length  $x$ . Merely from the point of view of the operation of Lambert's law, the laminar arrangement of the visual pigments in the retinal receptors<sup>1</sup> would

seem to be wasteful of available space. We have to remember, however, that for a given number of pigment molecules contained in a tube of fixed cross-sectional area, the optical density is proportional to the product  $\alpha c x$ . The particular merit of the lamellar organization of the visual pigment molecules is that, owing to their intrinsic three-dimensional asymmetry, their chances of capturing light quanta is appreciably enhanced<sup>2</sup> (but compare Weale<sup>3</sup>).

What would be the effect of such gaps on densitometry if they were parallel rather than perpendicular to the light beam (Fig. 1b)? Let us assume that we are still dealing with only one type of pigment, and that it is contained in tubules parallel to the incident light  $I_{\text{inc}}$ . Let the cross-sectional area of the light beam be unity and the total fractional cross-sectional area of the tubules be  $A$ , where  $A < 1$ . The light transmitted by the array will thus consist of two components: (a) one which has passed unattenuated through the gaps, and (b) the other reduced by absorption in the pigment. The measured transmissivity  $T_m$  is given by:

$$T_m = \frac{I_{\text{trans}}}{I_{\text{inc}}} = \frac{I_{\text{inc}}(1-A) + I_{\text{inc}} \cdot A \cdot T_p}{I_{\text{inc}}} \quad (4)$$

where  $T_p$  is the transmission factor of the pigment. Equation (4) reduces to:

$$T_m = (1-A) + A \cdot T_p \quad (5)$$

It is apparent that no matter how small  $T_p$ , if the pigment occupies only a small part of the measuring area its presence may go undetected. As densitometrists know, isolated specks of dust are mercifully without much effect on their results.

Suppose now that the above-mentioned array consists of  $n$  tubules of cross-sectional area  $A_{1-n}$ , each containing a pigment of transmission factor  $T_{1-n}$ . Furthermore, let there be inter-tubular spaces the total area of which is equal to  $1 - \sum_{i=1}^{i=n} A_i$ . Then the transmitted light  $I_{\text{trans}}$  is made up of the components:

$$I \cdot A_1 \cdot T_1 + I \cdot A_2 \cdot T_2 + I \cdot A_3 \cdot T_3 + \dots + I \cdot A_n \cdot T_n + I \left[ 1 - \sum_{i=1}^{i=n} A_i \right]$$

and the measured transmissivity is:

$$T_m = A_1 \cdot T_1 + A_2 \cdot T_2 + A_3 \cdot T_3 + \dots + A_n \cdot T_n + 1 - \sum_{i=1}^{i=n} A_i \quad (6)$$

Expressed in densities, equation (6) becomes:

$$D_{\text{measured}} = -\log_{10} \left[ A_1 \cdot 10^{-D_1} + A_2 \cdot 10^{-D_2} + A_3 \cdot 10^{-D_3} + \dots + A_n \cdot 10^{-D_n} + 1 - \sum_{i=1}^{i=n} A_i \right] \quad (7)$$

*Application to measurements in the living human eye.* A knowledge of the spectral properties of the visual pigments is of paramount importance to an understanding of many visual functions. But, owing to the presence of light-absorbing pigments other than those subserving vision (for example, blood, pigment epithelium), direct measurements of their transmission factors are unobtainable in



the living eye. One can, however, utilize the photolabile character of the visual pigments to detect and measure the changes in their transmission spectra that result from exposure to light, that is, bleaching. Within limits to be discussed below, such density difference spectra are independent of extraneous absorption or light-scatter. The cardinal point in this regard is that any measurement of density changes in the intact retina involves not equation (7) but the difference between two such equations. This density difference arises from the change effected in one or more of the values  $D_{i\lambda}$  by exposure to light of intensity  $I_\beta$ , where  $\lambda$  and  $\beta$  refer to the wave-lengths of the measuring and bleaching lights, respectively. If  $D_{i\lambda}$  represents the maximum receptor density of a pigment (that is, after complete dark-adaptation), and if it be assumed that following exposure to the bleaching light the receptor density is reduced to  $d_{i\lambda}$ , then the measured density difference  $\Delta D_\lambda$  for pigments  $i=1$  to  $n$  occupying areas  $A_{i=1}$  to  $A_{i=n}$ , respectively, is:

$$\Delta D_\lambda = \log_{10} \left[ \frac{A_1 \cdot 10^{-d_{1\lambda}} + A_2 \cdot 10^{-d_{2\lambda}} + A_3 \cdot 10^{-d_{3\lambda}} + \dots + A_n \cdot 10^{-d_{n\lambda}} + 1 - \sum_{i=1}^{i=n} A_i}{A_1 \cdot 10^{-D_{1\lambda}} + A_2 \cdot 10^{-D_{2\lambda}} + A_3 \cdot 10^{-D_{3\lambda}} + \dots + A_n \cdot 10^{-D_{n\lambda}} + 1 - \sum_{i=1}^{i=n} A_i} \right] \quad (8)$$

The situation is further complicated by the presence of products of the primary bleaching process<sup>5</sup>, and also by the regeneration of the original pigment. Both these factors tend, in general, to diminish the differences between  $D_{i\lambda}$  and  $d_{i\lambda}$  although to different degrees and depending on the value of  $\lambda$ . However, if it be permissible to neglect both regeneration and the effects of the products that arise from quantum absorption (compare Williams<sup>6</sup>), then  $D_{i\lambda}$  and  $d_{i\lambda}$  are related to the energy of the bleaching light by the expression:

$$d_{i\lambda} = D_{i\lambda} e^{-\alpha_i \beta \gamma I_\beta t} \quad (9)$$

where  $\alpha_\beta$  is the extinction coefficient of pigment  $i$  for the bleaching light;  $\gamma$  is the quantum efficiency, defined as the number of visual pigment molecules bleached per number of quanta absorbed; and  $I_\beta$  and  $t$  are the bleaching intensity and time of exposure respectively. Substitution of equation (9) in equation (8) gives:

$$\Delta D_\lambda = \log_{10} \left[ \frac{A_1 \cdot 10^{-D_{1\lambda}} \exp(-\alpha_1 \beta \gamma I_\beta t) + A_2 \cdot 10^{-D_{2\lambda}} \exp(-\alpha_2 \beta \gamma I_\beta t) + A_3 \cdot 10^{-D_{3\lambda}} \exp(-\alpha_3 \beta \gamma I_\beta t) + \dots + A_n \cdot 10^{-D_{n\lambda}} \exp(-\alpha_n \beta \gamma I_\beta t) + 1 - \sum_{i=1}^{i=n} A_i}{A_1 \cdot 10^{-D_{1\lambda}} + A_2 \cdot 10^{-D_{2\lambda}} + A_3 \cdot 10^{-D_{3\lambda}} + \dots + A_n \cdot 10^{-D_{n\lambda}} + 1 - \sum_{i=1}^{i=n} A_i} \right] \quad (10)$$

which may be written:

$$\Delta D_\lambda = \log_{10} \left\{ \frac{1 - \sum_{i=1}^{i=n} A_i [1 - 10^{-D_{i\lambda}} \exp(-\alpha_i \beta \gamma I_\beta t)]}{1 - \sum_{i=1}^{i=n} A_i (1 - 10^{-D_{i\lambda}})} \right\}$$

This equation underlies the measurement of difference spectra in an array of tubules containing various light-sensitive substances, the axes of the tubules being parallel to the light of the densitometer.

We propose to test equation (10) in two ways. First, we wish to determine how  $\Delta D_\lambda$  varies with  $I_\beta$  and compare

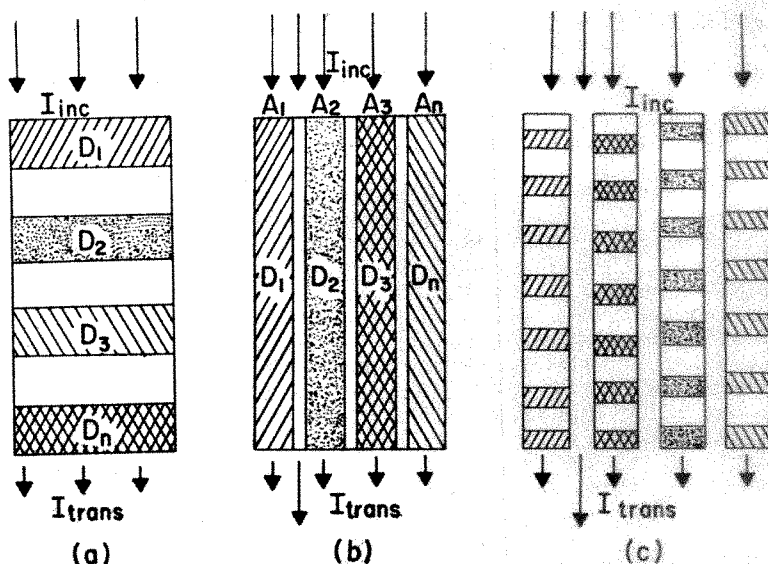


Fig. 1. Schematic representation of pigments located in containers with discontinuities (a) perpendicular, and (b) parallel to the direction of the measuring light. (c) The situation as it obtains in the retina (for details see text)

computed values with those observed in the living eye. Secondly, we should like to see to what extent the theoretical analysis predicts variations in the shape of the difference spectra obtained by partial bleaching of cone pigments in the human fovea. In addition, since equation (10) affords a means of estimating the *in situ* pigment densities, it is possible to compare density changes measured in the living eye with those recorded for individual receptors.

**Intensity function.** In the human periphery, where rods dominate the scene and the bleaching of cone pigments does not greatly affect the density difference measurements (but compare Weale<sup>7</sup>), equation (10) may be simplified by setting  $A_{2-n}$  equal to zero. An analysis of the relevant data would, however, lead outside the scope of this article, primarily concerned with cones. The trichromatic theory postulates the existence of three independent colour-mediating mechanisms in the fovea, populated exclusively by cones. If each is subserved by a specific cone pigment housed in separate containers, then solution of equation (10) requires a knowledge of the receptor densities of these pigments for the wave-length of measurement, their photosensitivities ( $\alpha_i \gamma$ ) to the bleaching light, and their respective distributions within the measuring area. Although there are several quantities in the right-hand side of equation (10) which cannot be precisely specified, it is possible to make reasonable estimates of their values based on the spectral characteristics of the foveal pigments determined experimentally and known anatomical features of this region.

It appears, both from reflexion measurements on the living eye<sup>8,9</sup> and microspectrophotometry of foveal sections<sup>10</sup>, that the foveal difference spectrum on total bleaching results principally from the density changes of two photolabile substances: the red-sensitive erythrolabe and the green-sensitive chlorolabe. The blue-sensitive pigment contributes little to the measurements for  $\lambda > 500 \text{ m}\mu$ <sup>10,11</sup>. For the purposes of the computations, therefore, the blue-sensitive pigment can be considered a photostable substance. Now, as regards the remaining pigments, Rushton<sup>8</sup> has indicated that the maximum density change recorded for erythrolabe ( $\lambda_{\text{max}} = 590 \text{ m}\mu$ ) is about 1.5 times that of chlorolabe ( $\lambda_{\text{max}} = 540 \text{ m}\mu$ ), and Brown and Wald<sup>10</sup> have confirmed on isolated retinas that the measured densities are in this ratio (although the  $\lambda_{\text{max}}$  of the



erythrolabe difference spectrum is said to be at a somewhat shorter wave-length; see following). As  $D_{\lambda} = \alpha_{\lambda} c x$ , this finding could result, for example, from either the concentrations or the extinction coefficients of erythrolabe being 1.5 times that of chlorolabe (for equal path lengths  $x$  and fractional areas  $A$ ). Let us assume arbitrarily that the extinction coefficients differ and the concentrations are equal. Then, for equal quantum efficiencies, the relative photosensitivities are as shown in Fig. 2, where the maximum of the P.540 curve is two-thirds that of P.590. The curves of Fig. 2 enable one to read off the relative densities  $D_{\lambda}$  of the two pigments at any measuring wave-length  $\lambda$ , and their relative photosensitivities ( $\alpha_{\beta}\gamma$ ) to any bleaching wave-length  $\beta$ . Thus, for a measuring wave-length  $\lambda = 540 \text{ m}\mu$ , and bleaching with  $\beta = 540 \text{ m}\mu$ ,  $D_{2\lambda}$  (the receptor density of erythrolabe) is taken to be equal to that of  $D_{1\lambda}$  (the density of chlorolabe), and  $\alpha_{1\beta}\gamma_1 = \alpha_{2\beta}\gamma_2$ . Similarly, for  $\lambda = 590 \text{ m}\mu$ ,  $\beta = 540 \text{ m}\mu$ , the photosensitivities are again equal, but  $D_{2\lambda} = 3.57 D_{1\lambda}$ . The expected values of  $\Delta D_{\lambda}$  can now be calculated as a function of  $I_{\beta}$  for various *in situ* pigment densities  $D_{1\lambda}$  and different fractional areas of pigment. It may be mentioned parenthetically that if the opposite assumption be made, namely equal photosensitivities but differing concentrations, the predicted values do not differ greatly from those given below.

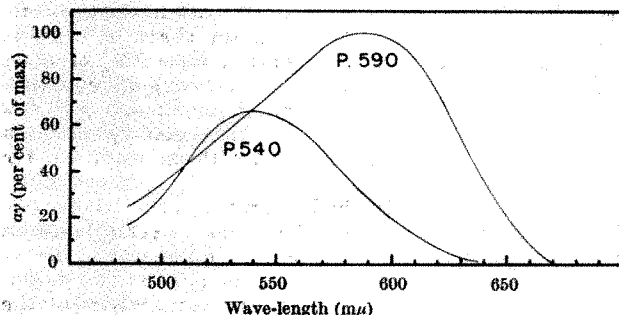


Fig. 2. The relative photosensitivities of chlorolabe (P. 540) and erythrolabe (P. 590), plotted on the assumption that density spectra are identifiable with density difference spectra (after ref. 8) and that the quantum efficiencies of the two substances are equal

If we assume that, owing to the close packing of cones within the fovea, chlorolabe, erythrolabe and photo-stable substances each occupy one-third of the total area, then solutions of equation (10) give the results shown in Fig. 3. The values of  $\Delta D(2)$  are for double transit through the retina in order to be compatible with *in vivo* measurements. It will be noted that, irrespective of the assumed values of  $D_{1\lambda}$  (the receptor density of chlorolabe), equation (10) predicts that measurable bleaching occurs over a range of merely 2 log units in intensity—a rather small range as compared with the billion-fold intensity gamut over which the eye responds before experiencing thermal injury. But the prediction agrees with the experimental data (Fig. 4) and is, in fact, similar to what one observes in connexion with rods<sup>12</sup>. If we next plot the experimental results for  $\lambda = 540$  and  $590 \text{ m}\mu$ , taken from the lower graph of Fig. 4, within the framework of predicted values (data points of Fig. 3), a maximum receptor density of about 0.15 for chlorolabe seems to be indicated. The corresponding density computed for erythrolabe is approximately 0.22.

**A spectral shift.** The experimental results of Fig. 4a show that with green bleaching light ( $\beta = 547 \text{ m}\mu$ ) the shapes of the difference spectra were, for measuring wave-lengths  $\lambda > 500 \text{ m}\mu$ , virtually independent of the illuminance of the bleaching light. Although  $\Delta D_{\lambda}$  increased as  $\beta$  was raised, the maxima of the difference spectra ( $\lambda_{\max}$ ) remained at approximately  $560 \text{ m}\mu$ . But bleaching with deep red light produced quite different results (Fig. 4b). Clearly, the curves no longer retain the same shape or position irrespective of bleaching intensity. For the

lowest values of  $I_{\text{red}}$ , the maximal density change occurred at about  $590 \text{ m}\mu$ . As the intensity was raised the  $\lambda_{\max}$  moved to shorter wave-lengths, and, for the highest intensity used, was at approximately  $560 \text{ m}\mu$ . These findings suggest that, whereas green light bleaches the red- and green-sensitive pigments in the same proportion for all intensities, the weak red light bleaches largely the red-sensitive pigment; as the red-sensitive pigment becomes exhausted increasing the intensity increases the proportion of green-sensitive pigment bleached<sup>11</sup>.

To what extent are the constancy of the shape of the difference spectra for green bleaches and its alteration with red bleaches predicted by equation (10)? It is readily seen that a shift in  $\lambda_{\max}$  carries with it a change in the ratio of the density changes obtained at two measuring wave-lengths. For example, in the case of a series of symmetrical curves—as is represented by the green bleaching data—the ratio  $\Delta D_{590}/\Delta D_{540}$  remains constant at all bleaching intensities. But a reduction in this ratio as the bleaching intensity is raised betokens a shift of  $\lambda_{\max}$  toward shorter wave-lengths. Now, from curves such as those of Fig. 3, which give the computed density changes at the measuring wave-lengths  $\lambda = 540, 590 \text{ m}\mu$  as a function of intensity, ratio values can be calculated for the green bleaching lights. In a similar manner, theoretical values of  $\Delta D_{590}/\Delta D_{540}$  may be obtained for orange bleaching lights ( $\beta = 590 \text{ m}\mu$ ). The continuous curves of Fig. 5 show the results of these computations for the same areal distributions as before, and a maximum receptor density of the green-sensitive chlorolabe  $D_{1\lambda} = 0.15$ . As expected, the two curves converge at high values of  $I_{\beta}$  when both pigments are nearly exhausted by either spectral bleaching light. However, at lower intensities, when the bleaching is partial, varying the intensity of a green bleaching light barely affects the density ratios, that is, the shapes of the difference spectra are very nearly constant. For orange light, on the other hand, the density ratio and bleaching intensity exhibit an inverse relation, that is, the  $\lambda_{\max}$  of the difference spectra move towards shorter wave-lengths as  $I_{\beta}$  rises.

The data points in Fig. 5 were taken from the experimental results shown in Fig. 4. For a green bleaching light ( $\beta = 547 \text{ m}\mu$ ), experimental values agree with theory very well indeed. The 'red' data ( $\beta = 680 \text{ m}\mu$ ), however, show a ratio change greater than that predicted. But it should be noted that the computed values are for orange light, the data in Fig. 2 not being sufficiently accurate at the longer wave-lengths for use in the computations. It would be expected, therefore, that bleaching with deep

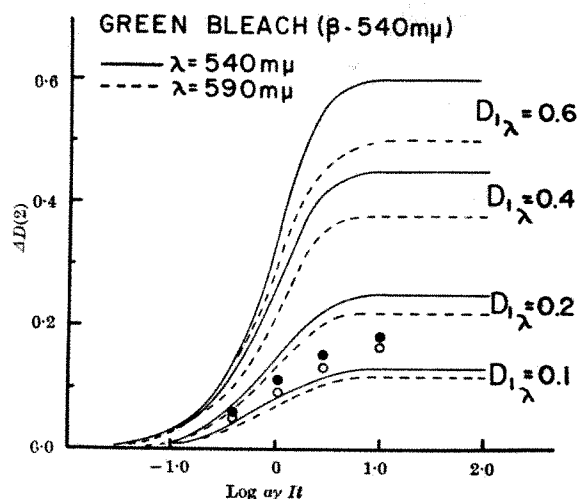


Fig. 3. Curves are the computed foveal density changes at two different measuring wave-lengths.  $D_{1\lambda}$  represents assumed maximum density values of the green-sensitive chlorolabe at the measuring wave-length  $\lambda = 540 \text{ m}\mu$ . For  $\lambda = 590 \text{ m}\mu$ ,  $D_{1\lambda} = 0.42 D_{1\lambda, 540}$  as estimated from Fig. 2. Experimental points give measured foveal density changes obtained from Fig. 4; open circles  $\lambda = 590 \text{ m}\mu$ , closed circles  $\lambda = 540 \text{ m}\mu$  (ref. 15)

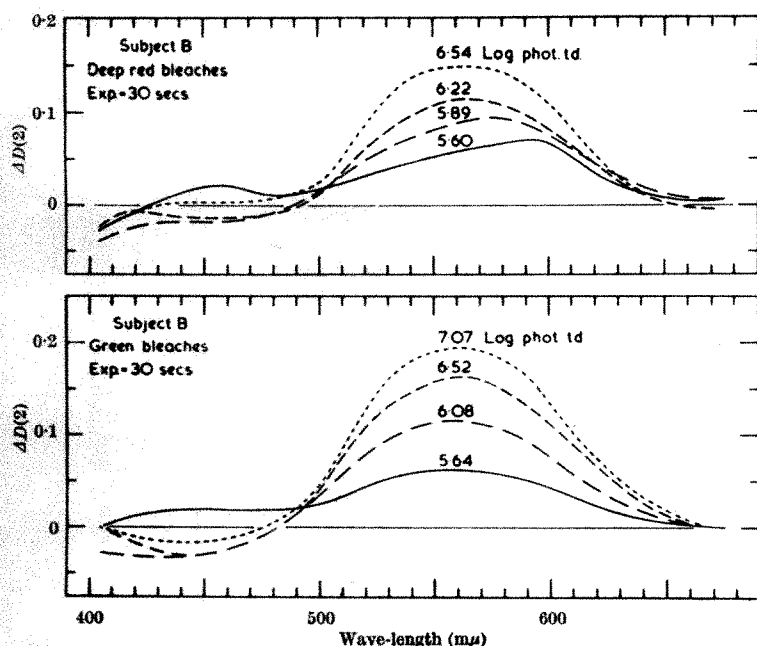


Fig. 4. Human foveal difference spectra obtained by fundus reflectometry. Vertical axis gives density changes referred to double transit through retina. (a) Results with green bleaching light ( $\beta = 547 \text{ m}\mu$ ); (b) bleaching with deep red light ( $\beta = 680 \text{ m}\mu$ ). The Ilford 206 filter used transmitted 1 per cent or less of the incident radiation at wave-lengths  $< 630 \text{ m}\mu$  (ref. 15; by courtesy of the editors of *Vision Research*)

red lights yields greater density ratios at low intensities owing to more selective bleaching of the red-sensitive pigment by the longer wave-length light. As indicated earlier, a rise in intensity bleaches an increasing proportion of the green-sensitive pigment, resulting in the marked decrease of the density difference ratio.

**Individual receptors.** The remarkable achievements of Marks, Dobelle and MacNichol<sup>13</sup>, and of Brown and Wald<sup>14</sup>, who have measured the transmissivities of individual receptors in isolated human retinae, represent a significant advance in the study of visual pigments *in situ*. Although only a few cones have so far been sampled, and these from non-foveal regions, it would not be amiss to comment on some of these findings in the context of this paper.

One notes, for example, that the  $\lambda_{\text{max}}$  of the red- and green-sensitive pigments are not in agreement with those derived from *in vivo* measurements<sup>15</sup> and, in fact, used in the earlier computations. It is interesting, in this connexion, that Marks *et al.* and Brown and Wald suggest the possibility that, in some instances, both the red- and green-sensitive pigments may be housed in a single element. Furthermore, it is difficult to assess the effect on the pigments that may result from morbid changes which occur in excised retinae. Unknown also is the influence that photo-products may have on the shape of difference spectra measured in single receptors and in the living eye. However, important though such factors may be to a comparison of results obtained with different techniques, their significance for the computations presented previously is minimized because results obtained in the living eye were used to predict other results also obtained in the living eye. For the most part, whatever correction may be applicable to computed values will also be applicable to the experimental values on which they are based. As such coherence may not extend to results obtained under greatly different conditions, comparisons cannot be pressed with too much force to cover such conditions. However, if this proviso is borne in mind, an attempt can be made to reconcile measurements on single receptors and those on the living human fovea.

With regard to cone pigment densities, Marks *et al.* give the range of differential absorption for measurements obtained on the receptor and a retinal area free of visual cells, respectively. Their results, therefore, are not directly

comparable with data which provide the density differences produced by bleaching. However, Brown and Wald's measurements of difference spectra are amenable to a more detailed analysis. One notes, in the first place, that there is considerable variance between the density measurements on single human cones and those in the living eye<sup>9,13</sup>. Whereas application of equation (10) to the *in vivo* data suggests a chlorolabe density of approximately 0.15 (Fig. 3), Brown and Wald give a density of 0.025 for this pigment. For erythrolabe the difference is even greater, the single cell measurements being lower by a factor of almost 17 as compared with the computed value. But these discrepancies may result from a variety of circumstances including, as the authors suggest, the possibility that some bleaching had occurred before the receptor measurements had begun. In addition, Brown and Wald used one or two flash exposures for bleaching, and we have no reliable evidence as regards the efficacy of brief flashes in bleaching cone pigments. Hagins<sup>16</sup> has established that, no matter how intense, flash exposure of rabbit rhodopsin bleaches approximately only one-half the available pigment. There are also morphological differences between the regions examined in the two studies;

parafoveal cones are shorter than foveal, so that on this score alone one would expect Brown and Wald's  $\Delta D$  values to be less than those obtained for the fovea.

Perhaps a more important consideration concerns the fractional area of the measuring field occupied by pigment. It will be recalled that, in arriving at an *in situ* density of 0.15 for chlorolabe, we assumed that photolabile pigments contributing to the measured values of  $\Delta D_2$  occupy two-thirds of the total measuring area. This estimate was taken in view of the tight packing of the 'rod-like' foveal cones. But in para-foveal regions, the diameter of the cone inner segment is at least twice as large as that of the outer segment<sup>17</sup>. If Brown and Wald's technique led to the isolation of the former rather than the latter, application of equation (10) shows that the corrected receptor density (at  $\lambda_{\text{max}}$ ) would be more than four times greater than their measured values. Finally, since much of the preliminary procedures are performed under red light, the

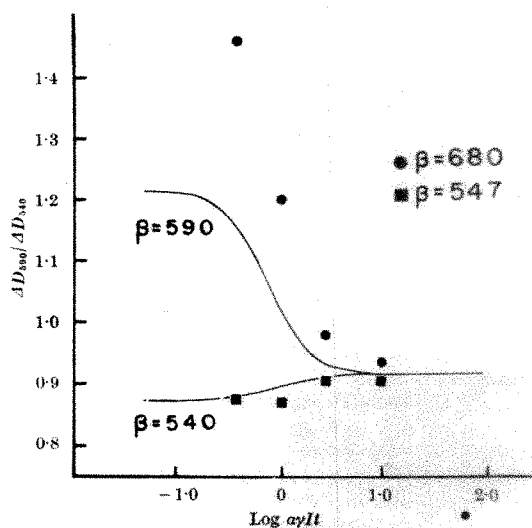


Fig. 5. Ratio  $\frac{\text{density difference measured with } \lambda = 590 \text{ m}\mu}{\text{density difference measured with } \lambda = 540 \text{ m}\mu}$  as a function of bleaching light intensity and wave-length  $\beta$

extremely low density value for the red-sensitive pigment may have been caused by selective bleaching of this substance prior to measurement. The upshot of these considerations is that much of the apparent discrepancy as between measurements on the living human fovea and isolated cones may be resolved when more detailed information on the aforementioned factors becomes available.

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## QUANTUM REQUIREMENT FOR ACETATE ASSIMILATION AND ITS SIGNIFICANCE FOR QUANTUM MEASUREMENTS IN PHOTOPHOSPHORYLATION

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TRUE photoassimilation of acetate under aerobic conditions is independent of the oxygen-evolving system of photosynthesis. This has been shown with the green member of the Volvocales, *Chlamydomonas*<sup>1,2</sup>, and with *Chlamydomonas mundana*<sup>3</sup>. Yet the dependence of the aerobic acetate assimilation on light implies an efficient mechanism to convert radiant energy into chemical energy, specifically into energy-rich phosphate, which is required for the transformation of acetate into cell material, carbohydrates<sup>1,4</sup>. A year ago we reported that *Chlamydomonas* assimilates acetate in near infra-red light<sup>5</sup>, which is known to be unsuitable for ordinary photosynthesis but not for photoreduction with hydrogen<sup>6</sup>. I have now determined the absolute quantum requirement for acetate photo-assimilation at different wave-lengths between  $\lambda$  450 and 740 m $\mu$ . Indirectly these measurements tell us something about the efficiency of photophosphorylation in an intact cell and its dependence on the light quality. The experimental arrangement was checked by measuring also the quantum yield of normal photosynthesis in *Scenedesmus obliquus*, where the light has to provide not only ATP\* but also the necessary NADPH<sub>2</sub>.

*Chlamydomonas stellata* Korschikoff was used in all experiments on acetate assimilation. The algae were cultivated in an acetate-containing medium in the light<sup>7,8</sup>. During the experiments the algae were kept in a closed cubic vessel inside a thermostat. The light sources were calibrated against the radiant energy of a standard carbon filament lamp (Eppley Laboratories, Inc., Newport, Rhode Island) measured with a large surface bolometer (H. Röhrig, Berlin, Germany)<sup>9</sup>. Interference filters (Schott and Gen., Mainz, Germany) were used to isolate narrow spectral regions. The light absorption of the algae was measured in an integrating sphere to minimize errors due to scattering<sup>10</sup>. The uptake of acetic acid by the algae was automatically recorded by titration with 0.01 N acetic acid at pH 7.0 ('Titrator TTT-1' with recording 'Titrigraph SBR2/SBU-1', Radiometer, Copenhagen). The quantum measurements on photosynthetic carbon dioxide fixation by the alga *Scenedesmus obliquus* D<sub>2</sub> were made using the same vessel at the same light intensities by retitrating the carbon dioxide consumed.

\*Abbreviations: NADPH<sub>2</sub>, reduced nicotinamide adenine dinucleotide phosphate; ATP, adenosine triphosphate; DCMU, 3-(3-dichlorophenyl)-1-dimethylurea.

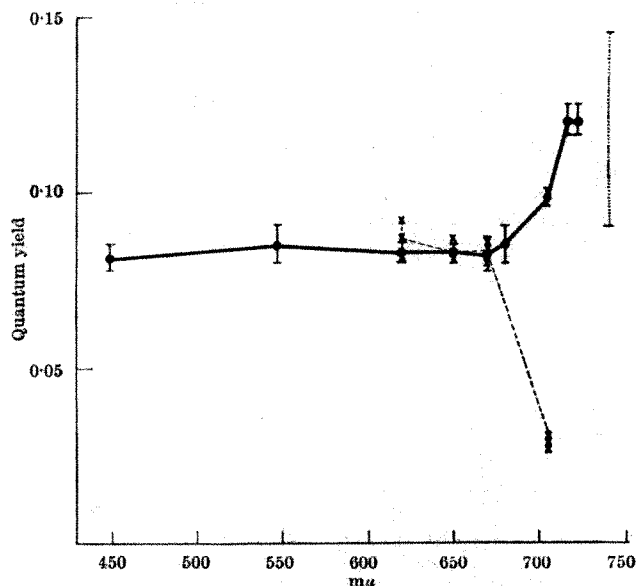


Fig. 1. Quantum yields for photo-assimilation of acetate and photosynthetic carbon dioxide fixation at different wave-lengths. ●—●, Acetate assimilation, *Chlamydomonas*; x — x, photosynthesis, *Scenedesmus* D<sub>2</sub>.

Fig. 1 is the plot of the quantum yields for photo-assimilation of acetate and for photosynthetic carbon dioxide fixation at different wave-lengths derived from several series including hundreds of single measurements. The yield for acetate assimilation stays constant in the spectral region between  $\lambda$  450 and 680 m $\mu$  with an average of 0.083. It rises in the longer wave-length region beyond  $\lambda$  680 m $\mu$  to an average of 0.12. At  $\lambda$  740 m $\mu$  our measurements become unreliable because of the difficulties in measuring a very weak absorption. The quantum yields for carbon dioxide fixation and for acetate assimilation overlap in the spectral region below  $\lambda$  630 m $\mu$ . Beyond  $\lambda$  700 m $\mu$ , however, they fall apart.

Table 1 presents the quantum numbers for acetate assimilation from which the quantum yields have been calculated. Below  $\lambda$  630 m $\mu$  the assimilation of 1 mole of acetic acid requires on an average 12 mole quanta. Readings as low as 10.6 at  $\lambda$  671 m $\mu$  were repeatedly

Table 1. QUANTUM NUMBERS FOR PHOTO-ASSIMILATION OF ACETATE BY *Chlamydomonas* AT DIFFERENT WAVE-LENGTHS

Wave-length (mμ)	No. of expts.	Average Mole quanta/mole CH <sub>3</sub> COOH	Lowest quantum No. measured repeatedly
450	20	12.3 ± 0.75	11.3
547	24	11.8 ± 0.81	11.0
620	45	12.1 ± 0.41	10.5
650	16	12.1 ± 0.31	11.6
671	30	12.2 ± 0.71	10.6
680	21	11.8 ± 0.81	11.0
705	36	10.2 ± 0.39	8.8
716	18	8.3 ± 0.27	7.5
723	40	8.3 ± 0.26	6.8

obtained. Above  $\lambda$  680 mμ fewer quanta are required per mole acetic acid assimilated, on an average 8.3. Several times figures as low as 7.5 and 6.8 were registered at  $\lambda$  716 and 723 mμ.

Table 2 contains the quantum numbers for the photo-synthetic carbon dioxide fixation by *Scenedesmus*, a typical member of the Chlorococcales, which is often used in many laboratories. At  $\lambda$  620 and 680 mμ the lowest number measured was 9; in the near dark red at  $\lambda$  705 mμ, however, not smaller than 29. Because these numbers fit into the range of the most reliable quantum number determinations reported in the literature<sup>11,12</sup> they serve here as an independent check on our experimental set-up.

Table 2. QUANTUM NUMBERS FOR PHOTOSYNTHETIC CARBON DIOXIDE ASSIMILATION BY *Scenedesmus obliquus* D<sub>2</sub> AT DIFFERENT WAVE-LENGTHS

Wave-length (mμ)	No. of expts.	Average Mole quanta/mole CO <sub>2</sub>	Lowest quantum No. measured repeatedly
620	6	11.5 ± 0.74	9.0
671	24	12.0 ± 0.50	9.0
705	9	32.8 ± 1.62	29.0

Acetate assimilation does not change in the presence of  $5 \times 10^{-6}$  M DCMU, a poison concentration that is known to stop carbon dioxide fixation. This has been reported already for white light<sup>1,2</sup>, but, as can be seen in Table 3, it holds true also in monochromatic light.

Table 3. PHOTO-ASSIMILATION OF ACETATE IN THE PRESENCE OF  $5 \times 10^{-6}$  M DCMU AT DIFFERENT WAVE-LENGTHS

$\lambda$ mμ	Mole CH <sub>3</sub> COOH/mg chlorophyll/h (average of 7 expts.)
equal incident intensities	Control + DCMU
620	20 20
671	39 40
680	38 38
723	8 8

All the foregoing can be summed up by saying that in *Chlamydomonas* the quantum efficiency for fixing one molecule of acetate happens to be the same as that for photosynthesis so long as the light contains only wave-lengths shorter than  $\lambda$  680 mμ, but that above  $\lambda$  680 mμ, where photosynthesis is poor, the acetate assimilation becomes considerably more efficient.

Actually this statement applies not for the overall mechanism of acetate fixation, but only for the light requiring part of it, which so far as we know is the pro-

duction of energy-rich phosphate<sup>1</sup>. It follows that in this alga (and possibly also in others) the efficiency of photo-phosphorylation varies with the wave-length of the absorbed light. In present-day theoretical terminology we say it is high when the light is absorbed mainly by chlorophyll system I (beyond  $\lambda$  700 mμ)<sup>13,14</sup> and low when part of it is absorbed also by chlorophyll system II. The insensitivity against DCMU proves that system II cannot be involved and consequently also not the so-called 'photosynthetic phosphorylation' assumed to be coupled to the interplay between the light reactions of system I and those of system II (refs. 13, 14 and 15). If such a phosphorylation could contribute ATP in significant amounts for the fixation of acetate, the quantum yield of the latter should be constant throughout the entire spectrum. The apparently higher energy requirement at wave-lengths shorter than  $\lambda$  680 mμ must therefore be attributed to losses in absorbed light energy, which cannot be circumvented by a physical light energy transfer from the carbon dioxide reducing mechanism to that which induces acetate fixation<sup>16</sup>. This independence of two photochemical systems in *Chlamydomonas* (which shows a diminished ability for fixation of carbon dioxide) does not rule out the possibility that ATP formed in the near infra-red aids the fixation of carbon dioxide in the visible light when carbon dioxide is the only substrate available to the alga.

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## AFLATOXINS AND 'ALBINISM' IN PLANTS

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SINCE the discovery of penicillin, a metabolite of *Penicillium notatum*, much effort has been devoted to the examination of fungal and microbial metabolites, with the view of finding new therapeutic drugs<sup>1</sup>.

A new aspect of the problem became apparent more recently, when metabolites of the fungus, *Aspergillus flavus*, Link ex Fries, known as aflatoxins, have been found in human and animal foods and been shown to possess exceedingly potent hepatotoxic and/or hepatocarcinogenic activity for several species of animals, including fish, birds and rodents<sup>2</sup>. These findings with their implications for human pathology exemplify the fact that metabolites of micro-organisms can have deleterious

effects not only on pathogenic microbes (which makes them useful as therapeutic agents) but also on cells of higher organisms, in which they can cause disease. It might be expected that some of these fungal metabolites might also affect plant cells, and interfere with the germination of seeds or plant growth, and cause wilting or blanching of leaves by interfering with some specific biochemical processes such as the biosynthesis of chlorophyll.

In 1938, Koehler and Woodworth<sup>3</sup> showed that 'virescence' (pallor of leaves) of corn seedlings, usually considered as a genetic characteristic, could be induced by inoculation with spores of *Aspergillus flavus* and *A. tamarii*. Similarly, Durbin<sup>4</sup> traced the 'albinism' of



Table 1. EFFECTS ON GERMINATION AND LEAF COLOUR OF SEEDLINGS OF CRESS, *Lepidium sativum* L.

Compound:	Control	Concentrations ( $\mu\text{g/ml.}$ )											
	Nil	100		50		25		10		2.5		1	
	G* (%)	G	A†	G	A	G	A	G	A	G	A	G	A
Aflatoxin	85	0		10	++	65	++	85	++	90	+	85	±
Carolic acid	85	75	+	80	+			100					
Citrinin	85	75	+			90	±	90	—				
Coumarin	75	0		15	±	35	—	80	—				
Frequentin	85	15	+	85	+			90	—				
Mycophenolic acid	75	80	±	85	±			90	—				
Rugulosin	85	65	+	90	+			85	+	100	±		
Streptomycin sulphate	75	100	+	90	±	80	±	85	—				
Terrein	85	40	+	75	—			85	—				
Ustic acid	90	65	—					95	—			90	—

G\*, percentage of explanted seeds which germinated. A†, albinism; ++ complete; + partial; ± doubtful.

seedlings of citrus plants to their contamination with *A. flavus*, and Perlberger and Reichert<sup>5</sup> found that 'albinism' could be eliminated when the seeds were disinfected with preparations containing heavy metals: mercury, copper, nickel and lead. On the other hand, Ryan *et al.*<sup>6</sup> induced 'albinism' in citrus and in certain other plants with the fungus *Alternaria tenuis*, or with its heat stable extracts, but could not reproduce such effects with the several species of *Aspergillus flavus*, which they tested.

Since the discovery of aflatoxins, various species of *Aspergillus flavus* have been subjected to intensive investigations, which revealed that only certain strains, under favourable conditions, produce the toxic metabolites, aflatoxins.

It was therefore of interest to find out whether aflatoxins may be the factors responsible for the inhibition of chlorophyll synthesis as observed in 'virescence' or 'albinism' of plants.

Crude extracts were prepared from two groundnut meals by a method (Method A) already described<sup>2</sup>. One meal was known to be toxic to animals and to contain aflatoxins, and the other was a non-toxic meal. These extracts were tested on the rapidly germinating seeds of cress *Lepidium sativum* L. The leaves of the seedlings that had the toxic extract (5 mg/ml. corresponding to approximately 0.5 g of meal) appeared yellowish, while those which had the same concentration of the non-toxic extract, or water, were bright green. Both extracts inhibited the germination of cress seeds at the concentration of 25 mg/ml., while only the toxic extract had some inhibitory effect on germination in concentration of 5 mg/ml. When crystalline preparations of aflatoxins became available the tests were repeated using various concentrations of aflatoxin(s). For comparison, similar tests were performed with other fungal metabolites, made available through the courtesy of Prof. J. H. Birkinshaw, and with certain other substances (for the structures of the various fungal metabolites see ref. 1). These included coumarin, a known inhibitor of germination, the action of which has recently been reviewed<sup>7</sup>.

For the test, twenty seeds of cress, *Lepidium sativum* L., were explanted on circles of surgical gauze placed in 2-in. Petri dishes containing 4 ml. of distilled water (controls) or of aqueous solutions, or suspensions, of the substances being tested. The Petri dishes were placed on a window sill, facing south-west, and left for seven days at room temperature. Germination usually became apparent on the third day. The appearance of the treated cultures was compared with that of the controls on the sixth or the seventh day; the conditions of the cultures deteriorated if kept longer. Alternariol and its methyl ether, asteric acid, 3,4-benzopyrene, citromyocetin, cycasin (a glucoside of methylazoxymethanol), cyclopolic acid, erdin, fuscine, kojic acid, luteoleosin, mineoluteic acid, quadrilineatin, retransins, sclerotiorin, spinulosin, sterigmatocystin, sulochrin, and dimethylnitrosamine had no detectable effect on the germination or colour of the seedlings when tested in concentrations up to 100  $\mu\text{g/ml.}$  under the conditions described.

Table 1 summarizes the effects of various concentrations of aflatoxin, coumarin and several other fungal meta-

bolites on the germination and on leaf-colour of cress seedlings.

Aflatoxin(s), like coumarin, inhibited the germination of seeds in concentration of 25  $\mu\text{g/ml.}$ , but its effect on the colour of leaves was more marked. Complete absence of the green colour occurred in the presence of 10  $\mu\text{g/ml.}$  of aflatoxin, and partial inhibition, with a characteristic bleaching of the edges of leaves, could still be recognized at 1  $\mu\text{g/ml.}$  Of the other compounds, some effect on germination and/or on colour could be discerned only with concentrations of 50–100  $\mu\text{g/ml.}$

It is of interest that compounds, representing several types of carcinogens, 3,4-benzopyrene, retrorsine, cycasin and dimethylnitrosamine, were completely inactive under the conditions tested.

The mechanism by which aflatoxins induce chlorophyll deficiency in plants can at present be only surmised; but this simple test could be used for the investigation of the biochemical processes involved, and of the factors which can modify them.

It would also be of interest to test whether strains of fungi, *Alternaria tenuis* and *Aspergillus tamaris*, which have been reported to induce 'albinism' in plants can also produce aflatoxins.

The striking effect of aflatoxin(s) in inhibiting the green colour of cress seedlings suggests that this effect could serve as the basis for a test for the presence of aflatoxins in foodstuffs suspected of being contaminated. The test requires neither special equipment nor technical skill, thus making it suitable as a field test under conditions where other methods are not feasible. The latter include: fluorescence<sup>2,8</sup> and ultra-violet absorption spectroscopy<sup>9</sup>, the 'duckling test'<sup>2</sup>, the 'egg test'<sup>10</sup> and tissue culture<sup>11,12</sup>. These methods require special apparatus and experience in handling and in interpreting the findings if misleading results are to be avoided.

The experiments recorded here demonstrated that aflatoxins can induce chlorophyll deficiency in cress seedlings and are possibly the cause of 'albinism' reported in certain other plants. This effect might be elaborated into a simple test for their detection in suspected materials.

We thank Prof. J. H. Birkinshaw for the crystalline specimens of the various fungal metabolites, and the Tropical Products Institute for the crystalline preparation of aflatoxins, containing mainly the B<sub>1</sub> aflatoxin.

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## LETTERS TO THE EDITOR

## ASTROPHYSICS

## Growth of Interstellar Grains

It is now generally accepted that interstellar grains can grow by some form of accretion from the surrounding interstellar gas (and other grains) given suitable conditions, and this growth has been used in several astrophysical theories<sup>1</sup>. A number of authors, for example, McCrea and McNally<sup>2</sup>, and Hoyle and Wickramasinghe<sup>3</sup>, have devised methods by which such growth of grains can occur, although none has been universally accepted. In this communication we shall assume that a fixed proportion of the colliding particles adhere to the grain and, using a simple kinetic theory model, calculate the rate of growth of a grain moving at any speed through the cloud.

We shall assume that the cloud is so large that the amount of material accreted is small and does not alter the number density,  $n$ , of particles (which we shall regard as 'molecules' for kinetic theory purposes) forming the cloud. We also assume that the grain is small compared with the mean free path of the gas, which enables us to use an undisturbed (equilibrium) velocity distribution for the oncoming particles, which we take to be Maxwellian. In astrophysical situations of interest both these conditions are likely to be satisfied. We also make the usual assumption that the grains remain spherical throughout and that the grain density,  $\sigma$ , stays constant.

Let  $M$  and  $a$  be the mass and radius of the grain at any time  $t$ , and  $U$  the velocity of the grain relative to the gas cloud. Take a set of spherical polar co-ordinates ( $r, \theta, \phi$ ) with origin at the centre of the spherical grain and polar axis in the direction of  $U$ . An element of area at the surface of the sphere is  $dA = a^2 \sin \theta d\theta d\phi$ . At this element, but stationary with respect to the gas, take a second set of axes ( $x, y, z$ ) with the  $y$ -axis along the outward normal and the  $z$ -axis intersecting the line of action of  $U$ .

The probability of a molecule of the gas having speed between  $v$  and  $v + dv$  along the  $y$ -axis is  $(\beta/\pi)^{1/2} \exp(-\beta v^2) dv$ , where  $\beta = m/2kT$ ,  $m$  being the mass of a gas molecule and  $k$  and  $T$  being respectively Boltzmann's gas constant and the kinetic temperature of the gas. Thus the rate at which mass is added to the grain by molecules with speeds in this range colliding with the area  $dA$  is:

$$\lambda(\beta/\pi)^{1/2} mn(v + U \cos \theta) \exp(-\beta v^2) dv dA$$

$\lambda$  being the proportion of colliding gas molecules that adhere ( $U = |U|$ ).

The rate at which mass is added to the whole grain is found by integrating for all possible collisions over the surface of the sphere. Now if  $v \geq U$  collisions occur for all  $\theta$  between 0 and  $\pi$  (that is, over the whole sphere), while if  $|v| < U$  collisions occur only if  $\cos \theta \geq -v/U$  and if  $v \leq -U$  no collisions occur. Integrating and inserting these limits, the total rate at which mass is added to the sphere is:

$$\frac{dM}{dt} = a^2 \lambda mn (\beta/\pi)^{1/2} \left( \mathcal{I}_1 + \mathcal{I}_2 \right)$$

where  $\mathcal{I}_1 = \int_U^\infty dv \int_0^\pi d\theta \int_0^{2\pi} d\phi (v + U \cos \theta) e^{-\beta v^2} \sin \theta$

and  $\mathcal{I}_2 = \int_{-U}^U dv \int_{\cos^{-1}(-v/U)}^\pi d\theta \int_0^{2\pi} d\phi (v + U \cos \theta) e^{-\beta v^2} \sin \theta$

Integrating over  $\theta$  and  $\phi$ , we have:

$$\mathcal{I}_1 = 4\pi \int_U^\infty v e^{-\beta v^2} dv = (2\pi/\beta) e^{-\beta U^2}$$

$$\begin{aligned} \text{and } \mathcal{I}_2 &= 2\pi \int_{-U}^+ U \left( \frac{v^2}{2U} + v + \frac{U}{2} \right) e^{-\beta v^2} dv \\ &= 2\pi \left\{ \left( \frac{U}{2} + \frac{1}{4\beta U} \right) \operatorname{erf}(\sqrt{\beta} U) - \frac{1}{2\beta} e^{-\beta U^2} \right\} \end{aligned}$$

where  $\operatorname{erf}$  denotes the error function (see, for example, ref. 4). Thus we have:

$$\frac{dM}{dt} = 4\pi a^2 \sigma \frac{da}{dt} = 2\pi mn a^2 \lambda \left( \frac{\beta}{\pi} \right)^{1/2} \left\{ \frac{e^{-\beta U^2}}{2\beta} + \left( \frac{U}{2} + \frac{1}{4\beta U} \right) \left( \frac{\pi}{\beta} \right)^{1/2} \operatorname{erf}(\sqrt{\beta} U) \right\}$$

It is convenient at this point to define a molecular speed ratio  $S = \sqrt{\beta} U = (2/\sqrt{\pi}) U/W$ , where  $W$  is the thermal velocity of the gas. We obtain:

$$\frac{da}{dt} = \frac{mn\lambda W}{8\sigma} \left\{ e^{-S^2} + \left( S + \frac{1}{2S} \right) \sqrt{\pi} \operatorname{erf}(S) \right\} \quad (1)$$

an expression for the rate of increase of the radius of the grain at any speed  $U$ .

Consider now the following limiting cases.

(i)  $S < 1$  ( $U$  small, of order less than the thermal velocity). Expansion of the error function (see ref. 4) and the exponential function in equation (1) gives:

$$\frac{da}{dt} = \frac{\lambda mn W}{8\sigma} \left( 2 + \frac{2}{3} S^2 + O(S^4) \right)$$

Retaining the first term in the bracket only, we obtain  $\frac{da}{dt} = \frac{\lambda mn W}{4\sigma}$ , which can be integrated to give:

$$a - a_0 = \frac{\lambda mn W t}{4\sigma}$$

where  $a_0$  is the radius at some given time,  $t = 0$ . This is the expression found by Williams<sup>5</sup> for the case of  $U = 0$ . Moreover, inserting the numerical values  $W = 2 \times 10^5$  cm/sec,  $\sigma = 5$  g/c.c., and  $\lambda = 1$ , as used by Lindblad<sup>6</sup>, we obtain the formula:

$$M = 5.7 \times 10^{33} (\rho t)^3 \text{ g}$$

where  $\rho = mn$  is the density of the gas and  $t$  is measured in years, which corresponds well with Lindblad's expression  $M = 10^{34} (\rho t)^3$  g.

(ii)  $S > 1$  ( $U$  large, of order greater than the thermal velocity). In this case the expansion of the error function in (1) gives:

$$\frac{da}{dt} = \frac{\lambda mn W}{8\sigma} \left( 2S + \frac{1}{S} + O(e^{-S^2}) \right)$$

Again retaining the first term in the bracket only, we have:

$$\frac{da}{dt} = \frac{\lambda mn W S \sqrt{\pi}}{8\sigma} = \frac{\lambda mn U}{4\sigma}$$

or

$$\frac{da}{dx} = \frac{\lambda mn}{4\sigma}$$

Integration gives:

$$a - a_0 = \frac{\lambda mn}{4\sigma} x$$

where  $a_0$  is the radius of the grain at a given point  $x = 0$ . This was also found by Williams<sup>5</sup> using another method for the case of very large  $S$  (thermal velocity  $W$  negligible).

From the general expression (1) (and assuming  $U$  is constant) it appears that the grain size is proportional to

age, suggesting that grain size increases without limit. It must be noted that our model is only valid while the radius of the grain is appreciably less than the mean free path of the gas, and the result is only meaningful while this condition is satisfied. Moreover, in a more accurate theory  $\lambda$  would depend on the grain size at any instant, but in the absence of detailed knowledge of the mechanism by which the molecules adhere to the grains we have confined ourselves to a constant value of  $\lambda$ .

In this calculation we have assumed that all the material in the cloud belongs to one accretable gas; but it will not alter the calculation if the cloud is assumed to consist of a number of non-interacting gases of different types and their contributions to the accretion (with the appropriate individual gas parameters) are added. (In particular, one of the 'gases' can be the other grains forming part of the interstellar cloud.) Thus the growth of a grain moving through a cloud of material composed of different gases with different accretion parameters  $\lambda$  could be found.

We thank A. S. Asebiomo for checking the calculations in this communication.

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### Neutrino Emission from Star-like Sources

In a previous communication<sup>1</sup> we reported some evaluations of the neutrino energy density in the universe taking into account the  $e^+ + e^- \rightarrow \nu + \bar{\nu}$  process in the pre-supernova stage of the stars. We found that this energy density may be higher than the energy density of the matter. Here we present a similar evaluation on the basis of the Mannino<sup>2</sup> hypothesis regarding the star-like objects. It is known that the optical power of these objects is about  $10^{46}$ – $10^{48}$  ergs/sec (ref. 3). According to Mannino<sup>2</sup>, this may be due to supernova explosions of as many as 4,000 a year. During the exploding stage the temperature of the stars goes up to  $10^9$ – $10^{10}$  K and the star begins to emit neutrino antineutrino pairs through annihilation of electron-positron pairs which at this high temperature are in equilibrium with the photons. The neutrino emission is of the same order and even higher of the photon emission<sup>4</sup>. Thus we can safely assume that the star-like sources emit  $10^{48}$  ergs/sec as neutrino power. Because the energies of these neutrinos are of the order of a MeV, we have an emission of  $\sim 10^{54}$   $\nu$ /sec/star-like source. (We can obtain these figures also supposing that each supernova inside the star-like object emits  $\sim 10^{45}$  ergs/sec as neutrino power; since we have  $10^{-4}$  supernovae/sec, and since the process goes on for about 200 days<sup>5</sup>, this is the same as saying that at each time we have  $10^{-4} \cdot 10^7 = 10^3$  supernova in the whole star-like, which means an emission of  $10^{45} \cdot 10^3 = 10^{48}$  ergs/sec from the star-like sources.)

Supposing that the star-like sources are young galaxies which are confined inside a shell out of the until now observed universe, we may have about  $10^8$  star-like (that is, one-tenth of the estimated number of galaxies<sup>6</sup>). Then we have  $10^{54} \cdot 10^8 = 10^{62}$   $\nu$ /sec. Since the process goes on for about  $10^8$  years (ref. 2), we have an output of  $10^{70}$  neutrino antineutrino pairs. On the whole universe, this makes a density of  $10^{70}/10^{84} = 10^{-14}$   $\nu$ /cm<sup>3</sup>. This figure is quite negligible with respect to the previous estimate<sup>1</sup> on the neutrino emission from the pre-supernova stage of the stars, and anyhow smaller than the energy density of the matter which is of the order of  $10^{-2}$  MeV/cm<sup>3</sup> (ref. 5).

If we suppose that our universe is pulsating we have an accumulation of neutrinos which are emitted in each phase of oscillation until an equilibrium is reached through the  $\nu + \bar{\nu} \rightarrow \gamma + \gamma$  process. But also in this case the density of neutrinos is smaller than that of the previous estimate<sup>1</sup>, although higher than the energy density of matter in the universe.

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### GEOPHYSICS

#### Metamorphism and Mineral Identification by Gamma Ray Methods

It is usually from the investigations of mineralogy that an estimation of the grade of metamorphism is made, since the appearance of some minerals and disappearance of others—or the change of composition in a solid solution series—is often symptomatic of the intensity of metamorphism. "For example, biotite is a common mineral of metamorphic rocks; however, it does not occur in very low grade rocks, its composition then being represented by mixtures of muscovite and chlorite. Minerals of the epidote group are characteristic of low grade rocks. At higher grades of metamorphism these minerals are largely converted to anorthite which enters into plagioclase. In lowest grade rocks the plagioclase is nearly pure albite and it increases in calcium content as the grade of metamorphism increases"<sup>1</sup>.

The mineral plagioclase has the chemical formula  $(\text{Na,Ca})\text{Al}_2\text{Si}_2\text{O}_8$ . The expression  $(\text{Na,Ca})$  in the formula means that sodium and calcium are interchangeable in the atomic structure of the crystals. If sodium is preponderant the mineral is called albite, and if calcium is preponderant, anorthite.

In order to find accurately which element predominates in the mineral, one can perform a chemical, petrographic, or spectroscopic analysis. These analyses suffer from the fact that the specimen may not be representative of the rock and that the specimen has to be destroyed before it can be analysed. This is disadvantageous especially when one also wants to investigate the thermal conductivity (heat flow), elastic constants and atomic weights (wave propagation), and conductivity and susceptibility (electric and magnetic properties) of the same specimen. A non-destructive analytic method is therefore proposed here.

When specimens are available in the laboratory,  $\gamma$ -ray attenuation methods may offer a simple and non-destructive method of analysis. An absorber the bulk density of which is  $\rho$ , made up of a mixture of elements the mass attenuation coefficients of which are  $(\mu/\rho)_1, (\mu/\rho)_2, \dots$  will have an overall narrow beam mass attenuation coefficient for the primary photons which is given by  $\mu/\rho = \sum p_i(\mu/\rho)_i$  where  $p_i$  is the fraction by weight of the  $i$ th element, and  $\mu$  the linear absorption coefficient<sup>2</sup>.

The computed  $\gamma$ -ray absorption coefficients for the minerals plagioclase  $(\text{Na,Ca})\text{Al}_2\text{Si}_2\text{O}_8$ , chlorite  $(\text{Mg,Fe,Al})_4(\text{Al,Si})_2\text{O}_{10}(\text{OH})_2$ , and epidote  $\text{Ca}_2(\text{Al,Fe})\text{Si}_2\text{O}_{12}(\text{OH})$  for the energy range 0.01–100 MeV using Grodzstein's tables<sup>3</sup> are shown in Table 1.

In the energy range 0.1–5 MeV, Compton effect is the predominant interaction and only the electron density can be obtained. However, at very low energies where the photoelectric effect is predominant, and high energies

Table 1. MASS ABSORPTION COEFFICIENTS FOR MINERALS AT ENERGIES FROM 0.01 TO 100 MEV (CM<sup>2</sup>/GM)

Energy (MeV)	Plagioclase		Chlorite		Epidote	
	CaAl <sub>2</sub> Si <sub>2</sub> O <sub>8</sub>	NaAl <sub>3</sub> Si <sub>3</sub> O <sub>8</sub>	Fe <sub>3</sub> Al <sub>2</sub> Si <sub>2</sub> O <sub>10</sub> H <sub>2</sub>	Mg <sub>3</sub> Al <sub>2</sub> Si <sub>2</sub> O <sub>10</sub> H <sub>2</sub>	Ca <sub>2</sub> Al <sub>2</sub> Si <sub>2</sub> O <sub>10</sub> (OH)	Ca <sub>2</sub> Fe <sub>2</sub> Si <sub>2</sub> O <sub>10</sub> (OH)
0.01	28.39	16.17	77.96	15.28	30.64	75.36
0.02	3.76	2.17	11.18	1.96	4.1	11.09
0.03	1.20	0.726	3.52	0.67	1.32	3.52
0.05	0.366	0.269	0.87	0.262	0.39	0.89
0.08	0.2	0.177	0.324	0.177	0.21	0.33
0.1	0.17	0.158	0.231	0.159	0.173	0.243
0.2	0.126	0.122	0.130	0.125	0.12	0.131
0.6	0.080	0.080	0.0785	0.081	0.077	0.079
1	0.063	0.063	0.0625	0.064	0.063	0.062
5	0.0287	0.0281	0.0296	0.0285	0.029	0.031
10	0.0229	0.022	0.0248	0.022	0.022	0.023
20	0.021	0.019	0.0239	0.0193	0.021	0.025
50	0.0216	0.0198	0.0261	0.0194	0.023	0.027
100	0.0236	0.0214	0.0290	0.0209	0.023	0.029

where pair production predominates, the interchangeable heavy and light elements in a mineral can be distinguished. This distinction does not seem to depend on the energy of the primary photons when once very low (< 0.1 MeV) or very high (> 5 MeV) energies are used. When the mineral is thus identified (for example, as anorthite or albite), the grade of metamorphism can be estimated and the specimen can be used to investigate its elastic, thermal, electrical and magnetic properties.

I thank the members of the Atomic Energy of Canada, Ltd., for helpful discussions and the National Research Council for financial assistance.

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### World-wide Propagation of Impulsive Micropulsation Activity through the Ionosphere

THE actual configuration in space-time of micropulsation activity has long been the subject of controversy, and no single model has been able to explain all the features of the different types of micropulsations. This communication concerns the method by which impulsive *Pi* 2 (ref. 1) micropulsations spread over the Earth. Such micropulsations are recognized more easily on the night side of the Earth, although they can be detected on the daylight side also if not masked by other daylight micropulsations such as *Pc* 3, *Pc* 4 and *Pc* 5 (ref. 1).

The origin of *Pi* 2 micropulsations is still uncertain. Some investigators believe that they are oscillations of the field lines caused by a change in particle pressure when a large bunch of trapped energetic particles is dumped<sup>2</sup>. On the other hand, charged particles impinging in high latitudes have also been suspected as the origin<sup>3</sup>, and in this respect several possible mechanisms have been considered whereby a beam of charged particles streaming through the magnetospheric plasma could trigger instabilities in the hydromagnetic frequency range<sup>4-7</sup>. Whatever the mechanism of creation may be, a certain kind of eigenoscillation, perhaps Dungey's<sup>8</sup> torsional mode of hydromagnetic oscillations of a bundle of magnetic lines of force, seems to work as an intermediate process. Despite the rather irregular appearance of *Pi* 2 micropulsations, one can still detect the existence of characteristic periods in most cases. The bundle of lines of force intersects the ionosphere in two magnetically conjugate regions in mid to high latitudes, resulting in the generation of two electrojets and the spread of ionospheric currents to all points on the Earth<sup>9</sup>. Fig. 1 (ref. 10) shows the distribution of ionospheric currents at a certain instant in the case of a typical *Pi* 2.

The figure suggests that the polar electrojet (in the northern hemisphere in this case) exists in the auroral

region in the meridian plane around 2,300 L.M.T., and that the stream lines of ionospheric currents spread both east and west along latitude circles. Herron<sup>11</sup> has actually observed that *Pi* 2 disturbances start from the 2,300 L.M.T. meridian and propagate eastward as well as westward. He has also observed significant phase differences in corresponding micropulsation signals over distances of hundreds of kilometres, although independent investigations by other groups often show no phase difference for events observed at points separated in longitude by thousands of kilometres<sup>12</sup>. According to the theory of electromagnetic induction, a periodic magnetic disturbance in a conducting material propagates with phase velocity  $T^{-1/2}/\sqrt{\sigma}$ , where  $T$  is the period (sec) of the disturbance and  $\sigma$  is the specific conductivity (E.M.U.). If one takes  $\sigma = 5 \times 10^{-16}$  as the daily mean in the *E* region and  $T = 100$ , the phase velocity is about 40 km/sec, which agrees with the results obtained by Herron<sup>11</sup>. The phase velocity has also been observed to be smaller for longer period disturbances. It would be interesting to determine whether, in fact, the velocity is actually inversely proportional to the square root of the period.

The foregoing calculation is a crude order of magnitude estimate for the one-dimensional case assuming an isotropic medium. However, the results are consistent with the experimental evidence, and do seem to indicate a strong possibility that the above mode of propagation for *Pi* 2 micropulsations is correct.

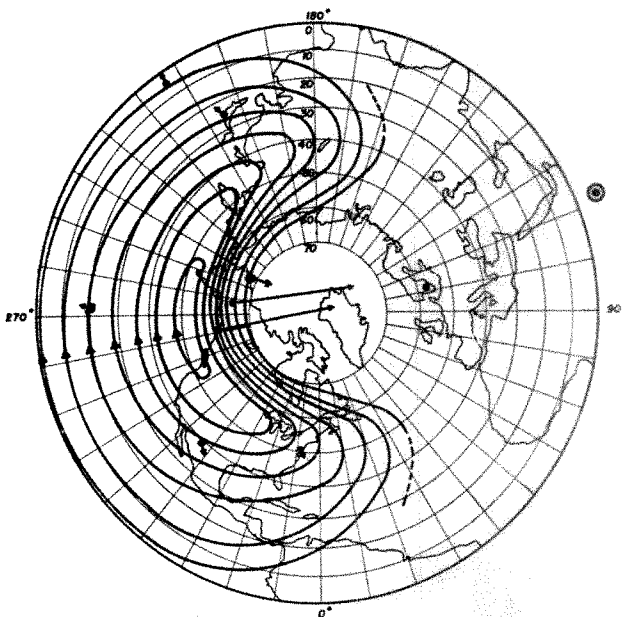


Fig. 1. Equivalent overhead current system for a *Pi* 2 at 0036 U.T., December 26, 1957. The intensity of the electric current between two adjacent contours is  $5 \times 10^2$  amp (Jacobs and Sinno, 1960<sup>13</sup>). \*In the previous paper<sup>10</sup>, the phenomenon is referred to as *Pi*. *Pi* is the older nomenclature for *Pi* 2, and the minus sign means that it occurred in association with a negative bay in the auroral regions.

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## PHYSICS

### Turbulent Flow Deposition of an Aerosol in Long, Thin Conduits

In relation to an air-sampling system installed in our high-activity handling building, we have carried out some investigations to determine the loss in mass of a solid-particulate cloud when passing, in a turbulent air flow, through different lengths of 10-mm inner diameter copper tubing.

The particulate cloud consisted of lithium hydroxide particles in the size-range  $0.05\mu$ – $1.5\mu$  with a mean size by mass of about  $0.5\mu$ .

The geometry of the tubing during a run was in the form of concentric rings (radius of curvature about 0.5 m) resting for the most part on a horizontal surface.

The tubing was thoroughly washed and dried after each run. We cannot offer any explanation for the peculiar results (Fig. 1). Moreover, a more or less thorough survey of available literature on micromeritics has

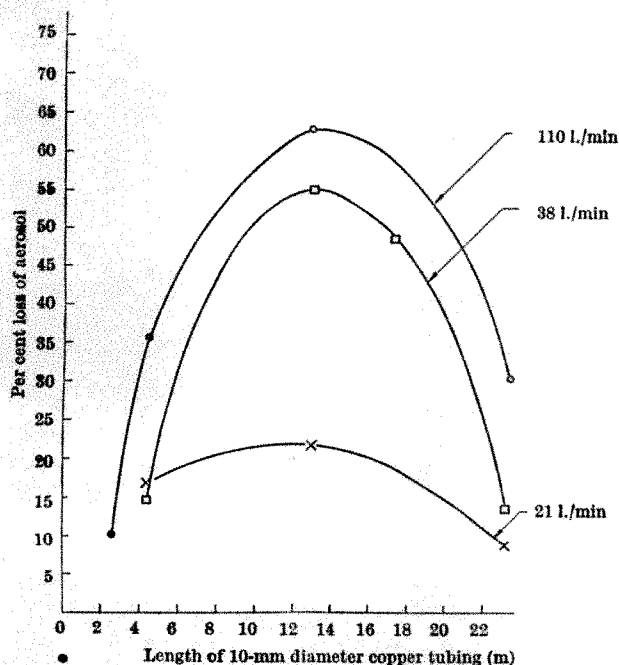


Fig. 1. Loss of  $\text{LiOH} \cdot \text{H}_2\text{O}$  particles in 10-mm diam. copper tubing during turbulent flow

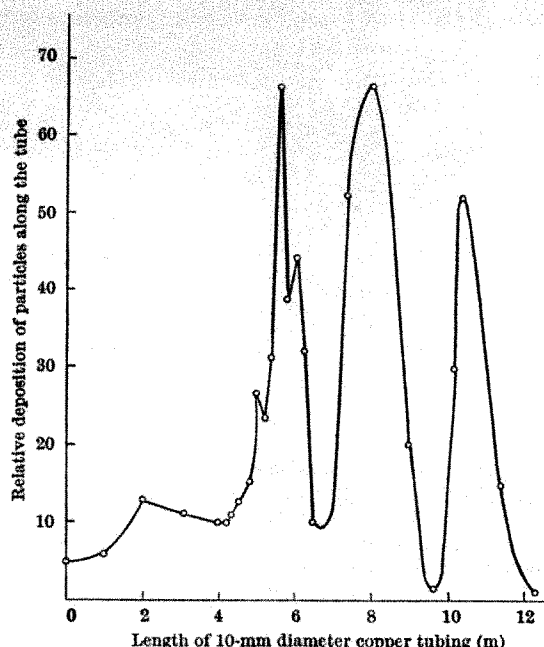


Fig. 2. Deposition pattern of particles along the 10-mm diameter tube of 12.7 m length

yielded nothing that would suggest the existence of such a phenomenon.

We have also been able to ascertain the existence of the same phenomenon for tubes with inner diameter of 5 mm, 4 mm and 3 mm.

From our findings we may state the following:

(1) The length of conduit for which maximum deposition takes place,  $L_{\text{max}}$ , is independent of the velocity of the turbulent stream.

(2) If  $r$  is the inner radius of a given tube, then for all tubes:

$$\frac{r^2}{L_{\text{max}}} = 1.8 \times 10^{-4} \text{ cm}$$

(3) If  $L_{\text{min}}$  is the length of conduit for which minimum deposition takes place, then for all tubes:

$$\frac{r^2}{L_{\text{min}}} = 0.9 \times 10^{-4} \text{ cm}$$

In order to observe the physical pattern of the aerosol deposits on the walls of the conduit for which a maximum loss had been observed (Fig. 1), we atomized a solution of  $\text{Na}_2\text{CO}_3$  with a tracer of sodium-24 and passed it through a 12.7 m length of 10-mm diameter tube. Fig. 2 shows the pattern of deposition along the length of this tube.

The same experiment with a 22-m tube failed to reveal any pronounced patterns such as in Fig. 2.

We are proceeding at this stage to (a) measure the phenomenon in straight conduits; (b) plan experimental arrangements with which we can describe the values and behaviours of all the parameters involved, for example, pressure, velocity, particle sizes, etc.; (c) determine the effect on the phenomenon of using aerosols with different densities and also gases of various densities and viscosities.

We are interested in hearing from any investigators who have come across this phenomenon or who can offer any information along these lines.

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## Nanosecond Heating of Aqueous Systems by Giant Laser Pulses

We have just been able to demonstrate heating of some pH-indicator systems within a few nanoseconds. We had suggested such very fast heating earlier<sup>1</sup>. At that time we were only able to demonstrate heating by a ruby-laser operating in its normal mode, associated with a flash of 600  $\mu$ sec duration.

To generate the heating pulse we used a ruby crystal in TRG laser, model 104 (Technical Research Group, Melville, New York). With the added rotating prism well aligned, we obtained an energy output of about one joule *Q*-switched, measured with TRG-power meter model 101. The shape of the output pulse was measured with a detection device containing an SD 100 photodiode of EG and G or a phototube RCA type 929 and connected to a travelling wave oscilloscope ('Tektronix 519') or to a fast-rise oscilloscope ('Tektronix 545' with *L*-plug-in unit). An illustration of the output pulse near threshold is shown on Fig. 1. Two pulses were generally obtained at full output (together 1 joule), about 100 ns wide and 200 ns apart, inherent to *Q*-switches operating with rotating prisms. Single pulses could have been obtained with a special attachment, which was not available to us, or by operation near threshold.

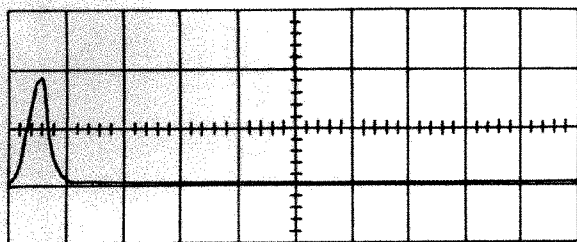


Fig. 1. Ruby laser pulse, *Q*-switched slightly above threshold, 50 mV per large vertical division, 100 ns per large horizontal division

The pulsed laser beam passed through a rectangular window and was slightly focused into a cell of 3 mm  $\times$  6 mm opening and 2 mm depth by two cylindrical lenses. The beam was directed so that no cell walls were touched. Perpendicular to the axis of the laser beam was the axis of the observation beam, such that it transversed a length of 6 mm. A well-aligned beam from a tungsten light source<sup>2</sup> was used. Its cross-sectional area within the small cell was about 1 mm. The monochromatic light (Schott double-band interference filter, peaking at 432 m $\mu$ ) was collected at the photocathode of a multiplier phototube (RCA type 7326). The voltage signal produced by the anode current of the multiplier phototube across the load resistor was d.c.-compensated and fed into 'Tektronix 535' with a *D*-plug in unit.

Fig. 2 shows an oscilloscope trace of an actual experiment. The aqueous solution in the cell contained 0.022 M CuSO<sub>4</sub>, 10<sup>-4</sup> M bromthymol blue, and 0.11 M triethanolamine, pH = 7.0 (23° C initially). A calibration experiment was conducted with available instrumentation<sup>3</sup>. It was found in the calibration experiment that a temperature change of about 3° C caused an equilibrium shift of about 20 mV (signal with mixture = 340 mV, with water alone = 4,500 mV, length of light path = 10 mm). Reduced to the 6-mm path length in the small cell for flash heating one should expect an equilibrium shift of the signal of 1.2 per cent/deg. C. The experiment resulted in a shift of 5 per cent (total signal with mixture = 60 mV, equilibrium shift = 3 mV). This corresponds to a temperature rise of 4° C.

The aqueous solution already mentioned had a transmission of 47 per cent, as measured with a Zeiss spectrophotometer at 694 m $\mu$  in a 2-mm cuvette. Then about 0.5 joule was absorbed in about 20 mm. One would

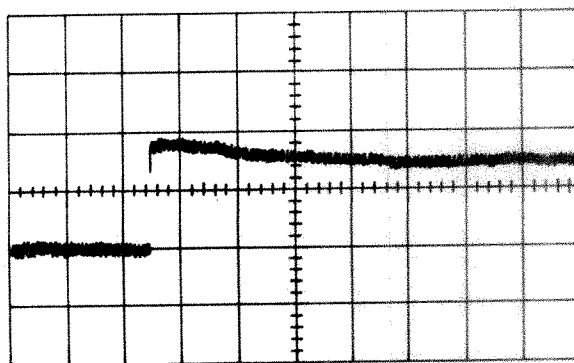
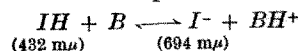


Fig. 2. Temperature jump experiment with a copper-complex absorbing the light pulse, 2 mV per large vertical division, 200 ms per large horizontal division. This large time scale was used to demonstrate the long duration of the change. These changes were measured thus far down to 10  $\mu$ sec/div.

therefore expect a temperature rise of 6° C. This value is somewhat above that derived before, but the deviation is acceptable on the grounds of the uncertainties involved in the measurements. The heating may also be less homogeneous than assumed, due to the structured pattern of the pulsed laser.

Fig. 3 shows an oscilloscope trace of another experiment, using 1.5  $\times$  10<sup>-4</sup> M methylene blue, 10<sup>-4</sup> M bromthymol blue, and 0.02 M glycylglycine (pH 7.3, 23° C). In a calibration experiment of 3° C temperature rise, an equilibrium shift of 6 mV occurred (240 mV signal, otherwise as before). A 6-mm path length in the small cell would result in a signal change of 0.4 per cent/deg. C. The experiment gave a change of 9.2 per cent, corresponding then to a temperature rise of 23° C. The spectrophotometric transmission at 694 m $\mu$  for a 2-mm path was 23.5 per cent, about 750 m joules are absorbed in 20 mm (ref. 3) solution, giving a 9° C temperature rise. As the experimentally derived temperature rise exceeds the theoretically possible one by a large amount, heating by the laser flash cannot account for the total change in transmission, measured at 432 m $\mu$ .

In an attempt to solve the lack of agreement, the absorbed laser energy was measured with a TRG-power meter, model 101. The absorbed energy was reproducible within 3 per cent (four flashes each) and corresponds to 30.5 per cent transmission for the methylene blue solution and 40 per cent for the copper-containing solution. While the transmission of the former solution increased by 7 per cent (photobleaching?), the transmission of the copper-containing sample decreased by 7 per cent. This decrease in transmission would follow directly from the heating effects. The temperature sensitive reaction is:



with *IH*, *I*<sup>-</sup> the two forms of bromthymol blue and

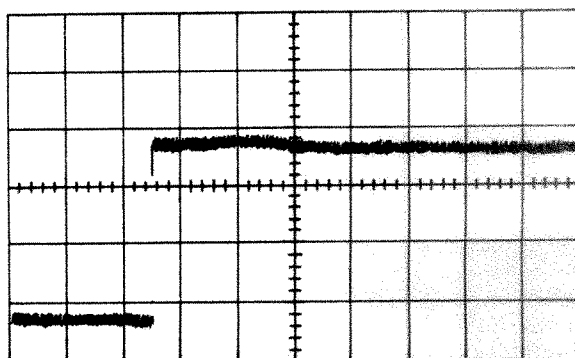


Fig. 3. Temperature jump experiment with methylene blue absorbing the light pulse, 2 mV per large vertical division, 200 ms per large horizontal division



$B$ ,  $BH^+$  those of the  $pH$ -buffer. The two wave-lengths indicate which form of the indicator absorbs there.

The experiments showing sudden rise in temperature demonstrated increased transmission at 432  $m\mu$  (Fig. 2), corresponding to production of  $I^-$ . The production of  $I^-$  is associated with a decrease in transmission at 694  $m\mu$ , as measured. Plotting extinction as a function of wave-length with  $pH$  as parameter shows that  $pH$  changes cause about equal extinction changes at both wave-lengths (but in opposite directions). The measured total shift of 5 per cent brings the observed transmission of laser pulses quite close to the expected value (42 per cent, neglecting absorption irregularities).

While nanosecond heating with the copper-containing solution seems fairly well established, such heating could only account for part of the effects of the methylene blue solution. Bleaching of the dye could account for most of the transmission increase at 432  $m\mu$  and at 694  $m\mu$ . This latter system is under further investigation.

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### Electrical Conductivity of Circumanthracene

CIRCUMANTHRACENE is the largest of the polycyclic aromatics having known crystal structure. X-ray crystal structure analysis of the grey-black prismatic needles showed that they are monoclinic with  $a = 23.77 \pm 0.01$  Å,  $b = 4.59 \pm 0.03$  Å and  $c = 9.98 \pm 0.01$  Å and  $\beta = 99^\circ 54' \pm 30'$  (ref. 1). The evaporated thin film of this thirteen ring aromatic ( $C_{14}H_{10}$ ), obtained easily by means of a vacuum sublimation, was brownish violet.

The electrical conductivity and its temperature dependence have been found for three forms of the aromatic hydrocarbon; needle-type single crystal having dimensions:  $30 \sim 50 \mu \times 30 \sim 50 \mu \times 1$  mm, surface-type thin film and sandwich type film, both being about  $1 \mu$  in thickness<sup>2</sup>. To avoid the effect of humidity, the specimens used were kept in a vacuum of  $10^{-5} \sim 10^{-6}$  mm mercury. Depending on the orientation of the large molecules in the monoclinic crystal, a substantial anisotropy was found as listed in Table 1 (ref. 3).

Table 1. THE ELECTRICAL RESISTIVITY AT  $15^\circ$  C,  $\rho_{11}$ , OF CIRCUMANTHRACENE OBSERVED FROM THE VARIOUS TYPES OF CELLS: ( $\rho_{11} = \rho_0 \exp(\Delta E/2kT)$ )

Specimen	Observed value $\rho_{11}$ ( $\Omega$ cm)	$\Delta E$ (eV)
Single crystal// b-axis	$6 \times 10^{12}$	1.8
Surface-type	$2.5 \times 10^{13}$	1.9
Sandwich-type	$10^{14} \sim 10^{17}$	1.7

A fairly strong photoconduction of circumanthracene was observed. An excellent agreement of spectral responses between the optical absorption and the photoconduction in solid state was found as drawn in Fig. 1.

An abrupt change of electrical properties of films of pure circumanthracene was observed, when they were prepared under high vacuum ( $10^{-7} \sim 10^{-8}$  mm mercury), produced by an ion pump. When the preparation of surface-type cell, the evaporation of two aluminium electrodes and of the organic specimen, were carried out at high vacuum, the observed resistivity was  $10^3$  times higher than that of an ordinary cell.

However, when a small amount of dried oxygen was introduced to the high vacuum cell, a sharp drop of the resistivity was observed and the value converged to that observed under ordinary conditions.

This behaviour may be explained by a combination of the hydrocarbon molecules with ambient oxygen. The

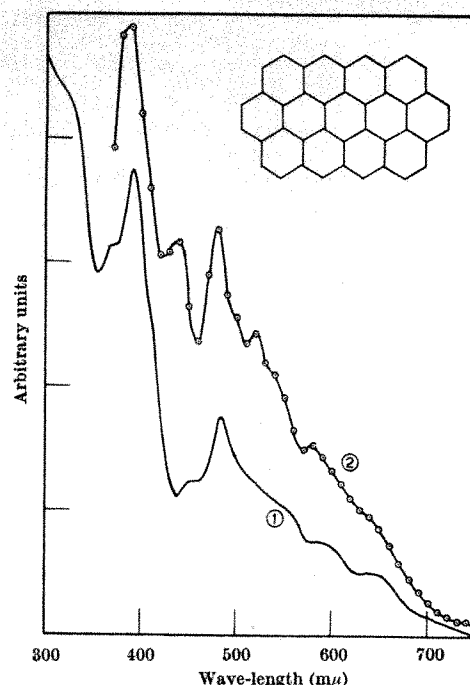


Fig. 1. Spectral response curves of optical absorption (1) and photoconduction (2) for circumanthracene thin film

combination was so strong that removal of oxygen from the organic molecule was difficult at room temperature: an adsorption of oxygen on the circumanthracene molecules may be classified as a weak chemisorption.

Furthermore, the value of electrical conductivity of the sandwich-type cell, prepared under high vacuum, changed by a factor of  $10^6$  in comparison with that of a cell prepared under a vacuum of  $10^{-5} \sim 10^{-6}$  mm mercury. This drastic change may be explained by the fact that the introduction of electrons to the conduction band of circumanthracene from the aluminium metal increases the conductivity of the cell. The effect of ambient oxygen on the contact is to remove the electrons from the conduction band of circumanthracene and the conductivity decreases to the normal value.

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### GEOLOGY

#### Marion Island, South Indian Ocean

MARION ISLAND lies in latitude  $46^\circ 49' - 46^\circ 59' S.$ , longitude  $37^\circ 35' - 37^\circ 55' E.$  It measures 12 miles east-west by 7 miles north-south, and rises to a maximum height of 3,890 ft. With the smaller Prince Edward Island, 12 miles to the N.N.E., it constitutes the Prince Edward Island group.

In contrast to many of the islands of the Southern Atlantic and South Indian Oceans, there is a lack of geological knowledge of these islands. Prince Edward Island has never been examined, and information on

Marion Island is restricted to the observations made during the short visits of the naturalists aboard H.M.S. *Challenger* in 1873<sup>1</sup> and of Jeannel<sup>2</sup> in 1938. By courtesy of the South African Navy, I spent several hours on the Island during the relief of the meteorological station there in April 1954. At that time, a thorough geological examination of the Island appeared imminent. As this has yet to materialize, the following note on the geology is submitted.

The Island is wholly volcanic. A younger and an older lava series are present, with intercalated agglomerate, and numerous large scoria mounds. Most of the Island is built up of the older lavas, chiefly dark-coloured olivine-basalts varying in their degree of compaction, and often carrying phenocrysts of olivine. There are also several light-coloured flows with a distinctive platy habit, for example on Skua ridge, 1½ miles north-west of the meteorological station. An agglomerate, with rounded fragments of olivine basalt up to 6 in. in diameter, was also noted interbedded with the normal olivine basalt.

Microscopic examinations of the normal basalt showed it to contain phenocrysts of magnesian olivine, and also of plagioclase and clinopyroxene, set in a dark matrix comparatively rich in iron ore. The platy lavas have a relatively even grain and contain less basic plagioclase. They are trachytes.

Several of the established valley floors have been inundated by flows of later olivine basalt. The most distinctive feature of Marion Island from offshore is the very numerous reddish scoria mounds dotted all over the Island. These rise for up to 700 ft. above their surroundings. Their conical shape is frequently perfectly preserved, and some retain crater-shaped depressions at their summits.

The age of the older lavas is uncertain. Behind the cliffed coastline the lower slopes of the Island locally exhibit a mature land surface. By analogy with other oceanic islands, they may date back as far as the Tertiary. The later lavas and scoria mounds are clearly of very recent age.

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## METALLURGY

### Effect of Oxygen Pressure on the Melting of Silver

OWING to the high solubility of oxygen in the liquid metal, the melting or freezing point of silver decreases considerably with increasing pressure of oxygen<sup>1</sup>.

For the present investigations in oxygen under pressure a high-pressure furnace was used. This consisted of a resistance element of platinum 10 per cent rhodium alloy, 1.2 cm in diameter and about 24 cm long, enclosed in a pressure vessel 5.5 cm in diameter. Furnaces of this type and ancillary equipment have already been described<sup>2</sup>.

For some of the measurements up to 20 atmospheres, 7 g of silver were heated in a silica crucible in oxygen to a temperature about 25° above the expected melting point, kept constant for 40 min, then cooled at the rate of 2° per min. The solidification was shown by an arrest in the plot of the E.M.F. from a silica-sheathed thermocouple immersed in the sample. Results agree fairly well with those of Allen<sup>3</sup>, who used a similar method up to 13.9 atmospheres. At higher pressures, possibly due in part

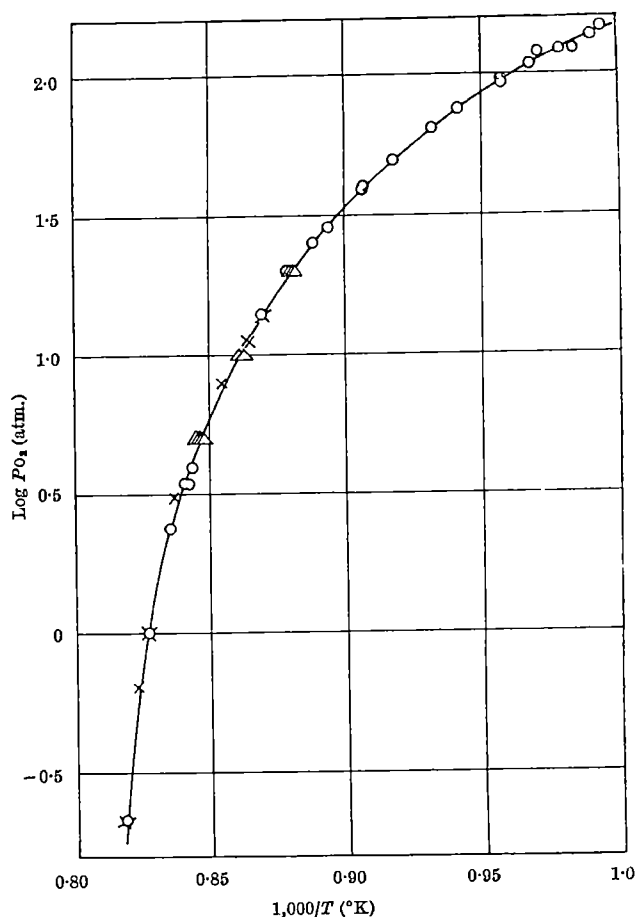


Fig. 1. Melting point of silver as a function of oxygen pressure. x, Allen: thermal arrest; Δ, present work: thermal arrest; O, present work: fusion of silver strip

to greater heat losses, the arrests were indistinct and an alternative method was used: a silver strip about 6 mm long, 1 mm wide and 0.09 mm thick was roughened with emery paper and the ends welded to two stout gold wires. A thermocouple (Pt-Pt + 13 per cent Rh) was placed alongside the strip. Through the strip a small direct current of about 5 m.amp was passed; this was recorded on a meter. With a heating rate of 1.5° per min, fusion of the silver was shown by a zero reading on the meter. A modification was tried in which the ends of the strip were welded to wires of platinum and platinum-13 per cent rhodium respectively and incorporated in a differential thermocouple unit by means of the thermocouple alongside it. Fusion was shown as a break in the trace of the differential E.M.F. Owing to oxidation of the welds at pressures above 60 atms., this method was less reliable than the former, which was principally used. Agreement between the last methods, however, was good.

At a pressure of oxygen of 1 atm. the melting point is 937° C. For pressures and temperatures in the range 0.213-2 atms. and 950°-928° C respectively the solution of oxygen in the liquid metal is dilute and the silver should behave practically ideally. From the freezing point depressions, taking the heat of fusion of silver<sup>4</sup> as 2,855 cal. mole<sup>-1</sup>, the atom fractions of silver ( $X_{Ag}$ ) have been determined. If the other atom fractions are assumed to be atomic oxygen, the relation  $X_0 = k\sqrt{P_{O_2}}$  is found to apply. The solubility of oxygen in the solid silver is too small to affect the relationship significantly. The mean value of  $k$  for the range is approximately 0.022. The same value is obtained by extrapolation of the higher-temperature data of Sieverts and Hagenacker<sup>5</sup> to this region.

Results for the pressure range 0.213–147.3 atm. are shown in Fig. 1. At the highest pressure, after allowance for a slight scatter, the melting point is approximately 736° C, showing a depression of 225°.

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## CRYSTALLOGRAPHY

### Layer Diameter in Graphite

THE X-ray method of estimating the layer diameter in samples of graphite, based on the breadths of the  $hk0$  reflexions, leads to apparent diameters of a few hundred Å. On the other hand, electron micrographs of the same sample show flakes the dimensions of which are some thousands of Å, nearly an order of magnitude larger. The discrepancy could arise from the simultaneous occurrence of strain broadening and particle-size broadening, or from the 'tangent-plane approximation'<sup>1,2</sup> being insufficiently good. The only  $hk0$  reflexion which can readily be examined is 1120, and measurements of integral breadths or half-widths require two reflexions in order to separate strain broadening and particle-size broadening. Measurement of the variance of a line profile, on the other hand, can in principle separate the two effects even when only one line is available for examination<sup>3</sup>. Use of the variance also makes it possible to allow for some departure from the conditions within which the tangent-plane approximation is valid.

The tangent-plane approximation leads to good results so long as the regions of high intensity in reciprocal space are concentrated near the points of the reciprocal lattice or, if not concentrated, at least have their directions of greatest extension making an angle other than 90° with the vector joining the reciprocal-lattice point to the origin. This limitation is well recognized when the disorder in layer stacking is complete<sup>4</sup>, but it has not always been taken into account for degrees of disorder of the kind encountered in samples of commercial graphite. In these the probability of a mistake in a layer sequence is of the order of one-fifth, and hence the  $hk0$  reflexions have a considerable extension in the direction of  $c^*$ , though they do not form continuous rods in reciprocal space. The tangent-paraboloid approximation<sup>4-6</sup> is appropriate for this problem. If  $S = 2 \sin \theta / \lambda$  is the distance from the origin of reciprocal space to any point in reciprocal space, and  $S_0$  is the distance to the  $hk0$  point, one can define a variable  $s$  by  $S = S_0 + s$ , so that:

$$S^2 = (S_0 + s)^2 = S_0^2 + 2S_0s + s^2 \quad (1)$$

If  $u, v, w$ , are the co-ordinates of a point in reciprocal space measured from  $S_0$ , then, with  $u$  parallel to  $S_0$ :

$$S^2 = (S_0 + u)^2 + v^2 + w^2 \\ = S_0^2 + 2S_0u + u^2 + v^2 + w^2 \quad (2)$$

These two equations for  $S^2$  must be equal, and a consideration of the relative magnitudes of the various terms shows that if  $w$  is measured in the direction of  $c^*$  the terms in  $u^2, v^2$ , and  $s^2$  are negligible in comparison with the rest. To a sufficient approximation, therefore:

$$s = u + w^2/2S_0 \quad (3)$$

The variance of  $s$  (Wilson<sup>7</sup>) is given by:

$$W_s \equiv \langle s^2 \rangle - \langle s \rangle^2 = \langle u^2 \rangle + \langle uw^2 \rangle / S_0 + \langle w^4 \rangle / 4S_0^2 - \langle u \rangle^2 - \langle u \rangle \langle w^2 \rangle / S_0 - \langle w^2 \rangle^2 / 4S_0^2 \quad (4)$$

The terms containing  $\langle u \rangle$  are practically zero, because  $u$  takes on positive and negative values with approximately equal probability. The remaining terms are the variance of  $u$  and the variance of  $w^2$  divided by  $4S_0^2$ :

$$W_s = W_u + W_w / 4S_0^2 \quad (5)$$

The tangent-plane approximation gives only the first of these terms. It is clear that if the observed variance  $W_s$  is attributed entirely to restricted particle dimension in the direction of  $u$ , the value obtained for the particle dimension will be too small, because: (1)  $W_u$  will result both from strain variance and particle-size variance; (2) the observed variance  $W_s$  depends also on the variance of  $w^2$ . The value of the variance of  $w^2$  required to correct the observed variance to give  $W_u$  only could be obtained, in principle, from the line profiles of the  $hkl$  reflexions, or from the theoretical expression for the line profile as a function of  $w$ . A tangent-paraboloid calculation for the integral breadth of the  $hk0$  reflexions would probably lead to a contribution from extension in the direction of  $w$  also, though Bacon<sup>6</sup> found that the half-width was relatively insensitive to variations in the perfection of the layer stacking.

We have made a preliminary examination of three samples of commercial graphite, for which the integral breadths of the 1122 reflexions indicate that mistakes in the layer sequence occur about every six planes, and the use of the ordinary Scherrer formula for particle size indicates layer diameters of 200 Å–500 Å. The line profiles of the 1120 reflexions were recorded with a counter diffractometer, and the variances calculated in a manner similar to that of Langford and Wilson<sup>8</sup>. A diamond-powder specimen, with approximately the same packing density as the graphite specimens, was used to correct for instrumental and wave-length broadening, and an approximate value for  $W_w$  was based on the integral breadths of the 1122 reflexions. The layer diameters obtained are subject to large error, since they depend on small differences between larger quantities, one of them being difficult to assess accurately at the present stage. Two of the samples showed no signs of heterogeneity, and gave apparent layer diameters of 3000 Å–4000 Å. The third graphite appeared to be composite, with the reflexions showing sharp and diffuse components simultaneously. The apparent layer diameter was 160 Å, in fair agreement with earlier X-ray measurements, and is presumably typical only of the component producing the diffuse lines. The intercepts of the variance-range curves indicated that some part of the breadth of the 1120 reflexions was caused by strain in the layers. However, the accuracy achieved was not high, and at the present stage of the analysis we do not feel justified in giving a numerical value for the strain.

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## CHEMISTRY

### Safety in Air Separation Plants : Determination of Atmospheric Pollutants by Gas Chromatography

MANY problems are associated with safety in air separation plants, and various theories have been proposed as to the cause, and avoidance, of explosions<sup>1-3</sup>. The primary cause is known to be the accumulation of atmospheric pollutants, condensed from the air stream at the very low temperatures involved in liquid air distillation, particularly in the presence of high oxygen concentration. The nitrogen plants designed by this company minimize possible hazard by limiting the maximum oxygen concentration to 31 per cent.

The levels and type of contaminant will obviously vary considerably from site to site, depending on the extent of industrialization and traffic concentration. Consequently, an investigation of safety in air separation must be preceded by a detailed analysis of atmospheric pollutants, and this initial part of the research programme consisted of analysing hydrocarbons up to *n*-pentane.

Bellar *et al.*<sup>4</sup> demonstrated that flame ionization detectors are capable of analysing atmospheric hydrocarbon pollutants to levels of 0.001 volumes per million (v.p.m.) without resorting to concentration techniques. An analyser constructed at these laboratories, incorporating a Perkin Elmer model 451 ionization amplifier and flame ionization detector, was used in our work. In contrast to Bellar, 5 ml. samples were analysed, and we would confirm that, even with this sample size, the suggested flows of 40 ml./min hydrogen, 140 ml./min oxygen and 40 ml./min carrier gas allow re-ignition of the flame without difficulty and the base line re-establishes itself before elution of the first component.

Past experience gained from the analysis of gases and liquids derived from thermal naphtha cracking had resulted in the development of a two-column separation from air, hydrogen, etc., to *n*-pentane. The separation of methane to propylene was satisfactorily attained on a 6-ft. × 0.25-in. outer diam. column of 3 per cent di-2-ethyl-

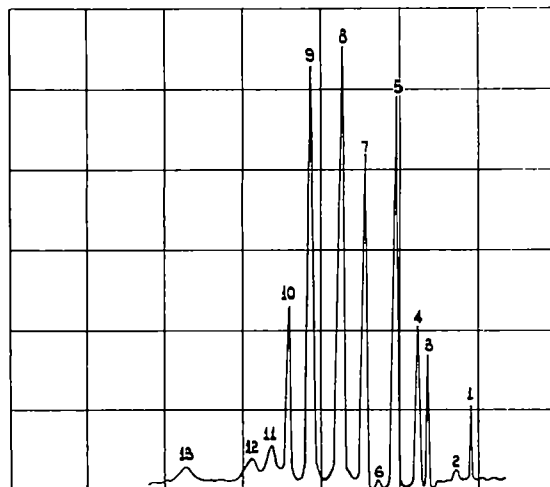


Fig. 2. 1, Methane; 2, ethane-ethylene; 3, propane-acetylene; 4, propylene; 5, iso-butane; 6, *n*-butane; 7, *n*-butene-1; 8, iso-butene-1; 9, *trans*-butene-2; 10, *cis*-butene-2; 11, 1:3-butadiene; 12, iso-pentane; 13, *n*-pentane

hexyl sebacate on 30-60 mesh Davison silica gel (column 1), while for the *C*<sub>4</sub>'s separation a 14-ft. × 0.25-in. outer diam. column of a 2:1:1 blend of the coated supports 20 per cent *bis* (2-methoxyethyl) adipate and a 20 per cent di-2-ethylhexyl sebacate on 'Chromosorb P' (ref. 5) (column 2) was used. The retention values for this column, previously unpublished, are given in Table 1, but it should be noted that a modified column is now available from Perkin Elmer<sup>6</sup>.

Table 1. RELATIVE RETENTION TIMES (*n* C<sub>4</sub> = 1.000)

Component	R.R. time	Component	R.R. time
Air-methane	0.155	iso-Butene-1	0.512
Ethane-ethylene	0.183	<i>trans</i> -Butene-2	0.813
Acetylene-propane	0.252	<i>cis</i> -Butene-2	0.691
Propylene	0.280	1:3-Butadiene	0.733
iso-Butane	0.358	iso-Pentane	0.805
<i>n</i> -Butane	0.401	<i>n</i> -Pentane	1.000
<i>n</i> -Butene-1	0.450		

As expected, high acetylene-levels were recorded, but wide fluctuations were found throughout the day, while the *C*<sub>4</sub>'s were surprisingly high and relatively consistent, and were often individually greater in quantity than the more commonly expected impurities. Fig. 1 is a chromatogram obtained at a period of high acetylene concentration, using column 1 at 50° C and a helium flow of 40 ml./min, while Fig. 2, using column 2 at 50° C and 40 ml./min helium, shows high *C*<sub>4</sub> concentrations. A comparison of the chromatograms indicates the very wide fluctuations found during analysis, since the samples were taken within 4 h of each other. Table 2 gives the actual concentration of each component.

Table 2

Peak No.	Component	Concentration (v.p.m.)	Peak No.	Component	Concentration (v.p.m.)
1	Methane	0.001	1	Methane	0.01
2	Ethane	0.001	2	Ethane-ethylene	0.003
3	Ethylene	0.004	3	Propane-acetylene	0.028
4	Propane	0.001	4	Propylene	0.036
5	Acetylene	0.220	5	iso-Butane	0.102
6	Propylene	0.008	6	<i>n</i> -Butane	0.002
7	<i>C</i> <sub>4</sub> 's	0.012	7	<i>n</i> -Butene-1	0.09
			8	iso-Butene-1	0.12
			9	<i>trans</i> -Butene-2	0.114
			10	<i>cis</i> -Butene-2	0.053
			11	1:3-Butadiene	0.018
			12	iso-Pentane	0.006
			13	<i>n</i> -Pentane	0.007

A comparison of these results with the now classical work of Altschuller and Bellar<sup>6</sup> shows major differences. The methane quantities recorded are very much smaller and the number and quantity of *C*<sub>4</sub>'s considerably higher.

The samples for all the results given in this communication were taken from the centre of Trafford Park, Manchester, which is one of the world's largest industrial

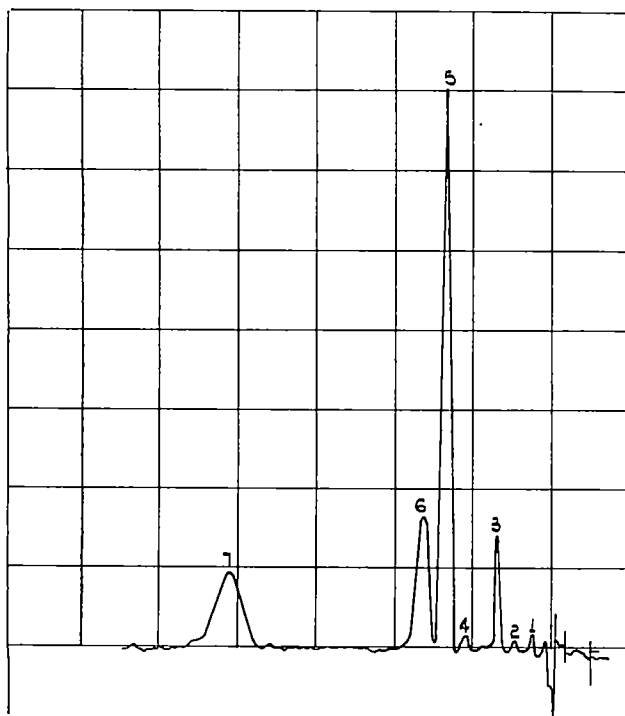


Fig. 1. 1, Methane; 2, ethane; 3, ethylene; 4, propane; 5, acetylene; 6, propylene; 7, *C*<sub>4</sub>'s

estates, and several large chemical manufacturing companies are situated very close to these laboratories. While the atmospheric pollutants found in this area are very different from samples taken in other less industrialized areas, the installation of air separation plants is more likely to occur in the industrialized areas, and thus the results obtained are of more interest to the plant designer than those from other cleaner districts.

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### Isolation and Preliminary Characterization of Soil Polysaccharides

SOIL polysaccharides, important components of humus<sup>1</sup>, are of microbial origin<sup>2</sup>. Their role in the formation of soil aggregates is of considerable significance in agriculture. We have, during the initial stages of our examination of the mechanistic role of polysaccharides in soil, concentrated on the isolation, purification, and the preliminary characterization of some components of the soil polysaccharide mixture.

Aliquots (200 ml. of liquid in all cases) of water, a 25 per cent aqueous suspension of 'Amberlite IRC-50(H)', 7 per cent disodium ethylenediamine tetraacetate, dimethylformamide, *N*-methyl-2-pyrrolidone, 8 M urea, and 0.6 N sulphuric acid were used to extract 100 g samples of the partially humified surface horizon of a Fenland soil (in cultivation for 20 years) with a pH of 6.0 and containing 80 per cent organic matter. The extract yields, calculated as organic matter, were 7.5 mg, 132 mg, 440 mg, 24 mg, 32 mg, 456 mg and 31 mg, respectively, for the different systems. A yield of 3 g was obtained when 100 g of soil was hydrogen ion saturated prior to extraction with dimethylformamide. The organic matter in the sulphuric acid extract contained 29 per cent polysaccharide which could be separated from coloured ( $\lambda_{\max}$  325 m $\mu$ ) components of the mixture by gel filtration through 'Sephadex G-100' (ref. 3). All other organic materials extracted contained less than 11 per cent polysaccharide. It was not possible to separate the polysaccharides in these from the coloured components on 'Sephadex'. After treatment of the organic matter extracted by urea with 0.1 N hydrochloric acid for 48 h at 0°, it was possible to separate 17 per cent of the material, mainly polysaccharide, from the dark coloured components on 'Sephadex G-100'. It was concluded that dilute acid was necessary to liberate polysaccharide materials in appreciable amounts from the coloured constituents of soil organic matter. Dilute sulphuric acid was used in subsequent extractions.

The soil (5 kg, containing 60 per cent water) was extracted for 24 h at 3° with 0.6 N sulphuric acid (10 l.). The filtrate was neutralized with sodium bicarbonate to give a solution *A* and a precipitate *B*. Solution *A* was exhaustively dialysed against water, then freeze-dried to give a crude polysaccharide mixture, *A* (4 g). Precipitate *B*, dissolved in 0.3 N hydrochloric acid, was dialysed successively against 0.3 N hydrochloric acid and water, then freeze-dried to give a crude polysaccharide mixture *B* (3 g). Only the first sulphuric acid extract was used in our experiments, though it was shown that appreciable amounts of polysaccharides were present in the sixth acid extract from the same soil sample. Separate frac-

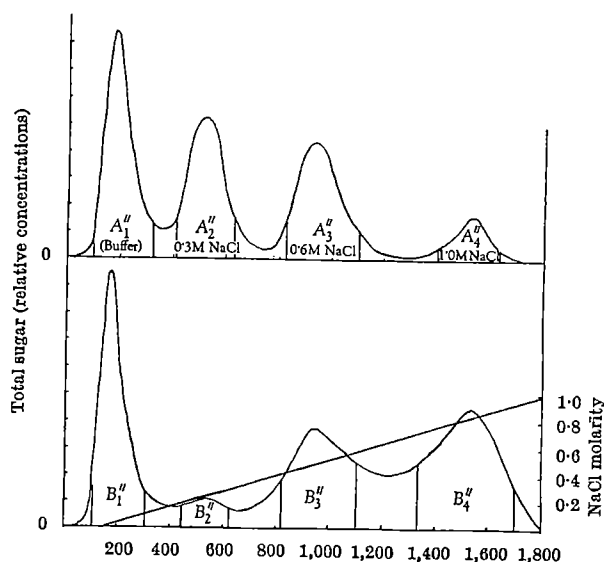


Fig. 1. Separation, by stepwise elution (*A''* fractions) and by gradient elution (*B''* fractions) with sodium chloride solutions, of soil polysaccharides on DEAE 'Sephadex A-50'

tions of aqueous solutions of *A* and *B* on 'Sephadex G-100' (5.5 cm × 100 cm) resolved colourless polysaccharide materials (*A'* 1 g and *B'* 0.6 g) in the 400–1,400 ml. eluate from the column from the coloured ( $\lambda_{\max}$  325 m $\mu$ ) lower molecular weight fractions (*A'* 2.6 g and *B'* 1.5 g) in the 1,400–2,300 ml. eluate. The investigations reported hereafter deal only with the high molecular weight polysaccharides *A''* (200 mg) and *B''* (150 mg) appearing in the 400–650 ml. eluates.

Free-boundary electrophoresis of saturated (0.7 per cent) solutions of *A''* in phosphate buffer (pH 7.0;  $\mu$  = 0.1) indicated the presence of one essentially neutral and of five negatively charged components. The relative descending mobilities were  $-0.14$ ,  $-0.52$ ,  $-1.05$ ,  $-1.46$ , and  $-2.11 \times 10^{-5}$  cm<sup>2</sup> volt<sup>-1</sup> sec<sup>-1</sup> at 0°. Stepwise elution (Fig. 1) of 75 ml. of 0.4 per cent *A''* solution from a 4 cm × 80 cm DEAE 'Sephadex A50' (Cl<sup>-</sup> form) column in phosphate buffer (pH 6.0;  $\mu$  = 0.05) with the buffer (350 ml.), 0.3 M sodium chloride (400 ml.), 0.6 M (500 ml.) and 1.0 M sodium chloride (550 ml.) gave, after dialysis and freeze-drying, polysaccharides *A''*<sub>1</sub> (80 mg), *A''*<sub>2</sub> (60 mg), *A''*<sub>3</sub> (120 mg) and *A''*<sub>4</sub> (40 mg). A previous experiment with a 0.2–2.0 M sodium chloride linear gradient had indicated that negatively charged polysaccharides could be removed with the sodium chloride concentrations selected. Electrophoretic examination of a saturated solution of *B''* (0.03 per cent at 4°) showed the presence of one major negatively charged component with a mean relative descending mobility of  $-1.45 \times 10^{-5}$  cm<sup>2</sup> V<sup>-1</sup> sec<sup>-1</sup> at 0° and also trace amounts of a fast moving component in the phosphate (pH 7.0;  $\mu$  = 0.1) buffer used. However (Fig. 1), elution at room temperature of 60 ml. of a 0.17 per cent solution of *B''* in phosphate buffer (pH 7.0;  $\mu$  = 0.05) with a linear gradient (0.1 M) of sodium chloride (1,800 ml. for a 3.4 × 65 cm column) gave, after dialysis and freeze-drying, fractions *B''*<sub>1</sub> (23 mg), *B''*<sub>2</sub> (6 mg), *B''*<sub>3</sub> (29 mg), and *B''*<sub>4</sub> (37 mg).

By means of paper chromatography glucose, galactose, mannose, xylose, arabinose, rhamnose, fucose and glucuronic acid were detected in the hydrolysates (1 N sulphuric acid for 6 h at 100°) of *A''* and *B''*. The molar ratios (rhamnose = 1) of sugars in fractions *A''*<sub>1</sub>, *A''*<sub>2</sub>, *A''*<sub>3</sub> and *A''*<sub>4</sub>, determined by microcolorimetric methods, are presented in Table 1.

Amino<sup>3</sup> and keto<sup>4</sup> sugars were not detected. The equivalent weights of the fractions, as determined by titration with 0.02 N sodium hydroxide of the polysaccharide fractions passed through 'Amberlite IRC-50



Table 1. RATIO OF SUGARS (RHAMNOSE=1) IN POLYSACCHARIDES  $A'_1$ ,  $A'_2$ ,  $A'_3$ , AND  $A'_4$ , FRACTIONATED ON DEAE 'SEPHADEX 4-50'

Sugar	Ratio in			
	$A'_1$	$A'_2$	$A'_3$	$A'_4$
Pentose <sup>a</sup> (expressed as xylose)	0.27	0.6	0.96	0.76
Glucose <sup>a</sup>	2.6	0.9	—	0.23
Galactose <sup>a</sup>	1.25	—	1.87	0.07
Mannose <sup>a</sup>	4.5	3.6	3.24	2.96
6-Deoxyhexose <sup>a</sup> (expressed as rhamnose)	1.0	1.0	1.0	1.0
Glucuronic <sup>a</sup>	0.11	0.32	1.67	1.13

(H)' resin, were 17,100, 1,860, 1,220 and 600 for  $A'_1$ ,  $A'_2$ ,  $A'_3$  and  $A'_4$ , respectively. Comparison of the uronic acid content and equivalent weight data for  $A'_4$  indicates that uronic acid was only a minor constituent of the total acid in this fraction.

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### Factors influencing the Formation of Some Polysaccharide-halogen Complexes

THE 'amyloid reaction' frequently used in the staining and identification of plant materials<sup>1</sup> has been modified by Gaillard<sup>2</sup> as a test to distinguish between linear and branched polysaccharides. Linear polysaccharides are precipitated from 1 per cent solution in 30 per cent aqueous calcium chloride by the addition of aqueous potassium iodide-iodine solutions, whereas branched polysaccharides are not. An examination of this reaction has been carried out to investigate its possible use in wood chemistry.

Various linear polysaccharides such as  $\alpha$ -cellulose, salep mannan, amylose, glucomannan from red alder<sup>3</sup> and ivory nut mannan gave dark blue or blue-black precipitates under the conditions of the test in agreement with Gaillard's results<sup>2</sup>. Pectinic and pectic acids did not form precipitates when iodine was added.

Extensive branching in polysaccharides prevented the formation of iodine complexes in agreement with Gaillard's results<sup>2</sup>. Examples of polysaccharides with filiform branches behaving in this manner include amylopectin, arabinogalactan from western larch, cherry gum and gum arabic. Polysaccharides with many short terminal branches such as guar gum and locust bean gum behaved in a similar manner. Galactoglucomannans from coniferous woods possessing terminal galactose branches similar to those of guar and containing galactose, glucose and mannose in molar ratios of 1 : 1 : 3 (refs. 4 and 5) to 0.5 : 1 : 3 (ref. 5), did not form precipitates, but coniferous galactoglucomannans containing molar ratios of 0.3 : 1 : 3 to 0.1 : 1 : 3 to 0.03 : 1 : 3 (refs. 4 and 6) formed heavy blue-brown precipitates, the browning cast of which was proportional to the amount of terminal galactose branches. Mixed solutions of galactoglucomannan (0.04 : 1 : 3) and guar gum in 30 per cent calcium chloride solution

having an average ratio of galactose, glucose, and mannose of 1 : 1 : 3 gave dark blue precipitates with iodine which were characteristic of the slightly branched component. Other polysaccharides with short terminal branches such as 4-O-methylglucuronoxylans from deciduous woods gave blue-black precipitates under the test conditions. Although softwood 4-O-methylglucuronarabinoxylans gave a doubtful test under these conditions, a 4-O-methylglucoarabinoxylan gave a dark blue precipitate with iodine.

A cellodextrin of low but undetermined molecular weight formed a precipitate under the test conditions, but none of the glucose-containing oligosaccharides from cellobiose up to cellohexaose gave precipitates. Decreasing the molecular weight of linear ivory nut mannan resulted in less complete complex formation. Xylobiose and xylotriose gave light brown, xylotetraose darker brown, and a xylodextrin mixture gave dark purple precipitates when iodine was added to their solutions in 30 per cent calcium chloride.

An examination of the effects of other multivalent salt solvents on the formation of iodine complexes showed that coniferous glucomannan (0.1 : 1 : 3) could not be precipitated from aqueous solutions of iron, copper, nickel or cobalt chlorides. This polymer could be precipitated from solutions in saturated or 30 per cent aqueous aluminium chloride, magnesium chloride, and barium chloride.

Precipitates of an iodine complex could be obtained when 45 per cent zinc chloride was used as a solvent for  $\alpha$ -cellulose, amylose, and ivory nut mannan. Hardwood glucomannans and salep mannan were not precipitated from this solvent when solutions of iodine were added, in contrast to the result in 30 per cent calcium chloride. Quantitative tests using 45 per cent zinc chloride as a solvent showed that glucans and galactose-deficient glucomannans were preferentially precipitated from crude coniferous galactoglucomannan preparations<sup>6</sup> when iodine was added.

The addition of bromine to a solution of elm 4-O-methylglucuronoxylan in 30 per cent calcium chloride resulted in the formation of a bright orange precipitate. Black spruce galactoglucomannan (0.1 : 1 : 3) gave a less well-defined test with bromine, while coniferous galactoglucomannan (1 : 1 : 3), gum arabic, 4-O-methylglucuronarabinoxylan, and 4-O-methylglucoarabinoxylan failed to form precipitates.

These results indicate that Gaillard's test<sup>2</sup> does not distinguish polysaccharides with a small number of single terminal branches from many linear polysaccharides. Polysaccharides with many filiform branches or with many terminal branches are not precipitated. Additional complications are introduced by the presence of carboxyl groups, the molecular weight of the polymer, and perhaps by the presence or absence of methylol groups on the anhydrosugar repeating units. The specificity of the reaction is influenced by the ionic nature of the solvent and by the nature of the halogen. In spite of its limitations, the test may be used under controlled circumstances to test for the presence of particular polysaccharides in mixtures, and if the hydrolytic and oxidative factors are eliminated or minimized, it may be used for preparative purposes.

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### Origin of Type-III Porphyrins in Nature

A RECENT article by Marks<sup>1</sup> has reviewed the present state of knowledge concerning porphyrin biogenesis. A part of the pathway which has stimulated much speculation is the step involving the condensation of porphobilinogen (I) to uroporphyrinogen III, IV (iii). A good deal of information is available on the enzymes concerned. Briefly, it seems that two enzymes are involved, uroporphyrinogen-I-synthetase and uroporphyrinogen-III-co-synthetase. The first, when incubated with porphobilinogen (I), produces uroporphyrinogen I, IV (i), while in the presence of the second enzyme, the product is uroporphyrinogen III, IV (iii). Uroporphyrinogen I is not a substrate for the co-synthetase. (Ref. 1 gives a comprehensive bibliography.) Most attempts to explain this unusual cyclization have started from the assumption that the enzymes would utilize a pathway which could be illustrated by chemical condensations *in vitro*—and all have been criticized on the ground that they produce a mixture of porphyrin isomers of uncertain composition, whereas the enzymatic condensations are much more specific.

An interesting variant was recently suggested by Mathewson and Corwin<sup>2</sup> in which they proposed that a tetrapyrane (III) (with type I order of side-chains) was the key intermediate. The structure might fold in several ways to give, in different circumstances, uroporphyrinogen I or III or the vitamin B<sub>12</sub> chromophore. The weakness of this proposal is that it requires at least two of the pyrrole rings in (III) to be in the  $\alpha$ -pyrrolenine form, in order that the chain is sufficiently flexible. There is no evidence that pyrroles with the type of sub-

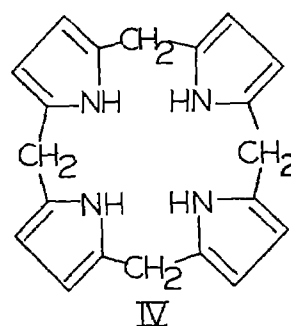
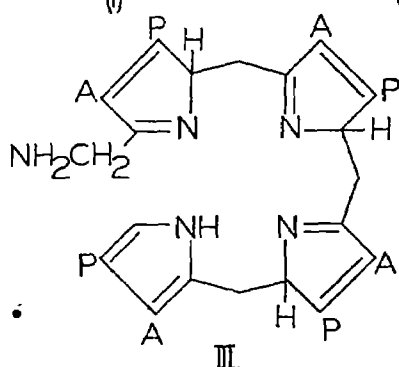
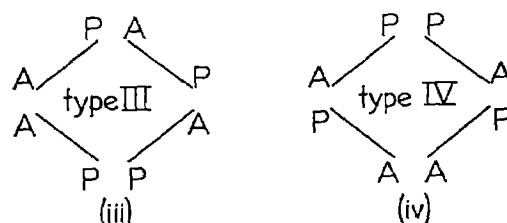
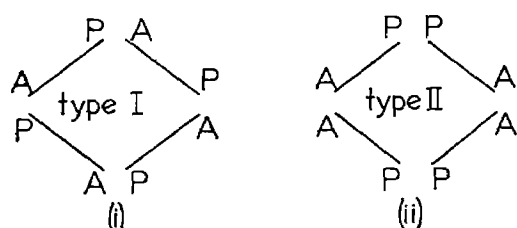
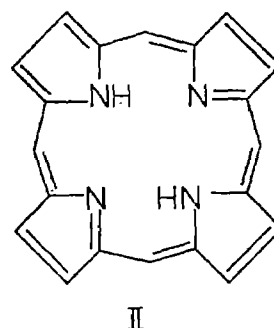
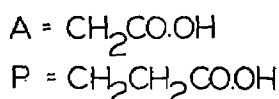
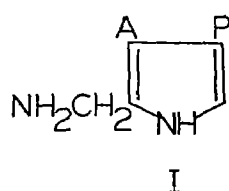
stitution required will exist in this form unless the nitrogen atom is protonated. The great merit of the proposal is that it focuses attention on the possible importance of stereochemistry in the cyclization.

Although the porphyrinogens are the important natural compounds, it is instructive to consider the symmetry of the porphyrins first.

The porphyrin ring is flat and hence all porphyrins have symmetry elements associated with the plane containing the macrocyclic system. However, the uroporphyrins, with the exception of type III, also have other symmetry properties. For example: type I, II (i) has a four-fold axis perpendicular to the plane of the ring, through the centre; type II, II (ii) has two planes of symmetry and a two-fold axis; type IV, II (iv) has one plane of symmetry. Thus, with the exception of the plane containing the porphyrin ring, uroporphyrin III, II (iii) is asymmetric and is the only uroporphyrin isomer with this property.

In the porphyrinogens, the bridging atoms each carry two hydrogens and the five-membered heterocycles are true pyrrole rings (IV). Consideration of models (Dreiding conformational models serve very well for this purpose) suggests that porphyrinogen rings are 'puckered'. Clearly, it should be possible to write the uroporphyrinogens types I, II and IV in forms which will retain all or some of the elements of symmetry outlined above for the corresponding porphyrins (though steric effects may place limitations on this in practice). For uroporphyrinogen III, however, there is no remaining element of symmetry.

Let us now arrange the molecules so that there is a minimum of interaction between the bridge CH<sub>2</sub> groups and the  $\beta$ -substituents. Assuming that the 'acetic' side-chain is the more bulky it is possible to arrange the mode



in such a way that the CH bonds of the meso-methylenes are staggered relative to the acetic side-chains. Thus we have a preferred conformation for the compound. The result is precisely similar if the 'propionic' side-chain is used; the only assumption necessary is that the two groups are not equally effective.

The simplest way to describe the conformation is to define the positions of the NH hydrogens as either 'up' or 'down' relative to the average plane of the macrocycle. Thus: (1) type I has two NH groups 'up' and two 'down' in a staggered sequence around the macrocycle; (2) type II has all four NH groups 'up' (or 'down'); (3) type IV has two NH groups 'up' on adjacent pyrrole rings and the other pair 'down'; (4) type III has three NH groups 'up' and one 'down'.

The purpose of this communication, therefore, is to suggest that uroporphyrinogen-III-co-synthetase has an asymmetric surface, perhaps with three necessary points of attachment, which can choose uroporphyrinogen III from a mixture.

It is now clear that chemical polymerization of monopyrroles (including porphobilinogen) to porphyrins generally produces mixtures of isomers. The composition of these mixtures has been the subject of some investigations<sup>3-5</sup> and the general conclusion is that acid-catalysed polymerization will produce mostly types III and IV and basic conditions will produce mostly type I (refs. 3, 4 and 6). A good deal has been written about the 'random' mixture of porphyrins produced from monopyrroles. This should give the isomers in the ratio I 1/8 : II 1/8 : III 1/2 : IV 1/4. It seems to me unlikely that such a random mixture could arise since the calculation of the ratio ignores the fact that the making and breaking of C—C bonds at the  $\alpha$ -positions of pyrroles is very dependent on the  $\beta$ -substituents. This is especially true in the series carrying the coproporphyrin side-chains ( $-\text{CH}_3$  and  $-\text{CH}_2\text{CH}_2\text{COOH}$  in the  $\beta$ -positions).

Thus, if uroporphyrinogen-I-synthetase, or part of its functional system, is a reversible deaminase enzyme (cf. ref. 7) which holds an equilibrium between porphobilinogen and a uroporphyrinogen mixture (plus ammonia in some form), then either the uroporphyrinogen I which has been prepared from this enzyme is not absolutely free of isomers or another part of the enzyme is concerned with removing uroporphyrinogen I from the mixture. The proportion of isomers in the uroporphyrinogen I need only be minute, provided that an equilibrium exists at the site of de-amination. Put in chemical terms, the de-amination is taking place under the equivalent of alkaline conditions.

To summarize, therefore, it is suggested that uroporphyrinogen-I-synthetase is a reversible de-amination enzyme which produces a mixture of isomers but with a very heavy preponderance of type I. The co-synthetase is a surface which picks out uroporphyrinogen III by its peculiar stereochemistry, thus forcing the equilibrium to give, finally, entirely the III isomer.

Several consequences of this hypothesis may be capable of experimental test.

(1) Uroporphyrinogen-I-synthetase may be a preparation with two distinct functions.

(2) The uroporphyrinogen I produced by this enzyme may not be absolutely homogeneous.

(3) The substrate for uroporphyrinogen-III-co-synthetase may be uroporphyrinogen-III itself and the enzyme may be capable of removing the isomer from a synthetic mixture.

(4) The stereochemical constraints postulated as governing the shape of porphyrinogen isomers must be large enough to be detected by chemical (or physical) techniques.

(5) The specificity of the enzymes after the formation of uroporphyrinogen III will decrease. The subsequent steps in the biogenesis of protoporphyrin IX all involve degradation of the side-chains which will then be less

effective in defining the conformation of the substrate. There is some evidence that this does occur, since all the uroporphyrinogens will form a coproporphyrin in the presence of the appropriate enzyme<sup>8</sup>. The specificity reappears with the enzyme responsible for the degradation of coproporphyrinogen III to protoporphyrin IX (ref. 9) and this may be the point at which the propionic side-chains take over the role of defining the conformation of the compound.

Attempts to determine the conformations of porphyrinogens have been initiated in this laboratory.

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### Crystal Structure of Potassium Diperoxotricyanochromate(IV)

IN the course of an extensive investigation of peroxochromates the crystal and molecular structure of potassium diperoxotricyanochromate(IV),  $\text{K}_3[\text{Cr}(\text{O}_2)_2(\text{CN})_3]$ , has been solved by three-dimensional Fourier methods and refined five cycles by the structure factor least-squares method using 2076 reflexions. The  $R$ -factor has dropped to 0.123.

The unit cell dimensions were determined from rotation and Weissenberg photographs using  $\text{CuK}\alpha$  radiation. The unit cell is monoclinic with  $a = 11.52 \text{ \AA}$ ;  $b = 7.60 \text{ \AA}$ ;  $c = 12.13 \text{ \AA}$ ;  $\beta = 111.0^\circ$ .

The measured density was  $2.15 \text{ g cm}^{-3}$ , indicating the presence of four molecules per unit cell. The systematically absent reflexions were  $h0l$  with  $h = 2n + 1$ . This is characteristic of the space groups  $P2_1/a$  and  $Pa$ . An intensity distribution curve revealed the presence of high symmetry and  $P2_1/a$  was therefore chosen. During the structure investigation it became obvious that a plausible structure could be obtained based on this assumption.

The structure investigation shows that chromium has co-ordination number seven and that the geometrical configuration of the ligands is a pentagonal bipyramid. The O—O bond distances are  $1.440 \pm 0.012 \text{ \AA}$  and  $1.455 \pm 0.012 \text{ \AA}$ , close to values found in  $[\text{Cr}(\text{O}_2)_2(\text{NH}_3)_3]$  (1).  $[\text{CrO}(\text{O}_2)_2\text{py}]$  (2–4) and in  $\text{K}_3[\text{Cr}(\text{O}_2)_4]$  (5). The four Cr—O bond lengths range from  $1.871 - 1.902 \text{ \AA}$  (e.s.d.  $0.009 \text{ \AA}$ ), and the three Cr—C bond lengths from  $2.081 - 2.102$  (e.s.d.  $0.012 \text{ \AA}$ ). The mean value of the carbon–nitrogen bond distances is  $1.137 \text{ \AA}$  (e.s.d.  $0.018 \text{ \AA}$ ) ( $1.15 \text{ \AA}$  is usually found in cyanide groups).

The complex ion  $[\text{Cr}(\text{O}_2)_2(\text{CN})_3]^{3-}$ , therefore, is built in the same fashion as  $[\text{Cr}(\text{O}_2)_2(\text{NH}_3)_3]$  (1,6) with the cyanide groups replacing the ammonia groups.

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### Electrical Resistivities and the Type of Bonding in Some Quinone Complexes

MOST molecular complexes are essentially of the non-bonding type and their infra-red spectra are quite similar to the superposed spectra of the components<sup>1,2</sup>. However, the infra-red spectra of some diamine-quinone complexes and azine-quinone complexes have been found to show the pattern of the semiquinone ion instead of the quinone. Such complexes have been characterized as essentially of the dative type. Thus charge transfer molecular complexes may be classified into two groups, namely, the non-bonding type and the dative type, based on their infra-red spectra. Using this method of classification, I have pointed out that most of the known complexes having resistivities of  $10^6$  ohm cm or less are of the dative type<sup>3</sup>.

In addition to those reported in ref. 3 there are many complexes which are semiconducting or which are likely to be semiconducting but for which only the electrical resistivity or the infra-red spectrum has been reported. Hoping to find a relation between the electrical resistivity and the type of bonding in these complexes, I have measured the necessary properties. Nearly all of the known and some new complexes of *p*-chloranil, *p*-bromanil, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone and its bromo analogue exhibiting resistivities of  $10^{11}$  ohm cm or less are included in the present work. These quinones and their semiquinone ions show infra-red spectra sufficiently different from each other for characterization of the complexes<sup>3</sup>.

Measurements of the electrical resistivities were made on compressed samples. The infra-red spectra were recorded as mineral oil mulls using a Perkin-Elmer Infracord spectrophotometer. The results are summarized in Table 1. The complexes are presented in the order of increasing resistivity. If two or more different resistivity values are available for the same complex, the lowest value is taken for this purpose. The quinones are indicated by the kind and number of the substituents: for example, Cl<sub>4</sub> for *p*-chloranil and Cl<sub>2</sub>Cy<sub>2</sub> for 2,3-dichloro-5,6-dicyano-*p*-benzoquinone. The mole ratio of acceptor to donor is given only when the ratio is known to be different from 1:1. Most of the others are of 1:1 composition, but some are of unknown composition. The infra-red spectra are classified by the pattern due to the acceptor component: Q indicates the pattern of quinone molecule and SQ indicates the pattern of semiquinone ion. In none of the cases examined was the co-existence of the quinone and the semi-quinone ion observed.

As reported before, the anomalously composed dibenzo [c,d] phenothiazine complexes, Nos. 1 and 3, are much more conducting than the corresponding 1:1 complexes, Nos. 7 and 29. All of them are of the dative type<sup>4</sup>.

It is rather surprising to find that the highly conducting 1,6-diaminopyrene complexes, Nos. 4 and 5, are of the non-bonding type. Kronick *et al.* have reported that the spectra of these two complexes in the visible region are characteristic of a large radical-ion component. Comparing the observed spectra with the computed spectra for mixtures of free components and radical ions, they estimated the mole fraction of ionic component for the complexes as 0.65 with *p*-bromanil and 0.90 with *p*-chloranil. It was concluded that the

Table 1. ELECTRICAL RESISTIVITIES AND INFRA-RED SPECTRA OF SOME QUINONE COMPLEXES

No.	ohm-cm	(ref.)	Quinone	Donor (A : D)	Infra-red	(ref.)
1	$10^1$	(4)	Cl <sub>4</sub> Cy <sub>2</sub>	Dibenzo [c,d] phenothiazine (1:2)	SQ	(4)
2	$10^2$	(5)	Br <sub>2</sub> Cy <sub>2</sub>	1,6-Diaminopyrene	SQ	
3	"	(4)	Br <sub>2</sub> Cy <sub>2</sub>	Dibenzo [c,d] phenothiazine (2:3)	SQ	(4)
4	$10^3$	(5,8)	Cl <sub>4</sub>	1,6-Diaminopyrene	Q	
5	"	(5,6)	Br <sub>2</sub>	"	Q	
6	"	"	Cl <sub>4</sub> Cy <sub>2</sub>	Benzo [c] phenothiazine	SQ	
7	"	(4)	"	Dibenzo [c,d] phenothiazine	SQ	(3)
8	"	"	"	Dibenzo [c,d] phenoselenazine	SQ	(3)
9	$10^4$	(7)	Cl <sub>4</sub>	Tetramethyl- <i>p</i> -phenylenediamine	SQ	(3)
10	"	(8)	"	Durenediamine	SQ	(3)
11	"	"	Br <sub>2</sub>	"	SQ	(3)
12	"	"	Cl <sub>4</sub> Cy <sub>2</sub>	Phenothiazine	SQ	(3)
13	"	"	"	Phenoselenazine	SQ	(3)
14	"	"	Br <sub>2</sub> Cy <sub>2</sub>	Phenothiazine	SQ	(3)
15	"	"	"	Benzo [c] phenothiazine	SQ	(3)
16	"	"	"	Dibenzo [c,d] phenoselenazine	SQ	(3)
17	$10^5$	(7)	Br <sub>2</sub>	Tetramethyl- <i>p</i> -phenylenediamine	SQ	(3)
18	"	"	Cl <sub>2</sub> Cy <sub>2</sub>	<i>N</i> -Methylphenothiazine	SQ	(3)
19	"	"	Br <sub>2</sub> Cy <sub>2</sub>	"	SQ	(3)
20	"	"	"	Phenoselenazine	SQ	(3)
21	$10^6$	(10)	Cl <sub>4</sub>	<i>p</i> -Phenylenediamine	SQ	(3)
22	"	(9)	Cl <sub>2</sub> Cy <sub>2</sub>	"	SQ?	(9)
23	"	(9)	"	Perylene	Q	(9)
24	$10^7$	(10)	Cl <sub>4</sub>	Tetramethylbenzidine	Q	
25	$10^8$	(7)	"	Dimethylaniline	Q	(1)
26	"	(6)	"	Perylene	Q	(2)
27	"	"	Br <sub>2</sub>	Tetramethylbenzidine	Q	
28	"	"	Br <sub>2</sub> Cy <sub>2</sub>	Perylene	Q	
29	"	(4)	"	Dibenzo [c,d] phenothiazine	SQ	(3)
30	$10^9$	(7)	Br <sub>2</sub>	Dimethylaniline	Q	(1)
31	$10^{10}$	(10)	Cl <sub>4</sub>	1,5-Diaminonaphthalene	Q	
32	"	(6)	Br <sub>2</sub>	<i>p</i> -Phenylenediamine	SQ	(3)
33	"	"	Cl <sub>2</sub> Cy <sub>2</sub>	Pyrene	Q	(9)
34	$10^{11}$	(6)	Cl <sub>4</sub>	"	Q	
35	"	(6)	"	Hexamethylbenzene	Q	(1)
36	"	(6)	"	1,8-Diaminonaphthalene	Q	
37	"	(6)	"	<i>p</i> -Anisidine	Q	
38	"	"	"	Diethoxydinaphthostilbene (1:2)	Q	
39	"	"	Br <sub>2</sub>	Perylene	Q	(2)
40	"	"	Br <sub>2</sub> Cy <sub>2</sub>	Pyrene	Q	

presence of large amounts of the oxidized and reduced forms of amine-quinone complexes appears to correlate with high conductivity<sup>5</sup>. The present characterization by infra-red spectra, however, does not support such a conclusion. Contrary to the high fractions of ionic component estimated by Kronick, these 1:1 complexes are of the non-bonding type. For example, the spectrum of the *p*-chloranil complex given in Fig. 1 is close to the superposed spectra of the neutral components.

Kronick *et al.* have reported that the diaminopyrene-dichlorodicyano-*p*-benzoquinone complex, No. 2, is about

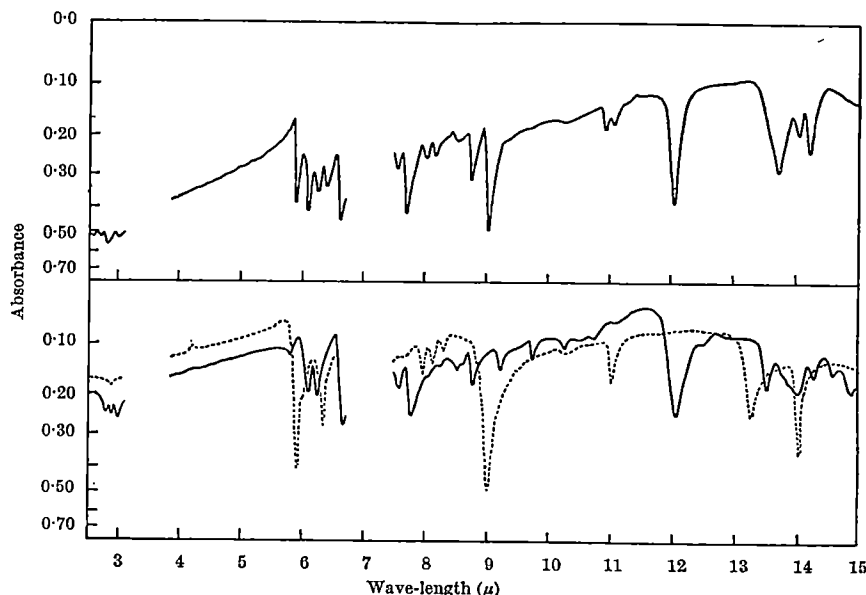


Fig. 1. Infra-red spectra of the 1,6-diaminopyrene-*p*-chloranil complex (top) and the component compounds (bottom), 1,6-diaminopyrene (—) and *p*-chloranil (-----)

95 per cent ionic. The infra-red spectrum indicates the dative nature of the bonding and is consistent with their conclusion.

In the case of the other diamine complexes, *p*-phenylene-diamine and its derivatives are known to form complexes of the dative type<sup>3</sup>; however, tetramethylbenzidine and 1,5- and 1,8-diaminonaphthalenes were found to give complexes of the non-bonding type, Nos. 24, 27, 31 and 37.

Most of the complexes with resistivities of 10<sup>7</sup> ohm cm or higher were found to be of the non-bonding type; there are two exceptions, namely, Nos. 29 and 32. Therefore, we must conclude that bonding of the dative type is neither necessary (Nos. 4 and 5), nor enough (Nos. 29 and 32), for the appearance of low resistivity in molecular complexes.

It is apparent that intermolecular interaction extended, at least one-dimensionally, throughout the crystal is required for transport of electrons or holes. In our semi-conducting molecular complexes such interaction may be provided by a strong charge ( $\pi$ -electron) transfer force. As mentioned above, the resulting complex does not need to be dative. Although the infra-red spectrum appears to be useful and reliable in classifying the type of bonding in molecular complexes, the spectrum does not give any information on how strong the interaction is. Consequently it may be possible that the charge transfer interaction in some complexes essentially of the non-bonding type is stronger than that in some complexes essentially of the dative type. Moreover, we do not obtain by the infra-red spectrum information as to whether the interaction is limited to within a pair of donor and acceptor molecules or extended farther. Perhaps, in the complexes of the dative type but with relatively high resistivities the interaction between donor and acceptor molecules may be only local or ionic rather than dative.

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## Evaluation of Diffusion Coefficients in Free-boundary Diffusion Experiments

IN free-boundary diffusion experiments a boundary between two solutions of different concentrations is formed in a cylindrical cell and the evolution with time of the concentration distribution is examined. For the evaluation of the diffusion coefficient from the experimental data several methods are available, for example, the moment method and the area method. In these methods the existence of an initially sharp boundary between the two solutions is in general presumed. However, it will be shown that the moment method is valid for very general boundary conditions, a fact that seems to have been overlooked in the past.

The second moment  $m_2$  of the normalized concentration distribution is defined by the equation:

$$m_2 = \frac{1}{\Delta c} \int_{-\infty}^{\infty} x^2 \frac{dc}{dx} dx \quad (1)$$

The time derivative of  $m_2$  may be written:

$$\frac{dm_2}{dt} = \frac{1}{\Delta c} \frac{d}{dt} \int_{-\infty}^{\infty} x^2 \frac{dc}{dx} dx = \frac{1}{\Delta c} \int_{-\infty}^{\infty} x^2 \frac{\partial}{\partial x} \left( \frac{\partial c}{\partial t} \right) dx \quad (2)$$

where it is assumed that the last integral is uniformly convergent and that:

$$\frac{\partial^2 c}{\partial t \partial x} = \frac{\partial^2 c}{\partial x \partial t} \quad (3)$$

From equation (2) and the diffusion equation (4) (in which  $D$  may be a function of the concentration):

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x} \left( D \frac{\partial c}{\partial x} \right) \quad (4)$$

one obtains on partial integration:

$$\frac{dm_2}{dt} = \frac{1}{\Delta c} \int_{-\infty}^{\infty} x^2 \frac{\partial^2}{\partial x^2} \left( D \frac{\partial c}{\partial x} \right) dx = \frac{1}{\Delta c} \left\{ \int_{-\infty}^{\infty} x^2 \frac{\partial}{\partial x} \left( D \frac{\partial c}{\partial x} \right) - 2 \int_{-\infty}^{\infty} x D \frac{\partial c}{\partial x} + 2 \int_{-\infty}^{\infty} D \frac{\partial c}{\partial x} dx \right\} \quad (5)$$

For well-behaved functions the integrated parts in (5) vanish and one obtains:

$$\frac{dm_2}{dt} = \frac{2}{\Delta c} \int_{-\infty}^{\infty} D \frac{\partial c}{\partial x} dx = 2\bar{D} \quad (6)$$

where  $\bar{D}$  is the mean diffusion coefficient. Integration of (6) gives finally:

$$\bar{D} = \frac{m_2(t) - m_2(0)}{2t} \quad (7)$$

Thus, the moment method is valid for an initial concentration distribution satisfying the very general conditions mentioned above (equation (3) and conditions for integration in (2) and (5)) and, in the case of concentration dependent diffusion, yields the mean value<sup>1,2</sup> of the diffusion coefficient over the concentration interval investigated.

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## Cleavage of *o*-Nitrophenylsulphenamides by Raney Nickel and Applications for Peptide Synthesis\*

THE *o*-nitrophenylsulphenyl (NPS) residue has recently been introduced as an amine-protecting group for amino-acids and peptides during peptide synthesis by Zervas *et al.*<sup>1</sup> It is readily cleaved by mild acidolysis using 2-3 equivalents of hydrochloric acid in ether. Although this treatment allows the selective cleavage of the *o*-nitrophenylsulphenamide group in the presence of acid-labile *tert*-butylesters in the peptide moiety under carefully controlled conditions<sup>2</sup> another cleavage procedure avoiding the use of acid altogether might prove useful.

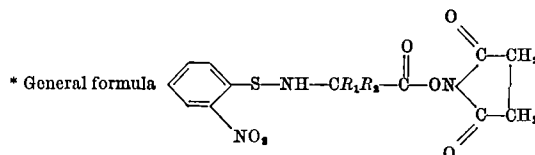
It has now been found that desulphuration by rancy nickel<sup>3</sup> provides a convenient procedure for the selective

\* Forty-second paper concerning peptides; compare with Melnikoff, J., *et al.*, *Z. Naturforsch.*, **18**, b. 1120 (1963). Abbreviations according to "Peptides", *Proc. Fifth European Peptide Symp.*, edit. by Young, G. T., 259 (Pergamon Press: Oxford, London, New York, Paris, 1963).



Table 1. *N*-HYDROXYSUCCINIMIDE ESTERS OF *o*-NITROPHENYLSULPHENYL-AMINO-ACIDS\*

Ester of	Recrystallization solvent	Yield (%)	M.p. (°C)	[α] <sub>D</sub> <sup>25</sup> c 1, dioxane	Formula	Mol. wt.	Anal. calc. (%)				Anal. found (%)			
							C	H	N	S	C	H	N	S
NPS-L-alanine	Ethylacetate/hexane	82	152-153	-102.5°	C <sub>13</sub> H <sub>13</sub> N <sub>2</sub> O <sub>6</sub> S	339.3	46.0	3.86	12.4	9.45	46.1	4.03	12.4	9.45
NPS-glycine	Dimethylformamide/water	59	192-194	—	C <sub>12</sub> H <sub>11</sub> N <sub>2</sub> O <sub>6</sub> S	325.3	44.3	3.41	12.9	9.86	44.4	3.55	12.8	10.0
NPS-L-proline	Ethylacetate/hexane	82	192-193	-42.0°	C <sub>15</sub> H <sub>15</sub> N <sub>3</sub> O <sub>6</sub> S	365.3	49.3	4.14	11.5	8.77	49.3	4.29	11.3	8.83
NPS-L-valine	Ethylacetate/hexane	75	138-139	-125.0°	C <sub>15</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S	367.4	49.0	4.66	11.4	8.73	49.1	4.78	11.4	8.64



Preparation from NPS-amino-acids and *N*-hydroxysuccinimide using dicyclohexylcarbodiimide.

removal of the *N*-*o*-nitrophenylsulphenyl group from peptides. The cleavage of the sulphenamide is easily accomplished by passing a solution of an NPS-peptide derivative in an organic solvent through a raney nickel column at room temperature. Any danger of an unwanted partial cleavage of the very useful acid labile *N*-*tert*-butoxycarbonyl<sup>4</sup>, *tert*-butylester<sup>5</sup>, *tert*-butylether<sup>6</sup>, or amine-protecting groups from β-dicarbonyl derivatives<sup>7</sup> is thus avoided. Other common protecting groups are also left intact, for example, alkylesters, *N*-toluenesulphonyl, *N*-triphenylmethyl, *N*-trifluoroacetyl, and *N*-formyl groups. (The benzyloxycarbonyl (Z) group is cleaved (reductively) by passing solutions of Z-peptides in similar manner through raney nickel columns.) For the preparation of NPS-peptide derivatives and for the stepwise elongation of the peptide chain *N*-hydroxysuccinimide esters<sup>8</sup> of NPS-amino-acids have been found to be useful reagents which on addition to eluates of raney nickel columns give the corresponding homologue NPS-peptides in high yields within a few hours at room temperature. Fig. 1 shows schematically the synthesis of NPS-Gly-Pro-Ala-Thr-OMe. In similar manner NPS-Gly-Pro-Ala-Thr(Bu<sup>t</sup>)-OBu<sup>t</sup>, NPS-Pro-Ala-Ser(Bu<sup>t</sup>)-OBu<sup>t</sup>, and Z-Leu-Val-Glu(OBu<sup>t</sup>)-Ala-N<sub>2</sub>H<sub>2</sub>BOC have been synthesized. Table 1 presents data of *o*-nitrophenylsulphenyl-amino-acid-*N*-hydroxysuccinimide esters. Two typical examples serve to outline the experimental details.

Example *a*: *o*-Nitrophenylsulphenyl-L-prolyl-L-alanyl-L-threonine methylester (1.14 g = 2.5 m.moles): [f.p. 159°-160°, [α]<sub>D</sub><sup>25</sup> = -105.0° (c 1, ethanol)] was dissolved in dimethylformamide (5 ml.) and placed on a raney nickel *W* 5 (ref. 10) column (2 cm × 5 cm) which had previously been washed with the same solvent. When the yellow solution was absorbed the column was washed with dimethylformamide (approximately 100 ml.) at a flow rate of about 30 ml./h until the colourless effluent was ninhydrin negative. (Raney nickel preparations which tended to pack too tightly in the column due to particle size were mixed with 10-30 per cent 'Hyflo Super Cell' in order to allow a reasonable flow rate.)

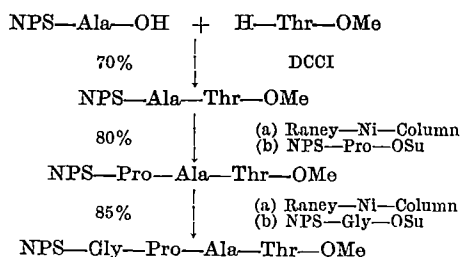


Fig. 1. Synthesis of sequence 153-158 of tobacco mosaic virus (vulgare) protein<sup>9</sup>. (NPS, *o*-nitrophenylsulphenyl; OSu, *N*-hydroxysuccinimide ester, DCCI, dicyclohexylcarbodiimide)

The eluate contained L-prolyl-L-alanyl-L-threonine methylester in paper chromatographically pure form (*R<sub>F</sub>* 0.35 in *sec*-butanol/formic acid/water, 75:13.5:11.5). *o*-Nitrophenylsulphenyl-glycine-*N*-hydroxy-succinimide ester (0.82 g = 2.5 m.moles) was added. After standing for 4 h at 20° C the solvent was removed *in vacuo*, the residue reprecipitated from ethanol/water (5:100 ml.), filtered off and washed successively with 1 M citric acid, 1 M sodium hydrogen carbonate and water. On drying 1.25 g (98 per cent) crystalline crude material were obtained. Recrystallization from ethanol/water gave 1.09 g (85 per cent) *o*-nitrophenylsulphenylglycyl-L-prolyl-L-alanyl-L-threonine methylester, f.p. 193°-195°, [α]<sub>D</sub><sup>25</sup> = -100.0° (c 1, ethanol), C<sub>21</sub>H<sub>20</sub>N<sub>2</sub>O<sub>8</sub>S (511.5); calc. C 49.3, H 5.71, N 13.7, S 6.28; found C 49.2, H 5.79, N 13.7, S 6.28.

Example *b* (essentially the same procedure was used as in example *a*): *o*-Nitrophenylsulphenyl-L-alanyl-*O*-*tert*-butyl-L-threonine-*tert*-butylester (6.0 g = 13.2 m.moles) [f.p. 110°-112°, [α]<sub>D</sub><sup>25</sup> = -35.0° (c 1, ethanol)] in 25 ml. dimethylformamide. Raney nickel column size: 5.5 cm. × 6.0 cm. Elution with approximately 1,000 ml. dimethylformamide. Reaction with *o*-nitrophenylsulphenyl-L-prolin-*N*-hydroxysuccinimide ester (4.8 g = 13.2 m.moles). 7.2 g (98.5 per cent) crude product, f.p. 142°-144°. Recrystallization from ether/hexane gave 6.86 g (94 per cent) *o*-nitrophenylsulphenyl-L-prolyl-L-alanyl-*O*-*tert*-butyl-L-threonine-*tert*-butylester, f.p. 145°-147°, [α]<sub>D</sub><sup>25</sup> = -83.8° (c 1, ethanol), C<sub>26</sub>H<sub>40</sub>N<sub>4</sub>O<sub>8</sub>S (552.7); calc. C 56.5, H 7.30, N 10.1, S 5.80; found, C 56.5, H 7.21, N 10.3, S 5.73. When NPS-Ala-Thr(Bu<sup>t</sup>)-OBu<sup>t</sup> was cleaved by three equivalents of hydrochloric acid in ether<sup>1</sup> not more than 60 per cent H-Ala-Thr(Bu<sup>t</sup>)-OBu<sup>t</sup> could be isolated (due to its solubility in most solvents).

With the procedure described many peptides can be prepared in a simple manner, except those with sulphur-containing amino-acids (Cys, Met). The reaction times are short (a few hours), the isolation of the products is easy and the yields are good. The complete removal of the *o*-nitrophenylsulphenyl group is easily visible from a colourless effluent of the raney nickel column. The quantitative elution of the peptide derivative requires relatively large volumes of solvent, which is a slight disadvantage. For the preparation of small peptides the raney nickel procedure seems to offer advantages over the acidolytic cleavage of the NPS-group in (a) giving higher yields and (b) providing an additional selective procedure that leaves intact other acid labile groups present in the peptide moiety. In several instances the procedure proved to be even superior to hydrogenation of the corresponding benzyloxycarbonyl-peptides in giving higher yields. Comparison of two Z-Pro-Ala-Thr-OMe preparations, one synthesized by the procedure described in this communication using NPS-Ala-Thr-OMe and Z-Pro-OSu<sup>8</sup> and the other starting from Z-Ala-Thr-OMe which was catalytically hydrogenated, gave identical melting points and optical rotations.

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## BIOCHEMISTRY

### Radioisotope Investigation of the Effect of Sunlight on Beer

THE formation of mercaptan on exposing beer to sunlight has been recognized for many years. It has also been shown that the mercaptan develops parallel with the occurrence of the sunlight flavour<sup>1,2</sup>. In the early report from this laboratory, according to the results of various model experiments with humulone and lupulone analogues, it was suggested that the flavour substance is a kind of mercaptan, especially prenyl mercaptan (3-methyl-2-butenyl-1-thiol)<sup>3,4</sup>. Kuroiwa *et al.*<sup>5</sup> have detected 3-methyl 2-butenyl mercaptan in 'sunstruck' beer as its 2,4-dinitrophenyl derivative by a chromatostrip method, and proposed isohumulones as the sole precursor of this mercaptan because when isohumulones in the model solution were replaced by humulones, lupulones and other related compounds, no 'sunlight' flavour could be detected.

The evaluation of sunlight flavour of beer has long been carried out only by organoleptic test. A potentiometric method has recently been proposed by Kuroiwa *et al.*<sup>5</sup>. However, the sensitivity of this method was limited by contaminant materials that were driven out from beer and consumed silver ions. When an air-injected beer bottle was exposed to the sunlight, Kuroiwa *et al.* observed that the amount of substances consuming silver ions increased markedly, but the odour was entirely different from the so-called 'sunstruck' beer.

The experiments reported here were initiated as an attempt to determine the precursors of mercaptan with the aid of compounds labelled with sulphur-35. It has now been shown that sodium sulphide is more active than cysteine in serving as the sulphur source of mercaptan.

The radioactive sulphur compounds were purchased from the Radiochemical Centre, Amersham, England. The humulones used were prepared by Mr. Haruo Tanaka. Lupulones, acetolupuphenone synthesized by Riedl's method<sup>6</sup>, and isohumulones prepared according to the procedure of Carson<sup>7</sup> were gifts of Dr. Mitsuo Koshika. L-Cysteine-<sup>35</sup>S was prepared by the reduction of L-cysteine-<sup>35</sup>S with tin-hydrochloric acid. 3-Methyl 2-butenyl mercaptan (b.p. 125°-127°) was synthesized from 3-methyl 2-butenyl bromide and thiourea<sup>8</sup>.

To 20 mg of humulones dissolved in 7 ml. ethyl alcohol was added 180 ml. of water saturated with carbon dioxide at 0° C and a solution of the sulphur-35 compound (0.01

Table 1. EFFECT OF COMPOUNDS ON THE INCORPORATION OF SULPHUR-35 INTO MERCAPTAN

Expt.	Compounds tested	Sulphur source	Total radioactivity in 2,4-dinitrophenyl deriv. (c.p.m.)
1	Humulones	Na <sub>2</sub> S	7,800
	Lupulones	Na <sub>2</sub> S	3,075
	Humulones	Cysteine	725
	Lupulones	Cysteine	425
2	Humulones	Na <sub>2</sub> S	6,625
	Lupulones	Na <sub>2</sub> S	1,825
	Isohumulones	Na <sub>2</sub> S	6,275
	Acetolupuphenone	Na <sub>2</sub> S	7,125

m.mole,  $1 \times 10^5$  c.p.m.). The mixture was bottled in colourless transparent bottles and exposed to direct sunlight. After the addition of 50 mg of prenyl mercaptan dissolved in 10 ml. of ethyl alcohol, the solution was extracted continuously with diethyl ether. The extract was washed, dried and finally concentrated to a small volume. A mixture of sodium bicarbonate (500 mg), 2,4-dinitrofluorobenzene (100 mg), acetone (20 ml.), and water (20 ml.) was added to the concentrate and kept in dim light at room temperature for about 24 h. The derivatives were extracted with benzene, and the benzene layer was thoroughly washed with 2 N sodium hydroxide to remove humulones and other phenol compounds and also the 2,4-dinitrophenyl derivative of hydrogen sulphide. The benzene solution was washed with water, dried and concentrated to 25 ml. 1 ml. (about 3 mg of the derivatives) of this solution was used for the determination of radioactivity.

A comparison of sodium sulphide S-35 and cysteine S-35 as precursors is presented in Table 1. When compared with humulones, isohumulones or acetolupuphenone, lupulone is a poor precursor of mercaptan. To obtain high incorporation of radioactivity into mercaptan, saturation with carbon dioxide was necessary; its omission caused a 50 per cent decrease in incorporation. Addition of vitamin C resulted in failure to obtain significant increase of sulphur-35 incorporation into mercaptan. These results are summarized in Table 2.

Table 2. EFFECT OF CARBON DIOXIDE AND VITAMIN C ON INCORPORATION OF SULPHUR-35 INTO MERCAPTAN

Saturation with carbon dioxide	Vitamin C (10 mg/180 ml.)	Total radioactivity in 2,4-dinitrophenyl deriv. (c.p.m.)
+	-	10,975
-	-	5,375
-	+	6,850
+	+	11,525

Kuroiwa *et al.*<sup>5</sup> considered isohumulones as the sole precursor of mercaptan. However, our experimental results, shown in Table 1 (Experiment 2), are entirely different.

It seems likely that light induces the formation of the prenyl radical or of a free radical intermediate by the abstraction of hydrogen from the carbon neighbouring the double bond of the prenyl chain in the molecule of humulones. Prenyl mercaptan might be formed by the combination of the prenyl radical and thiol radical initiated from hydrogen sulphide, cysteine, or glutathione. Also, this mercaptan can be formed by the fission of a thiol compound, probably produced by the combination of a thiol radical and a humulone free radical as described here. This is also possible in the case of isohumulones, acetolupuphenone, and lupulones.

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### Action of Non-penetrating Heavy Metals on the Catalase Activity of Yeast Cells

THE catalase activity of intact yeast cells which is 'patent' (that is, active *in vivo* against external substrate) is biologically distinct from the activity which is 'cryptic' (that is, hidden within the cell, detectable only after lysis of the cell). This conclusion followed from the demonstration that the cryptic enzyme was far more heat-labile than the patent fraction<sup>1</sup>.

Yeast catalase is an inducible enzyme<sup>2,3</sup>, its inducer being either oxygen or possibly  $\text{H}_2\text{O}_2$  formed by endogenous reduction of oxygen. When fully induced originally aerobic yeast was grown in anaerobiosis, the cryptic activity of the cell diminished due to dilution; the specific patent activity remained constant for one or more generations, due to flow of enzyme from the cryptic to the patent fraction<sup>4</sup>.

In order to account for these observations, I proposed a hypothesis which in its general form assumed that the patent enzyme was fixed at sites in the cell membrane, with its active centres available to external substrate, and stable to heat by virtue of being membrane-bound<sup>1</sup>. In a more specific form, the membrane model assumed that an equilibrium existed between the membrane-bound patent fraction and the cryptic fraction; mass action determined the level of patent activity for any given internal concentration of enzyme<sup>4</sup>.

In a series of elegant investigations begun in 1948<sup>5</sup>, Rothstein and his colleagues have investigated the nature of the binding to intact yeast cells and the physiological effects of a variety of heavy metals<sup>6-8</sup>. Of particular interest was his demonstration that uranyl ion ( $\text{UO}_2^{2+}$ ) inhibited both the fermentation of exogenous (but not endogenous) glucose and the activity of invertase (which in yeast is membrane-bound<sup>9,10</sup>) by binding to ligands in the cell membrane; this ion could not penetrate to the cell interior<sup>11</sup>. For example, disruption of the membranes of yeast increased by about 100 times the maximum binding of  $\text{UO}_2^{2+}$  by a yeast suspension<sup>9</sup>. Rothstein also showed that mercuric ion at low concentration was bound to membrane ligands, without appreciably modifying membrane permeability. At mercury concentrations of  $10^{-4}$  M and higher, the permeability characteristics of the membranes altered, as shown by their leakiness to intracellular potassium, and to external dyes. These observations suggested a test of the hypothesis that the patent catalase of aerobic yeast was membrane-bound; if so, it should be possible to inhibit this activity by non-penetrating heavy metals.

In these experiments, I used the same commercial brand of bakers' yeast (standard brands) as did Rothstein. Tris buffer at pH 5.1 was used routinely. Catalase activity was assayed by the titanium method<sup>12</sup>, with a final  $\text{H}_2\text{O}_2$  concentration of 0.02 M; yeast concentration in the assay was about 4 mg/ml. When uranyl ion was tested, it was included in the assay mixture. The effect of mercury was examined by pre-treating the cells with  $\text{HgCl}_2$ , usually at a concentration of  $8 \times 10^{-5}$  M, and then twice washing them with buffer before assay; aliquots of the mercury-treated and control cells were also treated with *n*-butanol prior to assay<sup>13</sup>, in order to determine the effect of mercury on the cryptic enzyme.

The effect of varying concentrations of  $\text{UO}_2^{2+}$  on patent activity is shown in Fig. 1, Curve A, and on that of lysed cells in Curve B. The patent activity was virtually abolished at a concentration of 0.1 M, whereas the enzyme of the butanol-lysed cells was completely inactivated at  $5 \times 10^{-4}$  M  $\text{UO}_2^{2+}$ , and was thus about 500 times more sensitive. The inhibition of patent activity could be reversed to some extent by high substrate concentration, as shown in Fig. 2, Curve A, but that of the lysed cells could not (Curve B). Since the latency of intracellular yeast catalase has been attributed to impermeability of the membrane to  $\text{H}_2\text{O}_2$  (ref. 14), it would

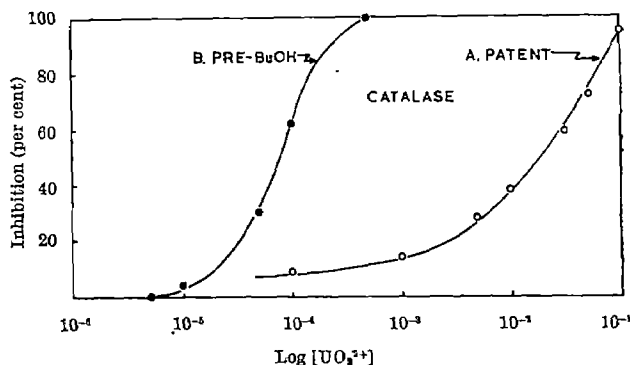


Fig. 1. The effect of concentration of uranyl ion on the patent catalase activity (Curve A) and the catalase activity of butanol-lysed cells (Curve B).

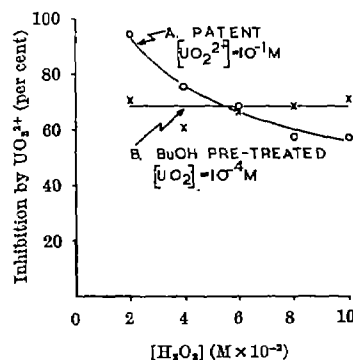


Fig. 2. The effect of concentration of  $\text{H}_2\text{O}_2$  on the inhibition of patent (Curve A) and lysed cell (Curve B) catalase activities caused by  $0.1 \text{ M } \text{UO}_2^{2+}$ .

seem that the site of reversal of the inhibition was at the cell membrane. The inhibition of patent activity by  $\text{UO}_2^{2+}$  could also be partly reversed (about one-third) by the subsequent addition of 0.05 M ethylenediamine tetraacetic acid to the assay mixture; this poly-carboxylic acid is unlikely to penetrate the yeast cell. Furthermore, the inhibition of patent activity could be reversed to a similar extent by  $5 \times 10^{-3}$  M inorganic phosphate in the assay; yet Rothstein has shown that the cytoplasm of this strain of commercial yeast contained inorganic phosphate at a concentration of  $10^{-2}$  M (ref. 6). Thus, the inhibition of patent catalase by  $\text{UO}_2^{2+}$ , and its partial reversal by high  $\text{H}_2\text{O}_2$ , by EDTA and by inorganic phosphate all suggest localization of the patent enzyme at the cell surface.

The effect of pre-treating yeast with varying concentrations of mercury prior to assay is shown in Fig. 3; the ordinate represents the ratio of mercury-treated cells: control cells. Thus a value greater than 1 indicates that mercury has increased the activity of the suspension, and a value less than 1 indicates an inhibition. Curve A shows that the patent activity was inhibited by concentrations of mercury of  $10^{-4}$  M and lower. At somewhat higher concentrations ( $3 \times 10^{-4}$  M to  $10^{-3}$  M) the activity of the treated cells was considerably higher than that of the controls. In this range of concentration of  $\text{Hg}^{2+}$ , the measured activity was the resultant of two competing effects of the heavy metal: (1) The activity of some cells was increased, due to the binding of mercury by certain membrane ligands, permitting increased permeability to  $\text{H}_2\text{O}_2$ , but not allowing entrance of the mercury itself. (2) Above a threshold amount of membrane-bound mercury, the total inactivation of both the cryptic and patent catalases of most of the cells occurred as the membranes became permeable to the mercury<sup>6</sup>. Since lysis induced by an agent (like butanol) which does not inactivate the enzyme results in a 60-fold increase in activity of the suspension<sup>1,4</sup>, it is clear from the observed 2- to 3-fold

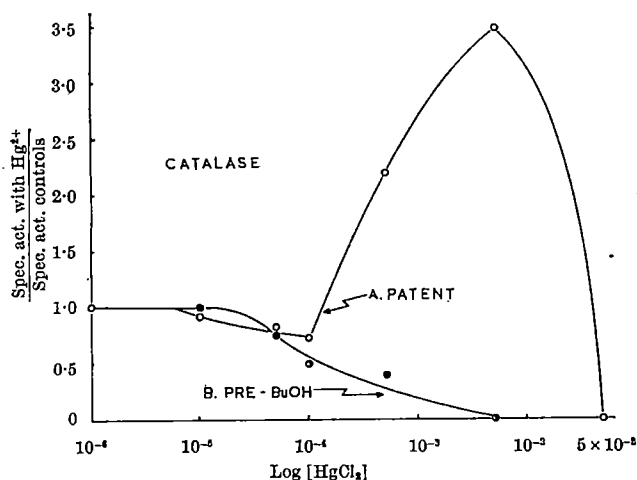


Fig. 3. The effect of concentration of  $\text{HgCl}_2$  on the catalase activities of A, intact cells, and B, lysed cells. Ordinate: ratio of activities of suspensions pre-treated with mercury: control suspensions

increase that both processes were occurring simultaneously but that the second was predominant. At concentrations of mercury higher than  $10^{-2}$  M, all activity was destroyed; the previously cryptic enzyme had also been completely inactivated as shown by subsequent lysis of the treated cells with butanol. Thus butanol could not reverse the inhibition of catalase by mercury.

Table 1. EFFECT OF MERCURY PRE-TREATMENT ON PATENT AND CRYPTIC CATALASE ACTIVITY

Intact cells were treated with  $8 \times 10^{-5}$  M  $\text{HgCl}_2$  (or in a few cases  $10^{-4}$  M) and twice washed with buffer. Their catalase activity was then assayed either directly or after treatment with butanol to expose the cryptic enzyme. Standard deviations are shown

A. Data from 15 out of 22 expts.; inhibition of patent activity > 15%:	
% Inhibition before butanol	% Inhibition after butanol
$29.1 \pm 8.0$	$1.4 \pm 3.8^*$
B. Data from all 22 experiments:	
% Inhibition before butanol	% Inhibition after butanol
$23.4 \pm 10.9$	$2.7 \pm 4.6^*$

\* Not significantly different from zero.

Table 1 summarizes the results of a series of experiments in which yeast suspensions were pre-treated with either  $8 \times 10^{-5}$  M, or  $10^{-4}$  M,  $\text{Hg}^{2+}$ , washed and then assayed either directly or after lysis. Inhibition of the patent catalase was of the order of 25 per cent; on the other hand, the cryptic enzyme was inhibited by only about 2 per cent, and this inhibition was statistically insignificant. Even if one were able to inhibit the patent activity completely, the activity of the butanol-treated cells, which increased by a factor of about 60 after lysis, would be inhibited by only about 2 per cent, which would not be detected by this method of assay. Several experiments similar to those described in Table 1 were performed on the patent catalase of anaerobically-grown, catalase-poor yeast; no inhibition of their patent activity could be detected at a mercury concentration of  $10^{-4}$  M.

The much greater resistance of the patent catalase to uranium (Fig. 1) is reminiscent of the finding<sup>15</sup> that the membrane-bound invertase of intact yeast was about 200 times more resistant to silver than was the extracted, purified enzyme. This has been interpreted as being due to preferential binding of the silver to inert membrane ligands with greater affinity for the metal<sup>16</sup>. But the catalase of the lysed cells, although immersed in a sea of exposed metal-binding groups of cell proteins and nucleic acids, etc., was extremely sensitive to uranium. Thus there exists the possibility that patent enzymes were stabilized against conformational changes by virtue of the way in which they were bound to the membrane. A possibly similar phenomenon was found in the case of a membrane-bound transport system for  $\beta$ -glucosidase in yeast, which was far more resistant to mercury than was

the  $\beta$ -glucosidase of lysed cells, despite the fact that the affinities of both transport system and enzyme for substrate and competitive inhibitors were identical<sup>17</sup>. Binding of crystalline liver catalase to various model surfaces was found to modify certain of its enzymatic properties<sup>18</sup>, so that it seems not unreasonable that membrane binding might alter the sensitivity of proteins to heavy metal inhibition.

It is therefore concluded that: (1) Uranyl ion, which has been found not to penetrate the cell membrane, could completely inhibit the patent catalase activity of yeast; the inhibition could be partly reversed by external inorganic phosphate at a concentration considerably lower than that which exists in yeast cytoplasm. The inhibition was also partly reversible by EDTA and by high substrate concentration. (2) Mercuric ion caused a significant inhibition of patent activity under conditions in which it was unable to penetrate the cell membrane, as shown by the fact that the cryptic enzyme was fully active, when assayed following lysis of the mercury-treated cells. (3) The patent catalase activity of aerobic yeast is due to enzyme molecules held at the cell membrane, or external to it in the cell wall.

I thank Mrs. Wanda Tacreiter for expert assistance, and the Medical Research Council of Canada for continued support of my work.

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## Keratin Protofilaments and Ribosomes from Hair Follicles

SEVERAL proteins, fibrous and non-fibrous, differing in morphology and chemistry, are synthesized by the hair follicle, not only in the hair itself, but also in the closely-associated inner root sheath<sup>1</sup>. Work on these proteins, particularly with regard to the distribution of the amino-acids cystine and citrulline, requires the development of an *in vitro* system similar to that used in the investigation of protein synthesis in a variety of other tissues<sup>2</sup>. We wish to report the practicability of such an approach for hair follicles.

Hair follicle tissue from the skin of albino guinea-pigs 8-15 weeks old was obtained by the wax method<sup>1,3</sup> and by this procedure the 'dispersed' tissue was brought together in an amount sufficient for investigation. Homogenization in a Potter-Elvehjem homogenizer was carried out for periods of 5-10 sec in a basic medium containing  $\text{tris-Mg}^{2+}$ -KCl. Sucrose was included when

necessary. Fractions were obtained by centrifugation at increasing  $g$  values, namely, 350 $g$ ; 10,000 $g$ ; 30,000 $g$  and 105,000 $g$ , and were examined by electron microscopy of sections and by negative-staining.

The material sedimented at low  $g$  values contained a high proportion of keratin fibrils. Protofilaments (formerly termed protofibrils<sup>4</sup>) of about 20 Å diameter were present and the proportion of these structures was markedly increased by brief ultrasonication in the presence of 90 per cent formic acid as swelling agent, a procedure which liberates them from the 80 Å-diameter keratin filaments (formerly termed microfibrils<sup>4</sup>, Fig. 1). The identification of these structures in the hair follicle fractions strongly supports the view, put forward from electron microscopy of sectioned  $\alpha$ -keratins, that 80 Å-diameter keratin filaments consist of a number (possibly 10 or 11) of protofilaments 20 Å in diameter<sup>5-7</sup>. This visualization of protofilaments is, therefore, contrary to recent claims that they are not real structures<sup>8-10</sup>. The protofilaments probably consist of 2 or 3 protein chains with a high  $\alpha$ -helical content<sup>11</sup>. Keratin synthesis presumably involves the formation of these elements followed by their aggregation into larger filaments and then fibrils, this last stage requiring the incorporation of the matrix protein.

In the evaluation of the contents of fractions obtained at higher  $g$  values, it has not been possible, so far, to obtain evidence of intact mitochondria; presumably the homogenizing conditions used (which included sucrose), and the effect of fibrous keratin during homogenizing, did not prevent disruption of the mitochondria. Material sedimented at 30,000 $g$  contained 'free' ribosomes and 'smooth' membranes. Typical microsome fractions would not be expected and were not found, because hair follicle cells have a cytoplasm rich in ribosomes, but virtually lacking in endoplasmic reticulum. At 105,000 $g$ , pellets were obtained, which, on examination in the electron

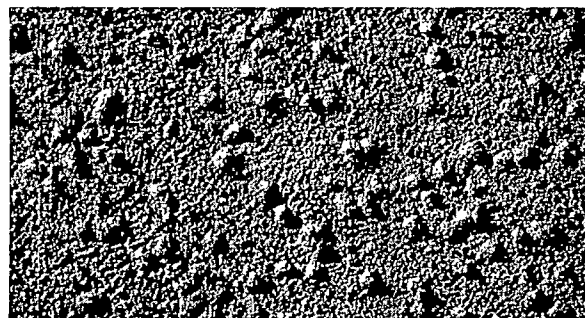


Fig. 3. Ribosomes sedimented at 105,000 $g$  shadowed with platinum ( $\times 80,000$ )

microscope, were found to consist entirely of ribosomes about 150 Å in diameter and presumably of the 70s type (Figs. 2 and 3).

When subjected to centrifugation on sucrose density-gradients, the optical density profiles of the ribosome fractions indicated the presence of polysomes. Moreover, we have found that the 105,000 $g$  supernatants will activate amino-acids and incorporate them into  $s$ -RNA (transfer-RNA) prepared from hair follicle tissue by the phenol method<sup>12</sup>. These results suggest that it should be possible to subject the hair follicle to a full investigation of protein biosynthetic pathways.

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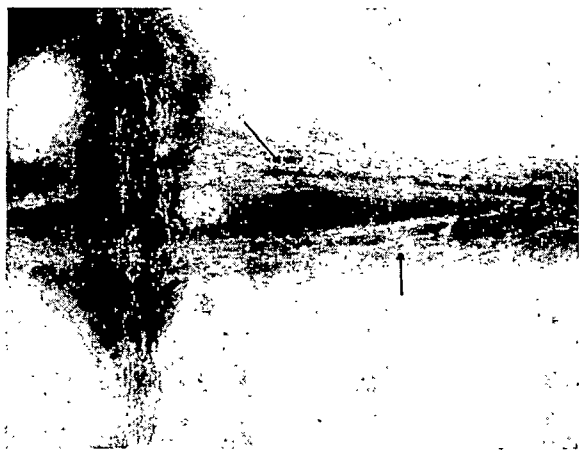


Fig. 1. Groups of protofilaments (20 Å in diameter) of keratin, negatively stained ( $\times 120,000$ )



Fig. 2. A section of ribosome pellet fixed in  $\text{OsO}_4$  and stained with lead hydroxide ( $\times 80,000$ )

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### Characterization of Plant Pigments by Gas Chromatography

PLANT pigments are compounds which are not volatile at the temperatures normally used in gas chromatography (up to about 250° C). The only reported work in this field has been carried out using the methyl esters of flavonoid compounds<sup>1</sup>. In this work characterization of the pigments was attempted by the technique of analysis of the products of pyrolysis. The original design of the apparatus used for this work was described for the analysis of phenolic resins and rubber polymers<sup>2</sup> and has since found many applications including the characterization of non-volatile alkaloids<sup>3</sup>, polystyrene<sup>4</sup>, purines and pyrimidines<sup>5</sup> and barbiturates<sup>6</sup>.

The apparatus used in this work was the Pye 'Panchromatograph' fitted with a 5-ft. column in which the stationary phase was silicone oil (20 per cent) supported on 'Celite'. The detector was the macro-argon type maintained at a potential of 500 V and the argon carrier gas flow was 60 ml./min at an inlet pressure of 20 lb./in.<sup>2</sup>. The column was fitted with a pre-column pyrolysis unit



manufactured by Pye and the compounds investigated, after preliminary purification where necessary by column chromatography, were applied to the filament by dipping it into a solution of the pigment in a suitable solvent and drying it in a current of warm air. The temperature of pyrolysis was approximately 800°C and was maintained for 10 sec.

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### 3·1-S Protein Fragment released from Reduced Wool by Trypsin

As one approach to the elucidation of the molecular arrangement in wool keratin, we have been using proteolytic enzymes under mild conditions to try to extract large molecules or aggregates of molecules from wool, which still preserve the configuration they had in the native fibre. In a previous communication<sup>1</sup> we described experiments designed to discover the cause of wool's resistance to proteolytic enzymes. An important reason may be the highly cross-linked structure which only allows the fibre to swell a small amount in aqueous solutions, so that the large enzyme molecules cannot easily diffuse into the fibre.

It was necessary, therefore, to find a treatment which would break disulphide bonds to allow the fibre to swell and the enzyme molecules to penetrate. At the same time, the treatment should preserve so far as possible the native arrangement of the molecules in the fibre. Elsewhere<sup>2</sup> we shall describe X-ray diffraction and deuterium exchange examination of keratin fibres which have been reduced with thioglycolic acid under various conditions. These experiments showed that it was possible to choose a reducing treatment (0·1 M thioglycolic acid, 0·1 M Na<sub>2</sub>HPO<sub>4</sub>, pH adjusted to 9·75, 18 h, 20° C) which did not irreversibly denature the fibre and yet permitted extensive (up to 50 per cent) digestion of reduced and rinsed Merino wool in 0·1 M Na<sub>2</sub>HPO<sub>4</sub> solution at pH 8·0, trypsin : substrate ratio 1 : 50, in 50 min at 20° C.

A further observation was that reduced fibres which had been re-oxidized by suspending them in a buffer at pH 7·0 and bubbling air through for several hours were no longer attacked by trypsin. If, therefore, trypsin did release large protein molecules or aggregates from reduced wool which were still in the native structure, then re-oxidation of these fragments in solution should prevent them from being broken down further by the enzyme. So for 2 h at 20° C air was bubbled through 0·1 M Na<sub>2</sub>HPO<sub>4</sub> solutions adjusted to pH 8·0 outside a dialysis bag containing the supernatant from centrifugation (1,250g for 5 min) of the reduced wool-enzyme reaction mixture. Dialysis was allowed to continue a further 18 h at 4° C without air bubbling before carrying out a run at 42,040 r.p.m. in the Spinco model E ultracentrifuge using a double sector synthetic boundary cell (Fig. 1). The asymmetry of the peak may mean that the keratin fragments released from the fibres were slowly broken down by the enzyme. If re-oxidation does prevent further attack on fragments in the native structure but does not prevent attack on denatured fragments, then it may prove possible to purify the native fragments by allowing the enzyme reaction to continue in the dialysis bag at 20° C after re-oxidation of the disulphide bonds.

An analytical ultracentrifuge run at 59,780 r.p.m. confirmed that, in fact, trypsin releases a large fragment from reduced Merino wool. The sedimentation coefficient of a 0·3 per cent solution in 0·1 M Na<sub>2</sub>HPO<sub>4</sub> solution at pH 8·0 was  $S_{20, \text{buffer}} \times 10^{13} = 3·1$ . This may be compared with light meromyosin<sup>3</sup>, molecular weight 100,000

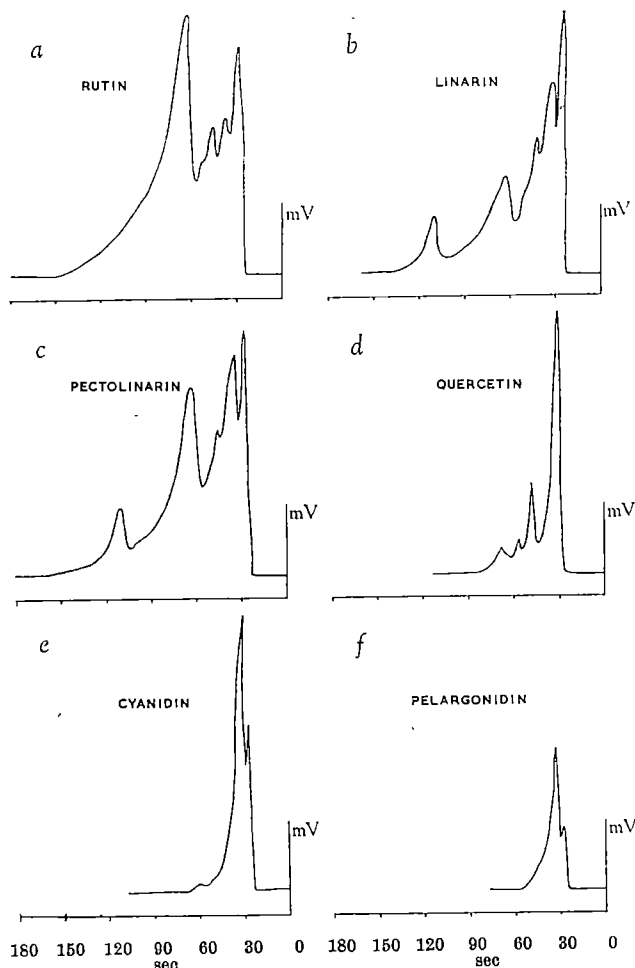


Fig. 1

The results obtained were found to be completely reproducible and the analyses were completed in a very short time; the pyrolytic products have not so far been identified. The flavonoid glycosides rutin (Fig. 1 (a)), linarin (Fig. 1 (b)) and pectolinarin (Fig. 1 (c)) were found to yield four characteristic peaks in each case; the flavonoid aglycone quercetin (Fig. 1 (d)) gave two major peaks only and the anthocyanin aglycones cyanidin (Fig. 1 (e)) and pelargonidin (Fig. 1 (f)) similarly gave two peaks; the peaks obtained from the two latter compounds were much closer together than those from quercetin. The retention times ( $R_t$ ) of the peaks obtained from the compounds investigated are shown in Table 1. It has thus been found that it is possible rapidly to assign a compound to its respective class, but this method has not so far afforded a means of identifying the pigment.

Table 1

$R_t$  of peaks obtained (sec)

Rutin	30	38	48	65	
Linarin	27	34	45	65	110
Pectolinarin	27	35	46	62	110
Quercetin	30	42	52*	62*	
Cyanidin	28	32			
Pelargonidin	28	32			

\* Minor peaks.

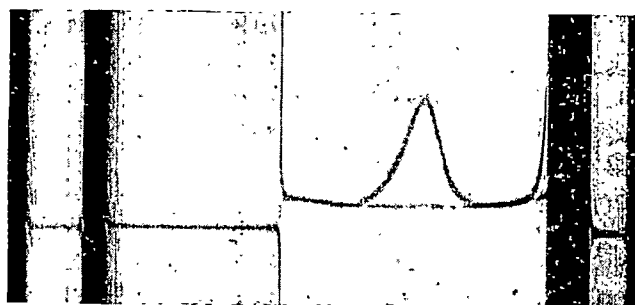


Fig. 1. Schlieren pattern from an ultracentrifuge run of a 1 per cent solution of protein released by trypsin from reduced wool. Buffer: 0.1 M  $\text{Na}_2\text{HPO}_4$ , adjusted to pH 8.0. Double sector synthetic boundary cell, bar angle  $75^\circ$ ,  $20^\circ\text{C}$ , 20 min after reaching 42,040 r.p.m.

$S_{20} \times 10^{13} = 2.8$ , or the  $\alpha$ -component from denatured tropocollagen<sup>4</sup>, molecular weight 100,000,  $S_{20,w} \times 10^{13} = 3.2$ . Purification and characterization of this high molecular weight constituent of wool keratin are proceeding.

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## PHYSIOLOGY

### Anæsthesia and Receptive Fields

Hubel and Wiesel<sup>1,2</sup> have described the responses of cells in the cat's visual cortex to precise stimulation of the retina. They say: "A comparison of visual responses in the anaesthetized animal with those in the unanaesthetized unrestrained preparation<sup>1</sup> shows that the main differences lie in the frequency and firing patterns of maintained activity and in the vigour of responses, rather than in basic receptive field organization." They found that responses declined in vigour with anaesthesia.

A comparison we have recently made of receptive fields of cells in the cat's visual cortex in the unanaesthetized *cervæu isolé*<sup>3,4</sup> with those in the anaesthetized preparation has shown dramatic differences. Recordings were made in the way described by Burns, Heron and Pritchard<sup>4</sup>. Two sorts of unit have been examined: those the presence of which could only be detected by responses to stimuli and those that were spontaneously active. Six of the first type have been seen. These showed no spontaneous activity before, during or after anaesthesia with 'Pentothal' (the anaesthetic used by Hubel and Wiesel) in doses of 2–8 mg/kg. They did not respond to stimuli before anaesthesia<sup>4</sup>, but did so within a few minutes of the administration of 'Pentothal'. Their responses to black bars moving in a specific direction on a white background had disappeared between 30 and 60 min after giving the anaesthetic. In 12 spontaneously active cells receptive fields could be plotted before, during and after administration of 'Pentothal'. Receptive fields changed considerably under anaesthesia, but reverted, usually completely, to their original form within 1 h.

For example, a unit with a receptive field  $5^\circ$  from the area centralis of the contralateral retina, and about  $5^\circ$  wide, initially only responded to fast horizontal movement of a vertical black bar from left to right on a white background. After the preparation had been given 5 mg/kg 'Pentothal' the unit responded to fast and slow move-

ments of the black bar from left to right and from right to left. The unit also responded to vertical movement of the horizontally orientated bar in an area outside, but contiguous with, its original receptive field and  $5^\circ$  by  $8^\circ$  in size. Thirty-five min after giving the anaesthetic the unit only responded to left-to-right movements in its original receptive field.

All the units recorded in the primary visual areas of four cats have shown similar differences. The changes observed have always been, at least partially and usually completely, reversible. In contrast to Hubel and Wiesel's findings, units in the visual cortex seemed easier to stimulate during anaesthesia and more responsive to stimuli identical with those used before the anaesthesia. Our impression was that anaesthesia in some way made peripheral stimuli more significant and easier to detect, perhaps by reducing or cutting off many of the other inputs to a cortical cell. It is interesting that whereas Burns, Heron and Pritchard<sup>4</sup> found that units not spontaneously active never responded to stimuli, many of the responses recorded by Hubel and Wiesel were from otherwise inactive units. This must reflect a difference between the unanaesthetized and anaesthetized preparations, as giving anaesthetic in our experiments revealed many hitherto unresponsive units.

It is also interesting that we have observed persistent changes in the frequency and distribution of maintained activity of cortical units during different levels of retinal illumination. Both Burns, Heron and Pritchard, and Hubel and Wiesel, saw responses only at the times of changes of illumination. Stimulation also caused changes in slope of both parts of the interval histogram described by Smith and Smith<sup>5</sup>.

Two of the possible explanations for the discrepancies between our results and those of Hubel and Wiesel are: (1) intact brain and *cervæu isolé* are not directly comparable; (2) Hubel and Wiesel never observed the same cell before and during anaesthesia, whereas all cells examined here were recorded in both states.

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### Mesencephalic Reticular Activating System and Cortical Acetylcholine Output

THERE is a great deal of indirect evidence<sup>1,2</sup> for the participation of cholinergic pathways in the electroencephalographic (EEG) arousal response. Parasympathomimetic agents, including acetylcholine (ACh) and cholinesterase inhibitors, cause EEG desynchronization similar to the EEG changes accompanying behavioural arousal or the stimulation of the mesencephalic reticular formation. Furthermore, atropine blocks EEG desynchronization produced by the above methods. Mitchell<sup>3</sup> has reported that stimulation of specific afferent pathways leading to the somatosensory cortex caused an increased release of ACh from this area. Using similar techniques we investigated the effect of mesencephalic reticular formation stimulation on cortical ACh output.

Cats weighing 2.5–3.5 kg were anaesthetized throughout the experiments with 1–1.5 per cent halothane administered by means of a respiratory pump. A bipolar electrode was placed stereotactically into the mesencephalic tegmentum (co-ordinates: A, 3.0; L, 4.0; H, -1.0) and this area was stimulated every 10 sec with trains of pulses of 8–12 V and of 0.1 m/sec duration for 1 sec at

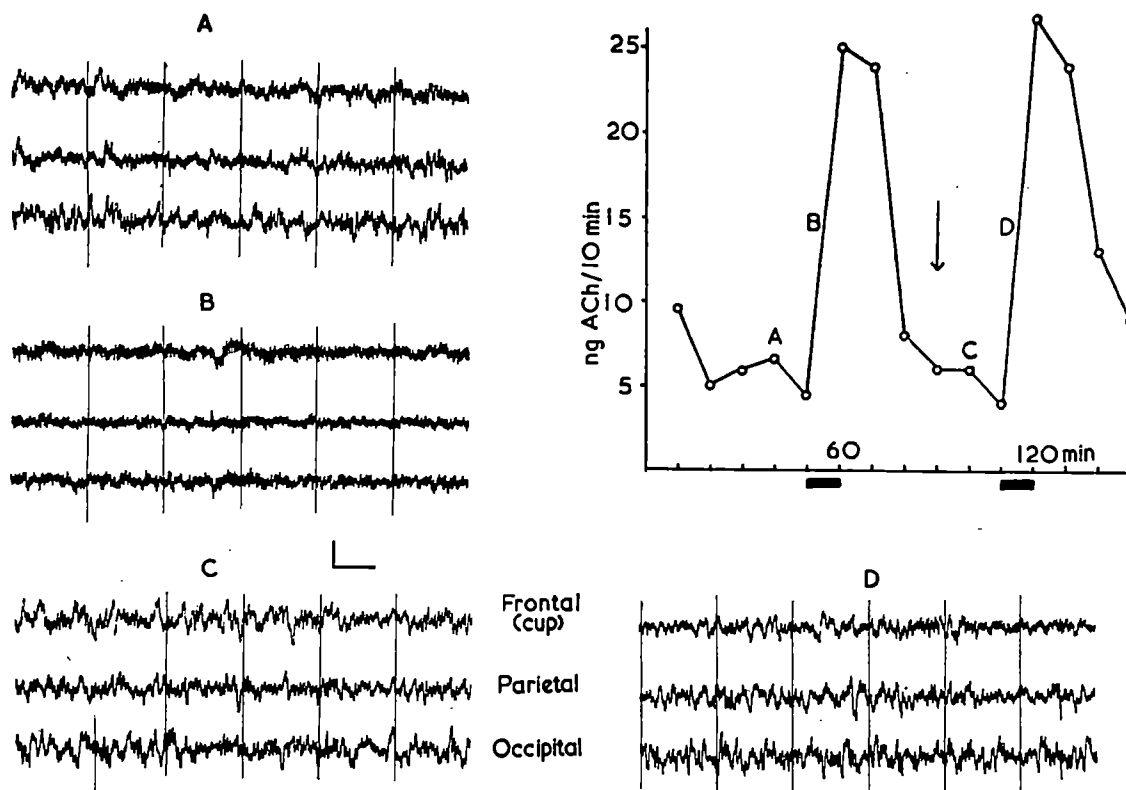


Fig. 1. Letters above EEG tracings indicate time of recording in relation to ACh output as shown in graph. During periods marked by thick line, stimulation of the reticular formation. At arrow, 1 mg/kg atropine sulphate injected i.v. EEG calibration: 0.1 mV, 1 sec

a rate of 100/sec. ACh was collected from the ipsilateral cortex in 0.25 ml. Locke's solution contained in 'Perspex' cylinders covering 0.75 cm<sup>2</sup> of the cortex. Collection of ACh was started after exposing the cortex for 30 min to 0.25 mg/ml. phospholine iodide. The Locke's solution placed on the cortex in which ACh release was estimated contained 1 µg/ml. eserine salicylate and the same concentration of atropine sulphate, a procedure shown previously<sup>4</sup> to enhance ACh output. The released ACh was assayed on dorsal strips of leech-muscle suspended in a microbath<sup>5</sup>. The EEG was recorded by means of small non-polarizing wick electrodes on a Grass electroencephalograph.

As shown in Fig. 1, in the absence of any stimulation of the reticular formation the EEG showed large, slow-wave sleep pattern, although the lead on the area exposed to the cholinesterase inhibitor frequently showed an activity of higher frequency. Stimulation of the reticular formation caused a continuous activation of the EEG during the 10–15 min collection period. In the same time ACh output from the cortex increased 5–6 times with large amounts of ACh still appearing in the period following the stimulation. Injection of 1 mg/kg atropine sulphate intravenously made the high voltage, slow-wave pattern more pronounced and practically abolished the EEG desynchronization resulting from the stimulation of the reticular formation. However, this dose of atropine did not prevent the increase in ACh output due to reticular formation stimulation.

In different experiments two cylinders were placed on the cortex, one on the posteroculic cortex, on the area of maximum evoked potentials resulting from the stimulation of the contralateral forepaw and a second, farther back, on the medial ectosylvian gyrus. Following the inhibition of cholinesterase in both areas, ACh output was measured concurrently. As shown in Fig. 2A, stimulation of the contralateral forepaw increased ACh output mostly from the somatosensory cortex, but there was a considerable increase in ACh output from the parietal cortex also,

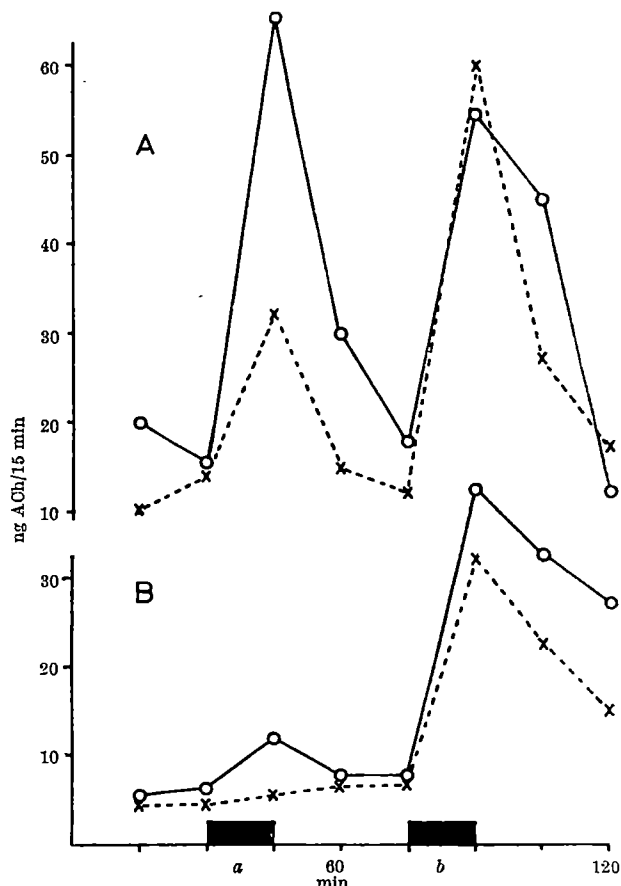


Fig. 2. A, 1 per cent, B, 1.5 per cent halothane anaesthesia. Solid line ACh output from posteroculic gyrus; broken line from medial ectosylvian gyrus; a, 1/sec stimulation of contralateral forepaw; b, stimulation of ipsilateral reticular formation

due probably to the activation of the reticular formation through the collaterals of the specific afferent pathways. When the anaesthesia was deepened (Fig. 2B) peripheral stimulation augmented ACh output only from the sensory cortex, although to a much lesser extent than under lighter anaesthesia. Stimulation of the tegmental reticular formation increased ACh output to about the same degree from both cortical areas.

These observations show unequivocally the participation of cholinergic neurones in the EEG arousal response. The finding that atropine blocked the EEG signs of arousal without affecting the increase in ACh output indicates that the enhanced output of ACh was not the result of a general increase in neuronal activity in the underlying cortex during EEG arousal. The simplest interpretation of the action of atropine on EEG therefore is that it blocks the postsynaptic effect of ACh in the cortex where, as has been shown by Krnjević and Phillis<sup>6</sup>, it also antagonizes the stimulating effect of electrophoretically applied ACh. The present observations also demonstrate that cholinergic pathways are part of at least two distinct cortical systems. One is the specific corticopetal system producing increased ACh release in the sensory area, as had been shown before by Mitchell<sup>3</sup>, and a non-specific system involved in the EEG arousal response, producing increased ACh release in a wide area of the cortex. This widespread cholinergic system has been demonstrated histochemically by Shute and Lewis<sup>7</sup>, who found important cholinergic tracts ascending from the mid-brain tegmentum to the cortex and to other parts of the fore-brain.

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### Effect of Tissue Size on Measurements of Sodium, Potassium and Water Contents of Isolated Rat Ventricular Tissue

DURING the course of experiments designed to examine the effects of inhalation anaesthetics on the sodium, potassium and water contents of isolated rat atrial tissue<sup>1</sup>, it was noted that the standard deviations of the sodium values were rather high. These values were thought to be unreliable and were therefore not reported. It was felt that part of the deviations in sodium values could be explained by the fact that uniform blotting procedures were not used on all tissues, thus removing variable quantities of sodium-rich bathing medium. Part of the difference could also be explained on the basis of contamination of the tissue or its digest from any number of sources, despite diligent care to use demineralized water and clean apparatus. Since the tissue was so small (about 50 mg) and its sodium content so small, it is easy to see that the slightest contamination would give erroneous results. As the experiments progressed, however, and greater attention was directed to the possibilities of contamination, the sodium values decreased and the standard deviations also decreased.

Since contamination did appear to play a part, it seemed desirable to use larger pieces of tissue so that the effects

of contamination would be minimal. The isolated rat ventricle was thus used. In order to assess the effects of tissue size on sodium values, small pieces cut from the right and left ventricle were compared with the intact ventricle which was split open before blotting.

Freshly dissected tissues were used in all cases. The hearts from rats killed by decapitation were placed in Krebs-Henseleit bicarbonate solution (modified to contain 1/2 the normal calcium concentration and 1 g glucose/l.). The auricles were removed and discarded. The ventricle was split in half and, when appropriate, small pieces of tissue from the right and left ventricle were removed. All tissues were blotted 20 times<sup>2</sup> on Whatman No. 44 filter paper, a fresh piece of filter paper being used after each 5 blottings. The tissues were then dried at 95° C for at least 24 h. The small pieces of tissue were then digested in 0.5 ml. concentrated HNO<sub>3</sub> in a plastic vial or bottle for at least 24 h. The volume was then brought to 25 ml. with demineralized water, and analyses for sodium and potassium were made with an Evans Electro-selenium, Ltd., flame photometer. The intact ventricles, which weighed about 10 times more than the small pieces, were digested in 5 ml. concentrated HNO<sub>3</sub> and diluted to 250 ml. with demineralized water before analysis. The results are given in Table 1.

Table 1. EFFECT OF TISSUE SIZE ON WATER, SODIUM AND POTASSIUM CONTENTS OF FRESHLY DISSECTED RAT VENTRICULAR TISSUE

	Sections of right ventricle (5)*	Sections of left ventricle (5)	Intact ventricles (10)
Mean wet weight (mg)	36.6	72.0	502.3
Per cent H <sub>2</sub> O	77.0 ± 0.65†	76.1 ± 1.23	76.9 ± 0.53
M.equiv. Na <sup>+</sup> /kg wet tissue	94.5 ± 13.0	74.8 ± 6.2	46.8 ± 3.8
M.equiv. Na <sup>+</sup> /kg dry tissue	412 ± 58	314 ± 28	203 ± 19
M.equiv. K <sup>+</sup> /kg wet tissue	71 ± 9	73.3 ± 5.6	83.5 ± 4.7
M.equiv. K <sup>+</sup> /kg dry tissue	309 ± 42	308 ± 18	360 ± 20
Na <sup>+</sup> /K <sup>+</sup>	1.33	1.02	0.56

\* Number of tissues.

† Standard deviation.

Several facts are evident from Table 1. (1) Sodium content decreased as tissue size increased and standard deviation of sodium values also decreased as tissue size increased. (2) Percentage water was not measurably altered by tissue size. (3) Potassium content was not altered as much as sodium content by tissue size, although the large intact ventricles showed higher potassium values than either of the 2 smaller pieces of tissue. This may be due to leakage of potassium from the cut surfaces of the smaller tissues, which surface is larger relative to the mass than is the case with the intact ventricle preparation. (4) The Na<sup>+</sup>/K<sup>+</sup> ratio was markedly reduced as the tissue size increased.

It is evident that the larger the tissue size (or the smaller its surface area in relation to its mass) the less the tissue content of sodium is affected by contamination or by the medium in which the tissues were dissected. Thus, sodium values from large tissues would more nearly reflect true sodium content of the tissue. Since the potassium values are higher for the larger tissues, they also probably more nearly reflect true tissue potassium content. For these reasons the intact ventricle preparation is to be preferred over small pieces of ventricular tissue for investigations involving tissue sodium and potassium determinations.

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### Effect of Indolyl-3-acetic Acid on the Permeability of Membranes in Storage Tissue

A STRIKING difference between the response of coleoptile tissue and storage tissue to applied auxin is that the former is immediate and the latter exhibits a lag period<sup>1-3</sup>. Sperling and Laties<sup>4</sup> have recently shown that in potato or Jerusalem artichoke tissue slices auxin response depends on the progress of metabolic changes associated with the lag period (ageing of tissue), and auxin had to be applied to fresh tissue to be effective. A similar characteristic feature of freshly cut storage tissue is that it requires the completion of a lag period before salt is accumulated. At least in the case of potassium this phenomenon was shown to be due to a reduction in the permeability of the outer cell membrane<sup>5</sup>.

Efforts to establish a coherent relationship between molecular auxin action and the induced cellular expansion have been inconclusive. Recently, doubts have been cast on promising hypotheses which involve plasticization of cell walls, dependent on auxin-induced methylation of galacturonic acids<sup>6</sup> or on the removal of calcium bridges from the wall (chelation theory)<sup>7,8</sup>. The observations presented here indicate that the primary site of auxin action may be revealed by examining its effect on the cell membrane.

Disks of beetroot tissue (15 mm diameter, 1 mm thick, average weight approximately 180 mg, var. 'Crimson Globe') were washed rapidly in three rinses of distilled water to remove cell debris before commencing the experimental treatment. Normally, fresh tissue was used but, when required, tissue was aged by placing the disks in vigorously aerated distilled water for 7 days, changing the aeration medium daily. Disks were incubated at 23°–24° C in batches of 25–75 per 150–500 ml. of aerated solutions to be tested. Each treatment was performed in triplicate. Potassium and calcium contents of the external solutions were determined by flame photometry.

Fig. 1 shows typical curves for the potassium concentration of the external solution when disks of beetroot were incubated in distilled water, in 0.1 p.p.m. or in 10 p.p.m. indolyl-3-acetic acid (IAA). After 15 h in distilled water, net movement of potassium ions was usually negligible, since potassium influx and efflux balanced each other<sup>9</sup> and a marked change at approximately 30–40 h from the start of the experiment due to a lowering of the efflux indicated the completion of the lag phase<sup>9</sup>. The IAA-induced changes in the potassium concentration of the external solution show that IAA did not change the duration of the lag period for salt uptake, although it increased the net leakage of potassium to the external solution. Rates of uptake and leakage in m.equiv./kg fresh weight/h (uptake shown +, leakage –) were + 0.008 ± 0.017, – 0.088 ± 0.013 and – 0.089 ± 0.006 for distilled water, 0.1 p.p.m. IAA and 10 p.p.m. IAA, respectively, during the period from 15 to 40 h, and + 0.171 ± 0.015, + 0.180 ± 0.017 and + 0.177 ± 0.025, respectively, during the period from 40 to 64 h. Thus, once the lag phase was completed rates of uptake remained unaltered by the auxin treatment.

Results presented in Table 1 show that IAA over a wide range of concentrations induced an increased leakage in fresh tissue only; it did not affect the amount of potassium associated with the apparent free space (A.F.S.). In aged tissue influx is much larger than efflux and therefore leakage of potassium can only be demonstrated with the help of tracers or by maintaining the potassium concentrations in the external solutions at less than 10<sup>-5</sup> M. Hence, the figures for leakage in aged tissue depend on experimental conditions but serve to indicate that IAA is effective only at a very high concentration (100 p.p.m.) in increasing the permeability of the cell membrane system: ageing appeared to reduce the sensitivity of the tissue towards applied auxin. Root tissues are very

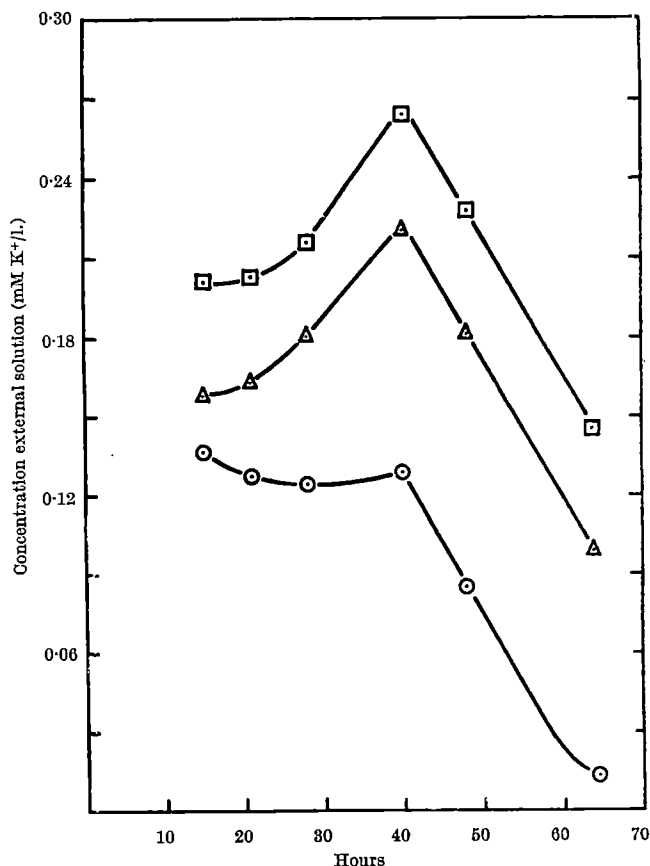


Fig. 1. Potassium contents of the external solution (mM/l.) due to uptake or release of ions by fresh cut beetroot tissue (14.0 g/500 ml.) against time (h). O, Distilled water; Δ, IAA 0.1 p.p.m.; ◻, IAA 10 p.p.m.

sensitive to auxin and their elongation is inhibited by concentrations as low as 10<sup>-11</sup> M (1.75 × 10<sup>-6</sup> p.p.m.)<sup>10</sup>, unless the tissue is depleted of auxin beforehand<sup>11</sup>. Generally, stem and coleoptile tissue seem to respond to concentrations of the order of 10<sup>6</sup> higher than those for root tissues. Therefore the decreased sensitivity of storage tissue on ageing may represent a change equivalent to a hypothetical transformation of root tissue into tissue with stem properties. According to Sperling and Laties<sup>4</sup>, the effectiveness of auxin depends on the changes in metabolism during the process of ageing. While this may be true, I suggest that the primary action of auxin is to bring about an immediate change in membrane permeability while the tissue is receptive. Jenkinson and Scott's<sup>12</sup> hypothesis of a feedback mechanism for the oscillations in bioelectric potentials observed in bean roots is based on a similar assumption.

Ethylenediamine tetraacetic acid (EDTA) is known to promote cellular expansion in certain tissues<sup>7</sup>. Like IAA it caused an increase in leakage when applied to storage tissue (Table 2); this leakage was strictly depend-

Table 1. The effect of IAA on the amount of potassium associated with the apparent free space (A.F.S.) and on leakage of potassium (m.equiv./kg fresh weight) in fresh and aged beetroot tissue, measured at intervals of 0.5, 1, 2, 4 or 8 h (1.8 g tissue/20 ml. solution; 10 ml. solution removed at each sampling and replaced by distilled water or appropriate IAA solution)

Treatment	A.F.S.	Fresh tissue Total leakage over:		A.F.S.	Aged tissue Total leakage over:	
		0–0.5 h	0.5–8 h		0–0.5 h	0.5–8 h
H <sub>2</sub> O (control)	1.74	0.22	1.45	0.110	0.003	0.02
0.001 p.p.m. IAA	1.73	0.26	1.66	0.112	0.004	0.03
0.01 p.p.m. IAA	1.76	0.28	1.80	0.100	0.003	0.02
0.1 p.p.m. IAA	1.72	0.30	1.90	0.103	0.005	0.03
L.S.D. 5 per cent	N.S.	0.085	0.23	N.S.	N.S.	N.S.
H <sub>2</sub> O (control)	1.21	0.37	2.76	0.137	0.036	0.27
1 p.p.m. IAA	1.27	0.43	3.13	0.148	0.005	0.04
10 p.p.m. IAA	1.28	0.51	3.79	0.131	0.005	0.04
100 p.p.m. IAA	1.27	1.41	36.5	0.127	0.150	5.55



Table 2. The effect of IAA or EDTA on rates of net potassium uptake (+) or leakage (-) in m.equiv./kg h before (8-30 h) and after completion of the lag phase (30-54 h) in beetroot tissue (4.4 g/150 ml. solution)

Treatment	8-30 h	30-54 h
H <sub>2</sub> O	-0.088 ± 0.002*	+0.205 ± 0.018*
5 × 10 <sup>-5</sup> M IAA	-0.335 ± 0.013	+0.195 ± 0.016
5 × 10 <sup>-5</sup> M EDTA	-0.167 ± 0.010	+0.185 ± 0.028

\* Standard errors of mean determinations.

Table 3. The amount of calcium removed from beetroot tissue by solutions of IAA or EDTA at various intervals after incubation (cumulative values), expressed as mg/kg fresh weight of tissue (calcium present in tissue at time of incubation 148 ± 30 mg/kg fr. wt.)

Treatment	2	Interval after incubation (h)				90
		8	30	54		
H <sub>2</sub> O	4.1	8.1	23.6	27.6	14.0	
5 × 10 <sup>-5</sup> M IAA	4.0	8.7	24.8	28.3	15.0	
5 × 10 <sup>-5</sup> M EDTA	91.0	93.0	93.0	87.0	78.0	

ent on the molar ratio of EDTA in solution to calcium present in the tissue. In contrast with the IAA-induced leakage, the effect of  $0.5 \times 10^{-5}$  M EDTA could easily be reversed by addition of an equivalent amount of calcium. Table 3 shows the amounts of calcium in solution, and thus removed from tissue by  $0.5 \times 10^{-5}$  M EDTA and  $0.5 \times 10^{-5}$  M (8.75 p.p.m.) IAA respectively. It is obvious that leakages induced by EDTA and by IAA respectively are based on different principles. It should be rewarding to obtain more detailed knowledge of the mechanism by which IAA causes the immediate increase of membrane permeability.

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## Effect of Cardiac Glycosides on Neuromuscular Transmission

BIRKS has recently suggested that raised intracellular sodium concentrations increase transmitter release from pre-synaptic nerve terminals<sup>1</sup>. This hypothesis is based on measurements of the amount of acetylcholine which was released from the perfused cat superior cervical ganglion and from cholinergic nerve terminals in frog sartorii when active sodium transport had been inhibited with the cardiac glycosides digoxin or ouabain. In the neuromuscular preparation an increased acetylcholine release was signalled by increases both in the quantal content of the endplate potentials (e.p.p.s) and in the frequency of spontaneous miniature endplate potentials (m.e.p.p.s.). These drugs have been tested at a mammalian neuromuscular junction in the present investigation in an attempt to demonstrate the phenomena at this synapse.

The rat phrenic nerve-diaphragm preparation, the bathing solution and the intracellular recording technique have been previously described<sup>2</sup>. Neuromuscular transmission was blocked by raising the magnesium chloride concentration of the bathing solution to 11-14 mM and the temperature was maintained at 33°-37° C. Intracellular e.p.p.s and m.e.p.p.s were recorded from the endplate region of muscle cells with glass microelectrodes filled with 3 M potassium chloride. The phrenic nerve, immersed in paraffin, was stimulated at 1- or 2-sec intervals through a pair of platinum electrodes and the sweep speed of the oscilloscope adjusted to allow simultaneous

recording of the elicited e.p.p.s and of the m.e.p.p.s. A faster oscilloscope sweep speed was used for recording the time course of e.p.p.s. The oscilloscope traces were recorded on moving film, which was later read using a photographic enlarger. Ouabain ('Strophanthin-G', British Drug Houses) was weighed dry and dissolved in control solutions to give concentrations of 1.0 to  $1.37 \times 10^{-5}$  M. 'Digoxin' (Burroughs Wellcome), supplied dissolved in ethyl alcohol, was added to control solutions so that the final concentration of digoxin was  $7.7 \times 10^{-6}$  M to  $1.3 \times 10^{-5}$  M and of ethyl alcohol 0.1-1.0 per cent v/v.

Fig. 1A shows that the amplitude of both e.p.p.s and m.e.p.p.s increases in ouabain ( $1.37 \times 10^{-5}$  M). The average e.p.p. and m.e.p.p. amplitudes are illustrated in the sequence: in control solution, after 32 and 60 min in the ouabain solution, and after 20 min in control solution. On the right of each e.p.p. is shown a typical m.e.p.p. photographed at the same oscilloscope sweep speed, and above, samples of m.e.p.p.s at a slower sweep speed and greater amplification. For calculation of quantal content, the m.e.p.p.s were recorded at the higher amplification and an average of 60 observations was used as an estimate of the size of a single quantum.

The effect of ouabain on the amplitudes of both e.p.p.s and m.e.p.p.s is shown in Fig. 1B. The amplitudes of e.p.p.s (filled circles) and m.e.p.p.s (open squares) are plotted as a ratio of the control amplitude (horizontal broken line), each point representing an average amplitude derived from 60 observations. One of the striking features of the graph is the parallelism between changes in e.p.p. and m.e.p.p. amplitudes. After 20-30 min there was a progressive increase in size until at 60 min both the e.p.p. and m.e.p.p. amplitudes were 1.7 to 1.8 times the control. In spite of the increased amplitude of the e.p.p. in ouabain, there was no significant change in the quantal content (Fig. 1C) which was calculated from the ratio of average e.p.p. to average m.e.p.p. amplitude and plotted as a ratio of the quantal content in control solution (horizontal broken line Fig. 1C). M.e.p.p. frequency per second was also relatively constant (Fig. 1D), the small increase being within the range often observed over a similar time in control solution. After only 3 min in ouabain, the half decay time of the e.p.p.s showed an appreciable increase in duration from 0.30 msec to 0.41 msec and then lengthened progressively until after 60 min in ouabain it had more than doubled (Fig. 1E). Each point is an average of the half decay times of 6-10 e.p.p.s ranging in size from the largest to the smallest observed. After 20 min in control solution the half decay time showed very little recovery towards the original duration. The amplitude of the m.e.p.p.s continued to increase for 10-20 min after removal of the ouabain and then began to fall, but after 20 min in control solution the amplitudes were still greater than they had been in the ouabain.

Thus the m.e.p.p. amplitude 9 min after the ouabain had been washed out was three times the control size, but 11 min later had decreased to 2.2 times the control amplitude (Fig. 1B). In this experiment e.p.p. amplitudes were not recorded 9 min after removal of the ouabain, but in similar experiments there was usually a partial reversal of the potentiation, the degree of reversal varying with the time of exposure to ouabain. The quantal content of the e.p.p.s (Fig. 1C) and the m.e.p.p. frequency (Fig. 1D) remained relatively constant following removal of the ouabain. The muscle resting membrane potential was stable at  $68 \pm 3$  mV throughout the experiment. Results similar to those shown in Fig. 1 were observed in ouabain concentrations ranging from 1.0 to  $1.37 \times 10^{-5}$  M. These concentrations of ouabain would be expected to inhibit active sodium transport<sup>3,4</sup>, yet produced no increase in the quantal content of the e.p.p.s or in the m.e.p.p. frequency and, hence, it can be concluded that there was no increase in acetylcholine release. On the other hand, an increased sensitivity of the muscle membrane to acetylcholine was shown by the growth in size of the m.e.p.p.s (Fig. 1B).

Further evidence of post-synaptic action is revealed by the increased half decay time of the e.p.p.s (Fig. 1E). The nature of these post-synaptic effects has not been pursued in the work recorded here, but it is of interest to note that ouabain has been shown to increase the sensitivity of ganglion cells and cardiac muscle fibres<sup>4,5</sup> to acetylcholine and other depolarizing agents. It is generally agreed that this is not due to an anticholinesterase action<sup>5</sup>. The increase in the amplitude and the lengthened decay time of the e.p.p.s can both be accounted for by postulating that ouabain increased the electrical resistance of the muscle membrane. This postulate is in accord with the recent observations that ouabain lowers electrical conductance in frog skin<sup>6</sup>.

In contrast, digoxin, even in the lowest concentration used, caused an appreciable increase in e.p.p. amplitude, but there was not an equivalent increase in m.e.p.p. amplitude. The quantal content of e.p.p.s in  $7.7 \times 10^{-6}$  M digoxin plotted as a ratio of the control quantal content (broken horizontal line) is shown in Fig. 2C and an increase is apparent as early as 10 min in the digoxin. After 34 min in digoxin, the average quantal content of e.p.p.s had increased by 40–50 per cent. Full reversal of this potentiation was evident 60 min after the digoxin had been washed out. Alcohol alone, in the same concentration (0.24 per cent v/v) in which the digoxin was dissolved, caused a similar increase in the quantal content of e.p.p.s and the potentiation followed a similar time course (Fig. 2C). In other experiments ethyl alcohol (1 per cent v/v) caused a more marked increase in e.p.p. amplitude. Fig. 2B shows the change in e.p.p. size graphed as a ratio of the control

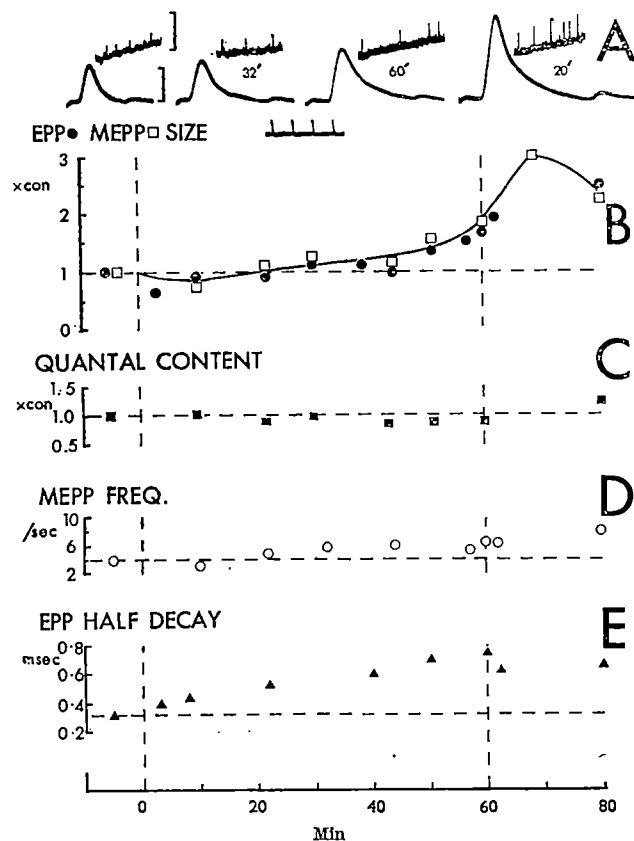


Fig. 1. Effect of ouabain ( $1.37 \times 10^{-5}$  M) on neuromuscular transmission. A, Illustrations of spontaneous miniature endplate potentials above, and intracellular endplate potentials below. Vertical calibrations: 0.5 mV above and 2 mV below. Time calibration: msec. B, Ordinate: ratio of endplate potential amplitudes (filled circles) and miniature endplate potential amplitudes (open squares) to their respective control sizes. The vertical broken lines in B, C, D and E represent changes of bathing solution, the first from control to ouabain, the second from ouabain back to control. C, Ordinate: ratio of quantal content of endplate potentials to control quantal content. D and E, Changes in miniature endplate potential frequency and half decay time of endplate potentials respectively.  $\text{MgCl}_2$  11 mM,  $34^\circ\text{C}$

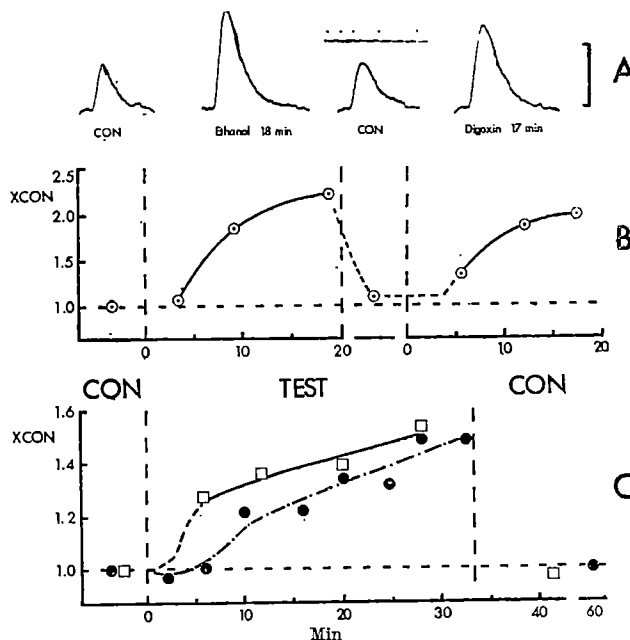


Fig. 2. The effect of ethanol and digoxin on neuromuscular transmission. A, Illustrations of average endplate potentials in the solution sequence: control, ethanol (1 per cent), control digoxin. Vertical calibration: 1 mV. Time calibration: msec. B, Ordinate: ratio of average endplate potential amplitude to control, amplitude. The vertical broken lines denote solution changes in the same sequence as in A.  $\text{MgCl}_2$  11 mM,  $33^\circ\text{C}$ . C, Quantal content of average endplate potentials in 0.24 per cent ethanol v/v (open squares) and digoxin  $7.7 \times 10^{-6}$  M in 0.24 per cent ethanol (closed circles) as a ratio of the control quantal content against time.  $\text{MgCl}_2$  14 mM,  $35^\circ\text{C}$

amplitude (horizontal broken line). In Fig. 2A these changes in e.p.p. amplitude are illustrated. The average e.p.p. amplitude has increased 125 per cent within 19 min exposure to the alcohol (Fig. 1A and B). Full recovery to the amplitude in control solution was observed 15 min after washing out the alcohol. Digoxin  $1.3 \times 10^{-5}$  M in 1 per cent ethyl alcohol (v/v) was then substituted for the control solution, and after exposure for 12 min to the digoxin the e.p.p.s had again begun to increase in amplitude. After 17 min in digoxin, the e.p.p. amplitude had increased 95 per cent. In further experiments increases in the quantal contents of e.p.p.s have been observed in ethyl alcohol concentrations as low as 0.05 per cent v/v.

Although ouabain and digoxin are presumed to have a common action in inhibiting active sodium transport across membranes and allowing an intracellular accumulation of sodium ions<sup>3,7,8</sup>, their effects at the rat neuromuscular junction are dissimilar. It is clear that the foregoing ouabain concentrations increased the e.p.p. amplitude not by a greater release of acetylcholine but rather by changes in the muscle membrane which made it more sensitive to acetylcholine. In contrast, digoxin in alcohol caused an early (within 10 min) pre-synaptic increase in e.p.p. amplitude (Fig. 2). It is likely that this increased acetylcholine release in digoxin was not an effect of the digoxin itself, but was due to the ethyl alcohol present as solvent. These results thus do not support the hypothesis proposed by Birks.

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## Afferent Discharges from Laryngeal Articular Mechanoreceptors

In previous communications<sup>1-3</sup> we have described the afferent nerve supply of the laryngeal joints, and the various types of articular nerve ending innervated thereby. We have also shown that electrical stimulation of the larger diameter fibres in the laryngeal articular nerves—which fibres terminate in corpuscular end organs in the capsules of the laryngeal joints<sup>2,3</sup>—gives rise to reflexly co-ordinated contraction and relaxation of the laryngeal muscles which can be detected visually and electromyographically<sup>4,5</sup>. We have further shown that similar reflexly co-ordinated changes in the tone of the laryngeal muscles are provoked by specific passive movements of isolated laryngeal joints<sup>4,5</sup>. We now wish to report that oscillographic analysis of the impulse traffic in the articular nerves innervating the cricothyroid joint from the recurrent laryngeal nerve, and in the recurrent nerve itself, has identified the mechanoreceptor afferent discharges responsible for the reflex muscular responses to passive movement of the joint.

For this purpose, the left cricothyroid joint was isolated from the lamina of the thyroid cartilage, and from the attachments of the cricothyroid and inferior constrictor muscles, in six cats lightly anaesthetized with pentobarbitone. The left thyroid lamina was then removed, to expose the intrinsic muscles and nerves of the larynx. Non-elastic threads were tied through the residual articulated fragment of the inferior cornu of the thyroid cartilage, and graduated passive movements of the cricothyroid joint were performed by attaching small weights to these threads, over pulley surfaces. Movements were performed in four planes mutually at right angles (that is, in cranial, caudal, anteromedial and anterolateral directions), as in previous experiments<sup>4,5</sup>. All responses were tested at various phases of the spontaneous respiratory cycle.

Bipolar, chlorided silver recording electrodes were placed in contact with one or more of the articular nerves supplying the joint, and with the trunk of the ipsilateral recurrent laryngeal nerve proximal and distal to the origin of its articular branches. Impulses from each recording site were fed in turn through a Grass pre-amplifier to the upper beam of a Tektronix oscilloscope, the lower beam being used for calibration and timing signals. Activity was photographed on film with an electrically controlled camera, triggered synchronously with the sweep mechanism of the oscilloscope.

Recordings were first made of the resting activity in the recurrent laryngeal nerve and in its articular branches in the absence of joint traction. The responses in the articular nerves and in the recurrent laryngeal nerve to displacement of the cricothyroid joint in four directions were then examined, with concurrent observation of the laryngeal muscular responses provoked thereby. The procedure was then repeated after bilateral section of the superior laryngeal nerves proximal to the origin of their external branches; after section of the ipsilateral recurrent laryngeal nerve distal to the origin of its articular branches; after section of the recurrent laryngeal nerve in the neck, proximal to the recording electrodes; and after section of the articular nerves distal to the recording electrodes. The effects of local anaesthesia of the joint capsule (with 2 per cent 'Lignocaine' hydrochloride solution), and of electrocoagulation of the joint capsule, were also examined.

In all experiments, the nerves and other exposed tissues were submerged in a pool of warm mineral oil. The animals were kept warm on a thermostatically regulated, heated operating table. Operative procedures were performed with microsurgical instruments and a Zeiss operating microscope.

Movements of the cricothyroid joint—but in caudal and anteromedial directions only—produced very brief bursts of rapidly adapting, high-amplitude potentials in

the articular nerves at the onset of movement, which passed proximally into the ipsilateral recurrent laryngeal nerve. No such responses were provoked by movements in other directions. No slowly adapting discharges were seen in the articular nerves during movement or sustained joint displacement; but release of the displaced joint gave rise to a second brief burst of rapidly adapting potentials in the articular nerves at the moment of release. These responses in the articular nerves were reversibly abolished (for about 40–60 min) by local anaesthetic infiltration of the joint capsule. They were irreversibly abolished by electrocoagulation of the joint capsule, and by section of the articular nerves distal (but not proximal) to the recording electrodes.

In the recurrent laryngeal nerve, the fast potential transients produced by joint movement were followed—after a short interval—by a more prolonged episode of irregular asynchronous potentials which was accompanied by a twitch of the adductor muscles of the larynx. This delayed asynchronous discharge (and the muscle twitch)—but not the initial rapidly adapting response—was irreversibly abolished by section of the recurrent laryngeal nerve proximal to the recording electrodes; and the same effect (but reversible) was produced by increasing general anaesthesia.

Bilateral section of the superior laryngeal nerves had no effect on the responses in the recurrent laryngeal nerve. Both the rapidly adapting and the delayed asynchronous discharges in this nerve (as well as the muscle twitch) were reversibly abolished by local anaesthesia of the joint capsule, and irreversibly abolished by electrocoagulation of the joint capsule and by section of the articular nerves. The muscle twitch, but not the rapidly adapting or delayed asynchronous discharges in the recurrent laryngeal nerve, was abolished by section of the recurrent nerve distal to the origin of its articular branches.

These experiments confirm that passive movements of the isolated cricothyroid joint in appropriate directions provoke reflex changes in the tone of the laryngeal muscles by impulses discharged into the ipsilateral recurrent laryngeal nerve through its articular branches<sup>4,5</sup>. They indicate that, after a brief central delay, these afferent impulses reflexly excite motoneurons in the brain stem from which efferent impulses are projected to intrinsic muscles of the larynx through motor fibres in the recurrent laryngeal nerve. They further indicate that the articular receptors responsible for the initiation of these reflex effects are rapidly adapting mechanoreceptors located in the capsule of the cricothyroid joint. Appropriate end-organs (Type II corpuscles) have been identified previously in the fibrous capsule of this and the other laryngeal joints<sup>1-3</sup>, and shown (in the case of the cricothyroid joint) to be innervated by large myelinated fibres in the articular branches of the ipsilateral recurrent laryngeal nerve<sup>3</sup>. The lack of slowly adapting mechanoreceptor discharges from the cricothyroid joint is explained by the absence of the appropriate category of end-organs (Type I corpuscles) from its capsule<sup>2</sup>—although a few such are present in the capsule of the crico-arytenoid joint<sup>3</sup>. The observations reported here indicate that the initial fast transient fired into the recurrent laryngeal nerve through its articular branches represents the response of Type II mechanoreceptors in the capsule of the cricothyroid joint to the stresses produced by movement of the joint; and that the delayed asynchronous discharge in the recurrent nerve represents the (polysynaptic) reflex response thereto in motor fibres innervating the intrinsic laryngeal muscles that provokes their changes in tone when the joint is moved<sup>4,5</sup>.

These findings provide further confirmation of the operation of articular mechanoreceptor reflexes in the larynx<sup>1,3-5</sup> contributing to the co-ordinated adjustment of the tone of its intrinsic muscles. They show that these reflex mechanisms are triggered primarily by very sensitive, rapidly adapting receptors in the laryngeal joint

capsules, from which impulses travel centripetally at high velocity in large myelinated afferent fibres in the laryngeal nerves. In addition, they support the view<sup>1,2,4</sup> that the principal innervation of the cricothyroid joint is by way of articular branches of the ipsilateral recurrent laryngeal nerve, and that the directions of movement of this joint that are critical for excitation of its capsular mechanoreceptors are those involving caudal and antero-medial displacements<sup>4,5</sup>.

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## Mechanism of Sugar Transport in Brain Slices

LITTLE work has been reported on the mechanism by which sugar is transported into brain cells. The results of the brain-perfusion experiments of Geiger *et al.*<sup>1</sup> suggest that a carrier-mediated transport process may be involved; but there are few quantitative data to support this possibility. This communication concerns the results of investigations of the transport of D-xylose into the cells of brain slices incubated *in vitro*.

Guinea-pig brain slices composed mainly of cerebral cortex were incubated for 30 min at 37° C in a bicarbonate medium similar to that recommended by Krebs and Henseleit<sup>2</sup>. The medium contained 12 mM sodium pyruvate to act as nutrient and 10 mM raffinose (see following), and was gassed with a mixture of 95 per cent oxygen/5 per cent carbon dioxide. Experiments showed that at the start of this incubation period the potassium content of the brain slice fell sharply while the sodium content increased. After some 20 min, however, potassium was re-accumulated and sodium extruded by the slices, so that by the end of the 30 min period the intracellular concentrations of these ions had stabilized at levels not differing greatly from those reported to occur *in vivo*<sup>3</sup>. The slices were then drained and quickly transferred to a second medium which was similar to the first, but contained, in addition, xylose or xylose and glucose or xylose and 2-deoxyglucose.

After a short period (usually less than 10 min because of the speed of the sugar-transport process) the slices were rapidly separated from the medium and analysed as follows. The total water content was determined from the wet and dry weights of slice samples. The xylose and raffinose contents of the tissues were estimated in protein-free solutions by the methods of Roe and Rice<sup>4</sup> and Cole<sup>5</sup>, respectively. The mean concentrations of xylose and raffinose in the tissue were expressed in terms of the spaces<sup>6</sup> occupied by these solutes. The tissue xylose was then partitioned between the apparent intra-

cellular (*Ic*) and extracellular (*Ec*) compartments, on the assumption that raffinose was entirely extracellular in distribution, as shown:

$$\text{Intracellular xylose concentration} = \frac{(\text{xylose space} - \text{raffinose space})}{(\text{total water} - \text{raffinose space})} \text{medium xylose concentration}$$

$$\text{Fractional penetration (f)} = \frac{Ic \text{ xylose concentration}}{\text{Medium xylose concentration}}$$

Values for the intracellular xylose concentrations expressed as fractions of the medium concentrations are given in Table 1 for a series of medium concentrations.

Increases in the intracellular xylose concentration failed to keep pace with increases in the medium concentration so that the fractional penetration of brain cells by xylose diminished as the medium concentration increased. If xylose were transported predominantly by a diffusion mechanism, the fractional penetration would be independent of the concentration of xylose in the medium. The results are rather what one would expect on the basis of a process which can be saturated such as carrier-mediated transport. The results further show that when the medium contains a low concentration of xylose the fractional penetration can exceed unity. This suggests that xylose can be accumulated in the cells of the preparation to concentrations exceeding that present in the incubation medium. When sets of slices from six brains were incubated independently in media lacking xylose, subsequent analysis showed that amounts of endogenous material, giving a mean value equivalent to  $10.7 \mu\text{g} \pm 3.1 \mu\text{g}$  xylose/g wet wt. tissue, were present. The experiments reported here involved the incubation of approximately 700 mg of tissue slices, and the slice xylose uptakes of approximately 120  $\mu\text{g}$  at the lowest medium concentration were corrected for the tissue blank. Although the error involved in the calculation of *f* at low medium xylose concentrations is very large, the small amount of endogenous material is insufficient to account for the increase in the tissue cell xylose concentration over the medium concentration. The results are taken to indicate, therefore, that the xylose transport process is an energy-requiring process permitting accumulation of sugar against a concentration gradient. The pronounced inhibition of the xylose uptake of brain cells by anaerobic conditions supports this conclusion.

The effects on xylose uptake of the addition of glucose and 2-deoxyglucose to the xylose-containing medium are also shown in Table 1. Both glucose and 2-deoxyglucose inhibit the uptake of xylose. The possibility that glucose is transported by the same system as the pentose was investigated. Lineweaver-Burk<sup>7</sup> plots of results from experiments measuring xylose-uptakes in the presence and absence of glucose are shown in Fig. 1. The results conform reasonably well to Michaelis-Menten kinetics. When glucose is present the slope of the regression line through the points is increased, but the intercept on the  $1/V$  axis is decreased. The difference between the intercepts of the two lines is not statistically significant, but more precise experiments are necessary to establish unequivocally whether or not glucose inhibits xylose transport competitively. The results give an apparent *K<sub>m</sub>* for xylose of 332 mM and a *V<sub>max</sub>* of 49 mM/l./min. If glucose is assumed to act as a competitive inhibitor the value of *K<sub>i</sub>* is 37 mM. The value of *V* corresponds to a

Table 1. XYLOSE PENETRATION OF INTRACELLULAR WATER OF BRAIN SLICES

Medium xylose concentration (mM)	1*	1	2	4	30	50	20	20 + glucose	50	50 + deoxyglucose
Incubation time (min)	10	10	10	10	10	10	4.5	4.5	4.5	4.5
Mean fractional penetration ( <i>f</i> ) ± S.E.M	0.89 (3) 0.06	1.81 (7) 0.36	1.73 (4) 0.22	1.20 (4) 0.18	1.05 (2) 0.05	0.82 (2) 0.04	0.74 (4) 0.12	0.35 (4) 0.08	0.50 (4) 0.15	0.20 (4) 0.09

Number of observations given in parentheses. The concentration of 2-deoxyglucose was 10 mM. The concentration of glucose was 50 mM.

\* Xylose-containing medium gassed with 95 per cent N<sub>2</sub>/5 per cent CO<sub>2</sub>.

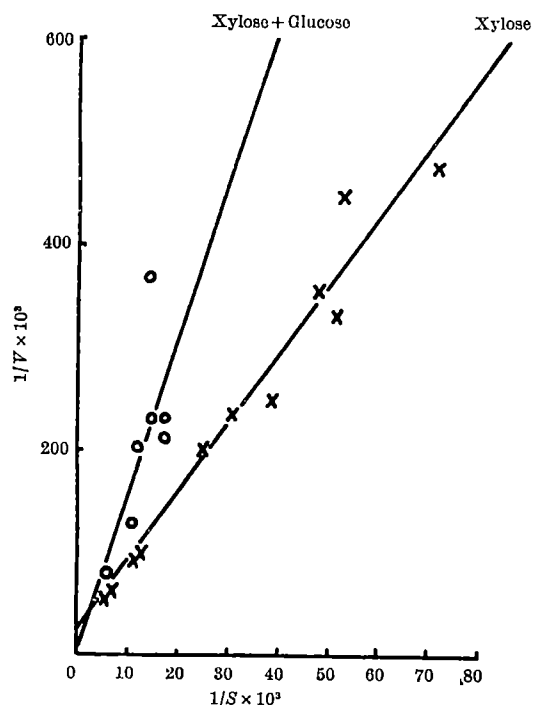


Fig. 1. Lineweaver-Burk plots of xylose uptake. Each point represents one experiment. The lines through the points were fitted by the method of least squares. The time of incubation with sugar was 4.5 min. Units:  $V$ , mM/l./min;  $S$ , mM/l.

maximal transport rate of approximately 750  $\mu$ M/g wet wt./h, and this is more than adequate to account for the highest rates of glucose utilization observed in cortical slices<sup>8</sup>.

The results presented here strongly suggest that a carrier mechanism of sugar transport operates in brain tissue, and that sugar can be actively transported by this mechanism. Experiments are now in progress to determine other characteristics of the transport system.

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### Effect of Norethynodrel on Pituitary Gonadotrophic Potency in Intact Male Rats

THE results described here were obtained during experiments on the mode of action of anti-gonadotrophic substances. They are reported because of the present-day interest in the action of steroids which suppress fertility, and to illustrate how assays of total gonadotrophin may be misleading in this context.

Adult male hooded rats with an average weight of about 350 g were injected subcutaneously with chemically purified norethynodrel dissolved in oil as described by Holmes and Mandl<sup>1</sup>, or with the oil alone. A total of 124 rats was used in six separate experiments in which 0.5 mg

norethynodrel was given daily for 7 days. The rats were killed on the eighth day, when it was found that in all the experiments norethynodrel had caused consistent and statistically significant reductions in the weight of the testes, seminal vesicles and prostate. In two further experiments using 30 rats, 0.1 mg norethynodrel was given daily for 4 days, the rats being killed on the fifth day. This dose caused less striking but still sometimes significant reductions in organ weights.

The anterior pituitary tissue from these rats was homogenized and precipitated as described previously<sup>2</sup>. Saline extracts of the dried material were assayed for follicle stimulating hormone (FSH) by the augmentation method using mice<sup>3</sup>, and for luteinizing hormone (LH) by a method based on published modifications of the method of Parlow<sup>4</sup>. Total gonadotrophin was assayed under the conditions of Brown and Billewicz<sup>5</sup>.

The lower dose of norethynodrel had little effect on the pituitary content of FSH (Table 1). The higher dose produced either little change or a considerable depression of FSH content: no reason for the variability was apparent.

Table 1. EFFECT OF NORETHYNOREL ON PITUITARY GONADOTROPHIC POTENCY

Experiment	Daily dose of norethynodrel (mg)	No. daily doses	Potency per gland of anterior pituitary tissue from rats treated with norethynodrel as % of potency per gland of tissue from control rats (95% fiducial limits)	
			Assayed as FSH	Assayed as LH
1	0.5	7	96 (38-240)	Not assayed
2	0.5	7	128 (74-240)*	170 (83-318)
3	0.5	7	44 (17-94)	Not assayed
4	0.5	7	39 (24-61)	166 (98-300)
5	0.5	7	52 (24-112)	Not assayed
6	0.5	7	51 (23-112)	127 (75-215)
7	0.1	4	106 (45-240)	139 (42-470)
8	0.1	4	87 (39-184)	143 (40-510)

\* This result was based on an assay of 2+1 design. All other assays were of at least 2+2 design.

In five of the experiments LH was assayed and in each case the pituitary tissue from rats treated with norethynodrel was more potent than tissue from rats treated with oil alone. The apparent increase in potency was not great in most instances and in experiments 2, 7 and 8 it was not associated with major changes in FSH content. In experiments 4 and 6, however, a rise in potency as LH accompanied a considerable fall in potency as FSH. The results suggest a change in the ratio of LH to FSH, but in many cases the fiducial limits were wide. Pituitary tissue from the first four experiments was, therefore, tested in the assay of total gonadotrophin to see whether a change in quality of the gonadotrophin might be reflected in a change in the slope of the dose-response lines (Fig. 1). In the first two experiments the lines for tissue

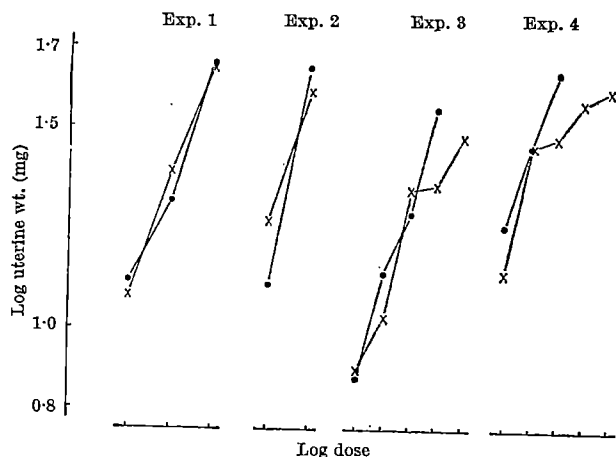


Fig. 1. Log uterine weight is plotted against log dose in the assay of total gonadotrophin. Lines for anterior pituitary tissue from rats treated with norethynodrel (x) are compared with lines for tissue from control rats (●) in experiments Nos. 1 to 4.



from treated and control rats were parallel. In Exps. 3 and 4, however, the lines were parallel at lower levels of response, but at the higher levels the lines for tissue from rats treated with norethynodrel tended to flatten out. This is compatible with an increase in pituitary LH relative to FSH<sup>5</sup>, the situation already suggested by the more specific assays.

These results are consistent with the hypothesis that norethynodrel in certain circumstances may prevent the release of pituitary LH. The findings in the assay of total gonadotrophin confirm a qualitative change in the pituitary gonadotrophin, and illustrate how potency estimates based on this method may be misleading if the design of the assay does not allow the detection of departure from parallelism. A similar and more striking example of this situation has been reported by Rothchild<sup>6</sup>, using material from rats treated with progesterone.

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## Phytate Metabolism in Animals

THERE has been interest for some years in the metabolism and availability of phytate phosphorus to animals. It has been reported<sup>1</sup> that phytate phosphorus was not detectable in the gut of ruminants below the reticulum, and it was concluded that micro-organisms were effective in hydrolysing phytic acid and its salts. Faeces of ruminants were capable of inducing the hydrolysis of the phytate of bran when mixed with it, and resulted in a reduction of 16 per cent of the total added phytate phosphorus after 7 days storage at 0° C.

In the case of poultry, the extent and cause of phytate hydrolysis are less well established. Common<sup>2</sup> concluded that phytate was not completely hydrolysed in the alimentary tract of pullets, and this finding has been confirmed for chicks<sup>3,4</sup>. He further showed that the recovery of phytate in the droppings of birds was greater when the diet was supplemented with calcium carbonate than when it was supplemented with calcium phosphate. Work carried out in this Department and in the Department of Agriculture<sup>5</sup> has shown that the recovery of phytate in the droppings of laying birds rises as the percentage of calcium in the diet rises.

Recently an experiment has been reported<sup>4</sup> in which droppings were collected daily and stored cumulatively

for periods up to 7 days at 35° F before analysis for phytate. In order to test whether or not phytate hydrolysis might occur during such a storage period, a bulk sample of droppings from a number of adult (mixed sex) birds was collected within 30 min of voiding, and mixed into a homogenous mass. Two sub-samples of about 3 g each were taken, dried in an oven at 100° C for 24 h, ground in a laboratory mill, and analysed for total phosphorus<sup>6</sup> and phytate phosphorus<sup>7</sup>. Further duplicate sub-samples taken over the following five days from the primary sample, which stood during this time in a loosely covered dish at 50°–60° F, were similarly treated. The initial sample contained 4.83 per cent calcium in the dry matter. The rise in the total phosphorus content suggests that micro-organisms were actively oxidizing organic matter, while the gradual rise in the proportion of phytate phosphorus to total phosphorus suggests that overall hydrolysis was not occurring but that on the contrary slight net phytate synthesis was taking place. The results of this preliminary trial suggest that changes in the total and phytate phosphorus content expressed as percentages of the dry matter and also of the phosphorus distribution, possibly caused by micro-organic activity, may occur if there is an appreciable delay between the time of collection of poultry droppings and their analysis.

This work also supports recent findings<sup>8</sup> that phytate synthesis occurred in the faecal samples collected from chicks fed a phytate-free, casein-gelatin based diet when kept under conventional conditions. When similar chicks were maintained in a germ-free environment, however, the faeces contained no phytate.

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## Pancreatic Function during Hypothermia in the Isolated Canine Pancreas

RECENTLY, Véghelyi *et al.*<sup>1</sup> have reported that pancreatic hypothermia leads to diminished pancreatic secretion and diminished enzyme output. Using the isolated denervated canine pancreas<sup>2</sup>, we have been able to perfuse the gland with a cooled perfusate and to analyse the pancreatic secretion for volume and protein output.

As can be seen from Table 1, there is no change in pancreatic juice flow or in protein output when stimulated with 'Pancreozymin' and 'Secretin'.

Table 1. COMPARISON OF NORMAL AND HYPOTHERMIC PERFUSED CANINE PANCREAS

	Normal	Hypothermic
Volume (ml./min)	0.40	0.38
Juice protein (mg./ml.)	68	66

The temperature of the perfusate was 27° C. The gland was stimulated by injection of 25 units of 'Secretin' and 25 units of 'Pancreozymin' obtained from Boots Pure Drug Co., Ltd., Nottingham, England.

These results, when taken in conjunction with those of Véghelyi, would seem to indicate that pancreatic responses to hypothermia are related to neural control. It must be emphasized that the isolated-perfused gland is denervated and that the local reflexes, which Grossman<sup>3</sup> makes mention of, are probably not operative. Furthermore, it is important to realize that the nature of 'Pancreozymin' and 'Secretin' released in the normal intact animal is different from the direct stimulation of the isolated

Table 1. CHANGES IN THE COMPOSITION OF FRESH POULTRY DROPPINGS WITH TIME

Time (days)	Total phosphorus (% of dry matter)	Phytate phosphorus (% of dry matter)	% Phytate phosphorus % Total phosphorus
0.0	1.50	0.368	24.5
0.5	1.52	0.367	24.1
1.0	1.54	0.379	24.5
1.5	1.55	0.379	24.5
2.0	1.62	0.361	22.3
2.5	1.63	0.402	24.7
3.0	1.64	0.425	25.9
3.5	1.75	0.442	25.3
4.0	1.70	0.452	26.6
5.0	1.74	0.467	26.8

gland. Nevertheless, this type of study gives insight into the differentiation of neural and hormonal activation in pancreatic physiology.

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## PHARMACOLOGY

### Renin-angiotensin System in the Spontaneously Hypertensive Rat

Okamoto and Aoki<sup>1</sup> have reported the separation of a strain of Wistar rats which exhibit spontaneous hypertensive cardiovascular disease. Some of these animals ( $F_2$  by their lineage) have been placed at my disposal, and I have confirmed the occurrence of hypertension in their offspring. I have also determined the activity of the renin-angiotensin system in the kidney by three different methods<sup>2</sup>. The left kidney was grafted on to a rat nephrectomized bilaterally 24 h previously and anaesthetized with pentobarbital sodium (40 mg/kg, intraperitoneally). Pressor activity of the renal venous effluent (renin release) was determined by the pressor response after graft with a definite period (10 min) of interruption of the blood supply to the kidney. The opposite kidney was kept frozen. Pressor activity of the crude extract (renin content) was also determined by injecting it into the bilaterally nephrectomized assay rat. The extract was then acidified to pH 3.0, and incubated at 0° C for 30 min to remove angiotensinase. Various amounts of the pre-treated extract were diluted to 1 ml. and incubated with an equal volume of non-haemolysed rat plasma and M/15 phosphate buffer (pH 6.5) containing 0.002 per cent of thimerosal and  $10^{-5}$  M of ethylenediamine tetraacetic acid at 37° C for 10 min. The angiotensin thus formed was determined by its pressor action in the rat anaesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) and treated with pentolinium tartrate (5 mg/kg, intravenously). The reaction constant ( $K$ ) was calculated to express the angiotensin-forming activity of renin in the kidney extract.

Four male and two female hypertensive rats, 15-50 weeks of age, with blood pressures of 160-210 mm mercury were examined. Three male and three female normal Donryu rats were used as controls. The rats used for assay were females of the Donryu strain, weighing 200-225 g. The results are shown in Table 1. A similar decrease in activity of the renin system has been found previously in other types of experimental hypertension<sup>3,4</sup> and is consistent with the observed decrease in the juxtaglomerular granulation<sup>5</sup>. Therefore, this investigation adds further support to the concept that renin plays no part in the pathogenesis of hypertension.

Table 1. RENIN-ANGIOTENSIN SYSTEM IN THE SPONTANEOUSLY HYPERTENSIVE RAT

	Control	Hypertensive
Renin release (mm Hg)	25 ± 3.8	13 ± 4.6
Renin content (mm Hg)	34 ± 2.6	21 ± 1.9
Angiotensin formation ( $K \times 10^3$ )	125 ± 13	55 ± 8.9

Figures are mean ± S.E.

I thank Profs. H. Kumagai and S. Ebashi for their advice.

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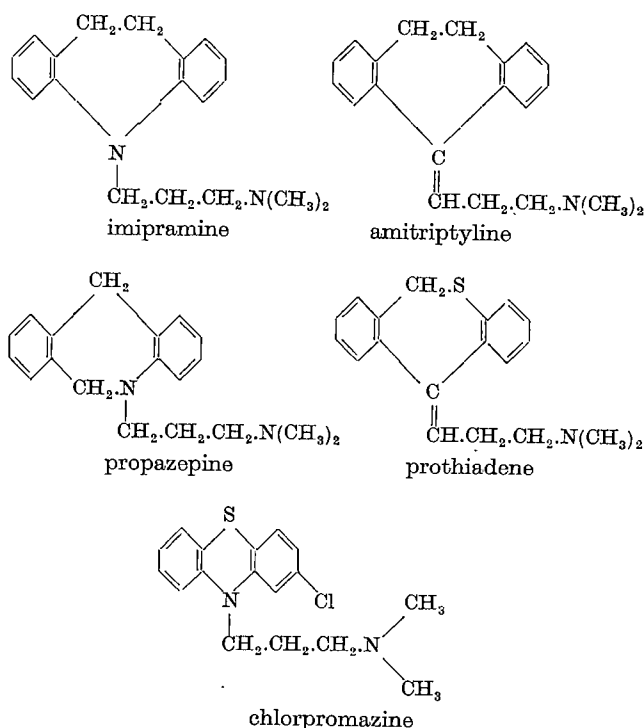
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### In vitro Interference of Antidepressants with Lipolytic Action of Adrenaline on Adipose Tissue

DURING an investigation of the known antidepressant imipramine ('Tofranil'), this drug was not found to inhibit amino-oxidase, which indicated that this enzyme is not a pre-requisite for antidepressant action<sup>1,2</sup>. The possibility has been raised that imipramine exerts its antidepressive effect by sensitization of a central nervous adrenergic mechanism<sup>3</sup>. Later experiments have shown that among a variety of drugs imipramine markedly reduced *in vivo* concentration of administered tritiated noradrenaline in the heart, spleen and adrenal gland, and concomitantly elevated the plasma level of administered tritiated noradrenaline for the first 5 min. These two facts probably reflected the inhibition of the uptake of catecholamine by tissue caused by imipramine<sup>4</sup>.

Since 1958 rat adipose tissue has been known to respond *in vitro* to catecholamines by enhanced free-fatty-acid mobilization<sup>5</sup>. The question has arisen whether and how this hormonally induced tissue response would be influenced by the presence of antidepressants.

Experiments were carried out with well-known antidepressants, imipramine, norimipramine, amitriptyline, noramitriptyline, including less widely used prothiadene, northiadene and propazepine of similar pharmacological properties<sup>6-11</sup>. In addition, the tranquillizer, chlorpromazine, was included in this investigation. Chemical relationship of all these drugs follows from their structural formulæ:



Norimipramine, nortriptyline and northiadene represent desmethyl derivatives of the parent substances.

Drugs under testing were added into 1.5 ml. of medium (Krebs-Ringer phosphate buffer with neutralized 2 per cent bovine plasma albumin, pH 7.6) where pieces of epididymal fat pad lying proximal to a visible blood vessel, weighing about 100 mg, were incubated. The final concentration of drugs was  $5 \times 10^{-3}$  M or  $1 \times 10^{-3}$  M. Epididymal adipose tissue was excised from bled albino rats. The tissue was incubated while shaking at 38° C for 2 h. Each drug was tested in 6 tissue samples without adrenaline and in 6 samples with adrenaline of final concentration  $3 \times 10^{-5}$  M. The amount of liberated long-chain fatty acids in the incubation medium was determined basically using Dole's method<sup>12</sup>. Means  $\pm$  S.E. are summarized in Table 1. All substances were found to decrease the lipolytic effect of adrenaline. By the decrease of this effect expressed in percentage of control values (Table 2), imipramine and its isomer propazepine are more potent than prothiadene and amitriptyline. What is remarkable is the structure-activity relation between the desmethyl derivatives and their parent drugs, the former having, by far, stronger antiadrenaline action in this simple test.

Table 1. EFFECT OF ANTI-DEPRESSANTS AND CHLORPROMAZINE ON BASELINE AND ADRENALINE-STIMULATED RELEASE OF FATTY ACIDS FROM ADIPOSE TISSUE

Exp. No.	Concen- tration	Drug	- Adrenaline	+ Adrenaline	Difference
1		Control	2.94 $\pm$ 0.86	16.15 $\pm$ 0.51	13.21
	0.005 M	Imipramine	2.77 $\pm$ 0.65	5.09 $\pm$ 0.56	2.32
2		Control	1.81 $\pm$ 0.32	12.16 $\pm$ 0.54	10.35
	0.001 M	Imipramine	1.84 $\pm$ 0.38	8.67 $\pm$ 0.501	6.83
	0.001 M	Norimipramine	3.54 $\pm$ 0.39	5.08 $\pm$ 0.38	1.49
3		Control	1.06 $\pm$ 0.22	17.77 $\pm$ 0.69	16.71
	0.005 M	Amitriptyline	2.27 $\pm$ 0.19	9.88 $\pm$ 0.602	7.61
	0.005 M	Nortriptyline	1.55 $\pm$ 0.14	1.80 $\pm$ 0.18	0.25
	0.005 M	Prothiadene	1.74 $\pm$ 0.16	8.17 $\pm$ 1.01	6.43
4		Control	2.5 $\pm$ 0.16	12.82 $\pm$ 0.83	10.67
	0.005 M	Northiadene	1.41 $\pm$ 0.208	1.80 $\pm$ 0.27	0.49
	0.005 M	Propazepine	1.35 $\pm$ 1.74	2.38 $\pm$ 0.93	1.03
	0.005 M	Chlorpromazine	2.23 $\pm$ 0.58	4.43 $\pm$ 0.68	2.20

Table 2. DECREASE OF ADRENALINE-STIMULATED FATTY ACID-MOBILIZATION EXPRESSED IN PERCENTAGE

Drug conc.	Parent substance	%	Monomethyl derivatives	%
	Control	100.0	—	—
	Propazepine	9.65	—	—
	Imipramine	17.5	—	—
0.005 M	Chlorpromazine	20.6	—	—
	Prothiadene	40.0	Northiadene	4.50
	Amitriptyline	47.6	Nortriptyline	1.56
0.001 M	Imipramine	65.9	Norimipramine	14.4

It is well known, and interesting in this connexion, that secondary amines (desmethyl derivatives) are more potent and more rapidly acting antidepressive drugs than their respective tertiary amines at the therapeutic dose. It is thought that desmethylimipramine, accumulated in the brain after demethylation of imipramine, represents the active antidepressive substance, its pharmacological action being dependent on the presence of free catecholamines in the brain. Even when it is hard to decide if the present studies might reflect in some reduced form the complex central stimulating antidepressive action, the results reported here seem to be consistent with the hypothesis that imipramine-like drugs considerably interfere with catecholamine metabolism<sup>4</sup>.

It is to be noted that amitriptyline and nortriptyline have also been found to be active anticholinergic agents. Their pharmacological action has been found somewhat atypical because amitriptyline suppressed salivation at lower doses than nortriptyline and, *in vitro* on guinea-pig ileum, amitriptyline was twice as active as nortriptyline<sup>16</sup>.

Chlorpromazine has been shown here and elsewhere<sup>4,17</sup> to possess similar properties to antidepressants despite its distinct clinical use as a tranquillizer. Bickel *et al.*<sup>18</sup> have shown, however, that tranquillizing and antidepressive pharmacological action is intimately related. Not only imipramine and amitriptyline—weak tranquillizers *per se*—but also phenothiazines, promazine and trifluorpromazine

—strong tranquillizers—could be changed into potent antidepressants by simple demethylation. Since such demethylation process occurs *in vivo*, considerable change of pharmacological properties of parent drugs could be expected if they should be metabolized by different rate and route.

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## HAEMATOLOGY

### Platelet Reaction to Adenosine Diphosphate *in vivo*

ADENOSINE diphosphate (ADP) causes agglutination of human<sup>1-3</sup>, cat<sup>4</sup> and rabbit<sup>5,6</sup> platelets *in vitro* and has also been shown to produce a rapid though transient fall in the number of circulating platelets when injected intravascularly<sup>4-6</sup>. Born and Cross<sup>4</sup> postulated that the intravenous injection of the nucleotide led to a drop in the platelet count because "platelets adhere to endothelium and form aggregates which are trapped in the smallest vessels". To test the hypothesis we observed platelet behaviour in small vessels following intravascular injection of ADP.

Transparent rabbit ear chambers were inserted in the left ear of 6 semi-lop-eared rabbits by the method of L'heru *et al.*<sup>7</sup>. Three to four weeks later the animals were anaesthetized with intravenous 'Nembutal' (50 mg/kg initially and 10-15 mg/kg as required afterwards) and the common carotid artery supplying the ear chamber was exposed. A 25-gauge hypodermic needle was inserted in the artery and connected by polythene tubing to a syringe in a slow infusion pump. Infusion of physiological saline was started immediately. Afterwards a  $10^{-2}$  molar solution of ADP (Sigma Chemical Co., St. Louis) in physiological saline (pH 7.3-7.4) was given for 10 min at the rate of 0.28 ml./min and then physiological saline was recommenced. Vessels in the ear chamber were observed at a magnification of 400.

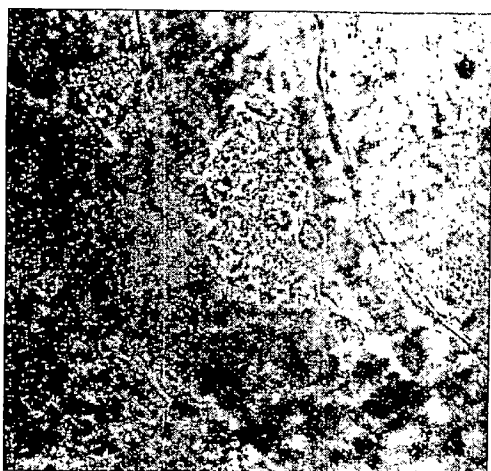


Fig. 1. Platelet aggregate incorporating leucocytes arrested in blood-stream. Flow from left to right. ( $\times 480$ )

All the ear chambers showed mild inflammatory changes characterized by leucocytes 'sticking' in some vessels; most adhered to the endothelium momentarily, but others were attached for long periods. Single platelets, rather than clumps, were found in the marginal blood flow. They adhered to the surface of leucocytes which either stuck to or rolled along the endothelium. Platelets did not roll along the vessel wall and only rarely stuck to the endothelium. When they did, their site of attachment was extremely localized and usually only one adhered. At times 2-5 platelets were attached at the same site, like pins to a magnet, before being swept away. Such were the findings observed during the infusion of physiological saline at the same rate as, or at a faster rate than, that used to administer the test agent.

Within 20-40 sec of commencing the ADP infusion the circulation slowed temporarily. The change varied in intensity from one animal to the next and may have been due to vasodilatation<sup>6</sup> and/or hypotension<sup>4</sup> induced by the agent. Within 2-4 min aggregates of platelets either alone or incorporating leucocytes and erythrocytes were seen in the stream (Fig. 1) adhering to leucocytes and endothelial spikes<sup>8</sup> or impacted in capillaries thereby obstructing flow. Some aggregates were undoubtedly swept into the chamber in the arterial stream, but others formed *de novo* in slow-moving blood columns.

Platelet thrombi and emboli were seen throughout the period of infusion and tended to disintegrate when washed into a fast-moving stream. Platelet adhesion to leucocytes was more frequent, but the tendency to adhere to endothelium was not increased. No morphological change was observed in platelets, leucocytes, erythrocytes or endothelial cells. After ADP infusion ceased blood flow improved and aggregates disintegrated; not all clumps disappeared simultaneously, as occurs *in vitro*<sup>3</sup>. In some instances disintegration was delayed in stagnant and impacted vessels; nevertheless all clumps had usually disappeared within 10 min.

Two animals were given an identical infusion of ADP 0.5 h later and one, a third, 0.5 h after the second. The changes observed were similar though not so prominent and their onset was slightly delayed.

The site of ADP infusion affected the intensity of the observed reaction. Infusion into the femoral or the marginal ear vein of 3 rabbits also caused platelet aggregation in the ear chamber, but it was much less prominent than when ADP was injected into the carotid artery.

In three animals (two with venous infusions and one with a carotid) 0.5 ml. blood was taken from a polythene cannula in the femoral artery at set periods before, during and after the infusion. The samples were collected in glass tubes containing 1 mg dried disodium ethylene-

diamine tetraacetic acid and platelet (Brecher-Cronkite method)<sup>9</sup> and leucocyte counts were made. A 30-50 per cent reduction in platelet count was recorded during the infusion, confirming the work of Regoli and Clark<sup>2</sup>; a parallel drop in the leucocyte count also occurred.

These experiments demonstrate that the intravascular administration of ADP is associated with intravascular agglutination of platelets and also with their adhesion to leucocytes. Platelet thrombi adhere to leucocytes and endothelial spikes and on other occasions become impacted in small vessels, but in the rabbit their adhesion to endothelium is not a prominent feature. The drop in platelet count recorded during ADP infusion is probably due to the trapping of aggregates in blood filters and also to their sequestration in small vessels, as occurs when other substances are injected intravenously<sup>10</sup>, rather than to their 'pavementing' on endothelium. Perhaps the incorporation of leucocytes in the platelet thrombi explains the simultaneous leucopenia recorded in this work.

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### Serum Delta-Globulin in the Young Fowl (0-29 Days) and in Other Avian Species

Fowl serum contains a protein which has a lower mobility than  $\gamma$ -globulin in agar-gel electrophoresis at pH 8.6 and was therefore designated  $\delta$ -globulin<sup>1</sup>. This component of serum is exceptional in that it has a molecular weight of only about 20,000 and that prolyl constitutes approximately 12 per cent of its amino-acid residues (Richards, to be published). A method for isolating  $\delta$ -globulin has been described<sup>1</sup>, but it is complicated by the fact that only small amounts of this component are present in adult fowl sera. For this reason, and in view of its unusual characteristics, its occurrence in young birds and in other avian species was investigated.

Immuno-electrophoretic analysis of the sera of young chicks (Fig. 1) showed that  $\delta$ -globulin was present in the serum of the newly-hatched chick and seemed to increase rapidly up to the age of 4 days, although none was found in the 1-day-old chicks. In the case of chicks up to the age of 2 days the sera of several birds were pooled. When sera of older birds, up to the age of 29 days, were investigated, it was found that  $\delta$ -globulin was always present, but the immuno-electrophoretic technique gave no clear indication of the amount in individuals, the occurrence and intensity of the  $\delta$ -globulin line being remarkably variable.

Fig. 2 shows the results of immuno-electrophoresis of turkey, duck, guinea-fowl and pheasant sera, using rabbit antiserum to fowl and turkey sera. A  $\delta$ -globulin line was obtained with guinea-fowl serum but not with the others. Similar experiments with pigeon, quail and goose sera and

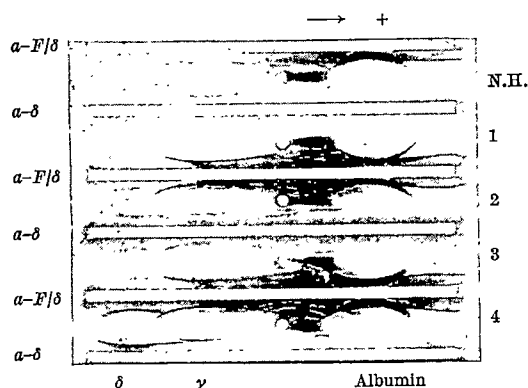


Fig. 1. Immunoelectrophoresis of sera of newly-hatched (N.H.) to 4-day-old chicks showing the presence of  $\delta$ -globulin. Rabbit antisera in the troughs:  $a-\delta$ , antiserum to fowl  $\delta$ -globulin.  $a-F/\delta$ , a mixture of  $a-\delta$  and  $a-F$ .

with fowl egg-yolk and egg-white suggested that  $\delta$ -globulin was present in goose serum and in egg-yolk. This does not prove that  $\delta$ -globulin was absent in those cases where a precipitation line was not observed. In order to obtain a reasonably strong line in immuno-electrophoresis of fowl serum it is often necessary to use serum which has been concentrated by a factor of three<sup>1</sup>. No  $\delta$ -globulin line was observed when concentrated turkey plasma was used in immuno-electrophoresis, but absorption of rabbit anti-fowl serum with turkey plasma completely abolished the line produced by the reaction of concentrated fowl serum with unabsorbed anti-fowl serum. It may be that fowl  $\delta$ -globulin has antigenic determinants in common with some other protein of turkey plasma, but it is much more likely that turkey plasma contains a very small amount of  $\delta$ -globulin which has not yet given rise to an observable precipitation line in immuno-electrophoresis. This conclusion is supported by the presence of antibody to  $\delta$ -globulin in rabbit anti-turkey serum; the line given by this antibody with the  $\delta$ -globulin of guinea-fowl is clearly seen in Fig. 2.

Its low molecular weight and its electrophoretic heterogeneity (Fig. 3) suggested that  $\delta$ -globulin might be a molecular fragment of one of the immune globulins. Attempts to demonstrate cross-reactions between  $\delta$ - and immune-globulins have shown no such relation. Sera of

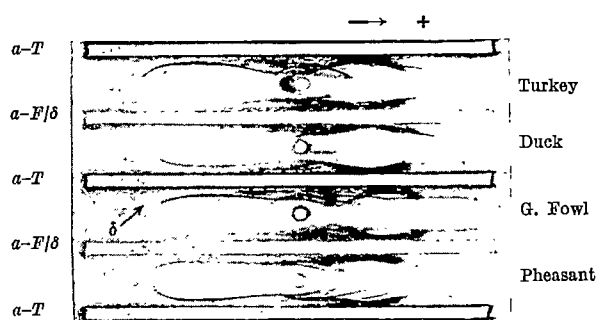


Fig. 2. Immunoelectrophoresis of sera of several species showing the presence of  $\delta$ -globulin in guinea-fowl serum and of antibodies to this component in the anti-turkey ( $a-T$ ) serum.

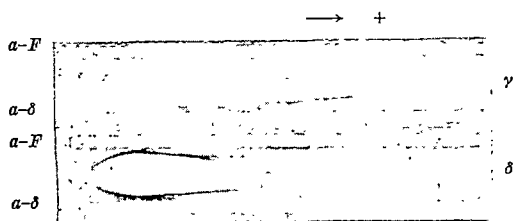


Fig. 3. Comparative immunoelectrophoresis of fowl serum  $\gamma$ - and  $\delta$ -globulins illustrating the electrophoretic heterogeneity of both proteins.  $a-F$ : rabbit antiserum to fowl serum.

bursectomized fowl in which the amount of  $\gamma$ -globulin was greatly reduced<sup>2</sup> were found to contain as much  $\delta$ -globulin as normal controls.

The presence of  $\delta$ -globulin in the serum of the newly hatched chick and even in the yolk of the new-laid egg, together with its occurrence in other avian species, supports the opinion that  $\delta$ -globulin is a normal component of serum. Its purpose and function have yet to be determined.

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### Quantitative Estimation of Human Serum Haptoglobins by an Immunological Method

ALL quantitative estimations of haptoglobin (Hp) are based on the measurement of its haemoglobin- (Hb-) binding capacity, and errors may arise from a decreased or inhibited affinity of haptoglobin for haemoglobin. The results of the peroxidase activity method<sup>4</sup> may be influenced by serum factors inhibiting the peroxidase activity of the Hb-Hp complex. Robert, Bajic and Jayle<sup>1</sup> have been able to show that certain chemical substances, such as heparin and glycine, will not only decrease the affinity of the haptoglobin and the peroxidase activity of the Hb-Hp complex but will also break down Hb-Hp complexes that have already formed. Analogously the influence of serum components on the Hb-Hp complex has been taken as a possible explanation for the often decreased serum Hp level in liver diseases<sup>2</sup>. The development of a method for direct determination of Hp was therefore of interest.

We have been able to estimate human serum haptoglobins quantitatively by an immunological method and to correlate the results with Hp values determined indirectly by the peroxidase activity (activation) method<sup>3,4</sup>. Antisera against human serum haptoglobins were produced in rabbits. Highly purified haptoglobins from a pooled serum containing the different types of haptoglobin prepared by the method of Kluthe and Isliker<sup>5</sup> were used as antigen. In addition we used a preparation which was kindly put at our disposal by Dr. Steinbuch of Paris. Sensitization was started by intravenous injections of the antigen followed by intracutaneous injections with Freund's adjuvant. The development of precipitating antibodies against human Hp was observed by the technique of Ouchterlony<sup>6</sup> and by immuno-electrophoresis<sup>7</sup>. Traces of antibodies directed against other serum proteins were easily removed by absorption with small amounts of a haptoglobinaemic human serum.

For quantitative immunological determination a dilution of the serum was mixed with an excess of antiserum and the amount of antigen-antibody complex was measured by the nephelometric method of Schultze and Schwick<sup>8</sup>. On a number of sera the results were checked by a chemical method (Kunkel and Ward<sup>9</sup>) and good agreement was found. Photometric readings were calibrated against dilutions of the purified haptoglobins.

Fig. 1 shows the results in 82 sera of apparently healthy persons in which haptoglobin was determined simultaneously by the immunological and by the peroxidase activity (activation) method. A linear relationship was observed in concentrations from 25 to 160 mg/100 ml. Differences between the regression coefficients for the three genetically determined types were not obvious.



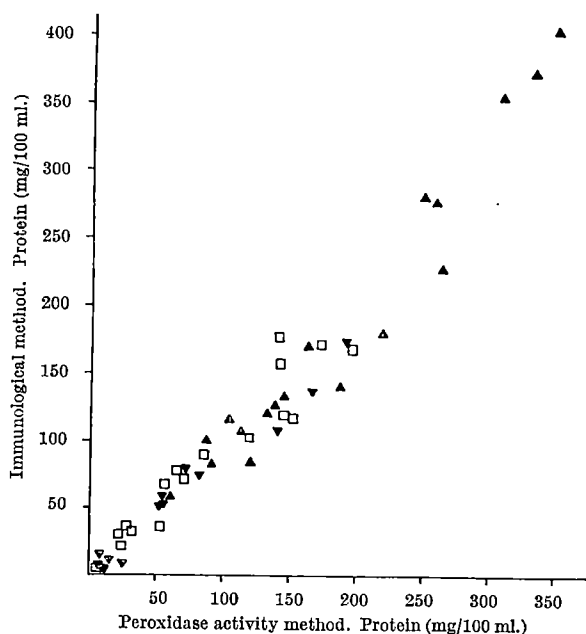


Fig. 1. Serum haptoglobin in 82 normal sera, determined by the immunological and by the peroxidase activity methods

▲ Renal diseases:  $y = 1.11x - 14.7$ ;  $r = 0.97$   
 □ Liver diseases:  $y = 0.98x + 1.4$ ;  $r = 0.93$   
 ▼ Blood diseases:  $y = 0.85x + 3.1$ ;  $r = 0.97$

( $P(b_{1-1} - b_{2-2}) > 0.1$ ;  $P(b_{1-1} - b_{2-1}) > 0.3$ ;  $P(b_{2-1} - b_{2-2}) > 0.3$ .) The types were kindly estimated by Dr. Hummel, Hygieneinstitut, Freiburg. Similar results were obtained with sera of patients suffering from different conditions which are apt to influence the Hp content of serum (Fig. 2).

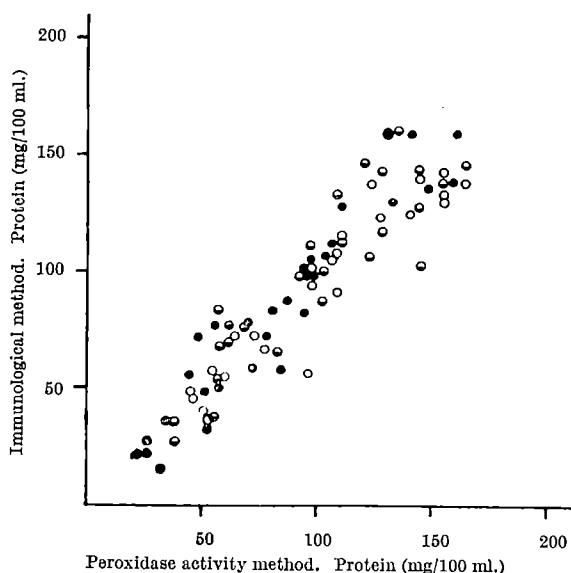


Fig. 2. Serum haptoglobin in 51 patients with various diseases, determined by the immunological and by the peroxidase methods

Type 1-1 ○ :  $y = 0.89x + 2.1$ ;  $r = 0.93$   
 Type 2-1 ● :  $y = 0.97x + 4.4$ ;  $r = 0.94$   
 Type 2-2 ● :  $y = 1.06x - 3.3$ ;  $r = 0.96$

The regression coefficient of the pathologic sera as a whole was not significantly different from that of the normal group. Slight differences were observed between the regression coefficients of the renal diseases ( $P = 0.06$ ), the hæmatological diseases ( $P = 0.01$ ) and the normal sera. These findings may be explained by the uncertainty of the determination of very low, and high, Hp values by

the peroxidase activity (activation) method. The good agreement between the immunological and peroxidase activity values in normal sera, as well as in sera from patients with various diseases, strongly suggests that Hp levels measured by indirect methods are actually caused by the real haptoglobin content. Analogously, it is unlikely that low Hp levels in liver diseases<sup>2,3,11</sup> are the result of an impaired formation of the Hb-Hp complex or of chemical inhibition of its peroxidase activity.

The immunological determination of Hp has also a practical importance in measuring very small amounts of haptoglobin. Traces of the protein which give no significant values with the indirect methods could be detected by testing the sera against antiserum in Ouchterlony plates. In hæmolytic disorders with greatly elevated hæmoglobin turnover in which insignificant peroxidase activity was found, the exhaustion of haptoglobin was proved by immunological technique (Kluthe and Heimpel<sup>10</sup>).

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### An Improved Amperometric Procedure for the Rapid Determination of Reduced Glutathione in Red Cells

THE determination of sulphhydryl groups in protein by amperometric titration in neutral aqueous solution has been reported by Benesch, Lardy and Benesch<sup>1</sup>. The use of this method for the estimation of glutathione in animal tissue has been considered in detail by Thomson and Martin<sup>2</sup>. One disadvantage of this amperometric titration technique is the transient life of the agar-salt bridge which connects the reference electrode to the reaction vessel containing the rotating platinum electrode. This communication describes a modification of the technique in which the agar-salt bridge is replaced by a ceramic plug which permits direct contact between the reference electrode and the reaction solution. In addition to this, the use of a motor which rotates the platinum electrode at a constant speed has given improved reproducibility of the galvanometer deflexion.

A diagram of the apparatus is shown. The rotator (Mullards, Ltd., London) is connected to the platinum electrode which rotates in *tris* buffer containing potassium chloride as described by Thomson and Martin<sup>2</sup>. The constituents of the reference electrode are the same as those of Benesch *et al.*<sup>1</sup>. The ceramic plug (Electronic Instruments, Ltd., Richmond, Surrey) is in direct contact with the reaction solution. When not in use the platinum electrode is stored in de-ionized water and the plug is covered with a rubber teat filled with the reference electrode solution. Before use the plug is washed vigorously with distilled water.

The apparatus is used for the routine determination of red-cell reduced glutathione as follows: fresh whole blood (0.1 ml. from a finger prick) is added to 0.5 ml. of distilled water and mixed gently to hæmolyse the cells. Sulphosalicylic acid (0.4 ml. of 6 per cent w/v) is added and the

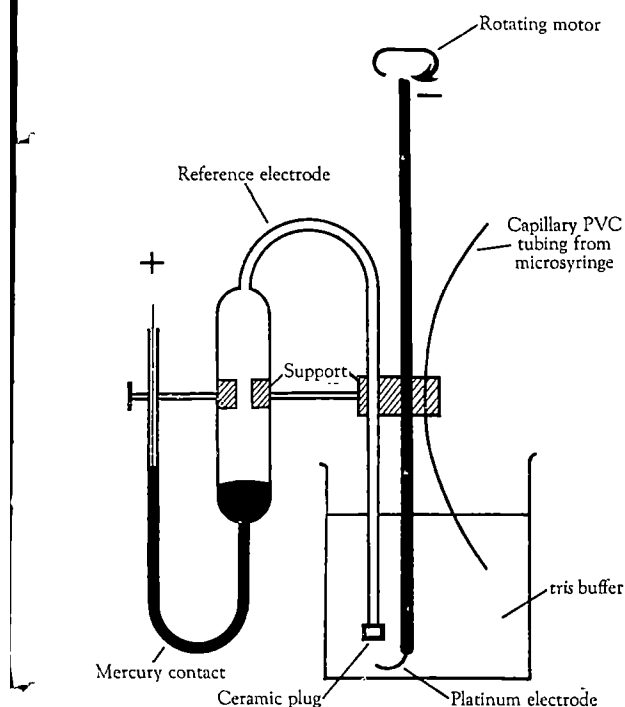


Fig. 1. Diagram of apparatus showing essential components

suspension carefully but thoroughly mixed to precipitate protein material. After standing for 2 min the suspension is centrifuged rapidly and a 0.5 ml. aliquot of the clear supernatant is added to 15 ml. of tris buffer (0.17 M) containing potassium chloride (13 mM) at pH 7.3. The titration is carried out as described by Thomson and Martin using silver nitrate solution (1 mM) dispensed from an 'Aglar' microsyringe fitted with PVC capillary tubing leading directly into the solution. Galvanometer deflexions are plotted against volume of silver nitrate added. The galvanometer deflexions are somewhat greater for a given amount of silver nitrate than those reported in the article of Thomson and Martin.

I thank Drs. S. G. Semple and A. H. Bottomley and Mr. R. W. Halls for their advice.

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## PATHOLOGY

### Reversal of Osteoporosis in Lactating Female Rats by Tricalcium Phosphate

PHYSIOLOGISTS as well as astronomers have difficulty in dealing with three bodies, and it is much simpler to consider only two at a time. Calcium, vitamin D and phosphate made bone growth so complicated that paediatricians neglected phosphate. But recently Widdowson *et al.*<sup>1</sup> have shown that phosphate may be a limiting factor. For 40 years, one of us has used the rat, which does not require vitamin D. In the rat, lack of phosphate stops the calcification of bone, but lack of calcium stops the formation of osteoid tissue. Albaum<sup>2</sup> showed that low calcium in the medium caused loss of ATP in bone cultures. Montorsi and Morisi<sup>3</sup> and others have shown that ATP favours the production of osteoid. Osteoid is the characteristic of rickets, and suppression of its pro-

duction by loss of ATP might change the rickets into osteoporosis. A weaned rat, placed on a low calcium diet, shows signs of rickets for a short time only. The rickets change into osteoporosis without a change of diet. To avoid this reversal, we used adults. Chossat<sup>4</sup> showed that a wheat and water diet would produce osteoporosis in birds.

We used lactating female rats so that the demand for calcium would be high, and males of the same age were used as controls. Six lactating female rats and 2 males were placed on the following diet, containing 0.04 per cent calcium: 79 per cent freshly ground yellow maize, 10 per cent dry beef liver, 10 per cent maize oil and 1 per cent sodium chloride.

The two males were each given 250 mg of tricalcium phosphate per day for 110 days and were then killed. Both had normal bones.

The 6 lactating females were kept on the low calcium diet for 60 days, when 3 were killed. These 3 showed basophilia of the bony trabeculae, fibrosis and increased clasmatoctytic activity (all characteristics of osteoporosis) of femur and spine. The degree of osteoporosis varied from 2+ to 3+ on a 4-point scale.

The remaining 3 lactating females were kept for a total of 110 days on the diet, but during the last 50 days were each given 250 mg tricalcium phosphate per day, and then killed. Two of them showed normal structure of femur and spine; the other had osteoporosis to a '1+' degree.

We therefore conclude that it may require about as long a time to cure osteoporosis as to produce it; when 60 days on a low calcium diet was used to produce osteoporosis, 50 days on a high calcium diet cured two rats and almost completely cured the third.

Calcium phosphate was used instead of other forms of calcium because in previous experiments, when calcium lactate or carbonate were used, kidney stones resulted. To flood the body with excess calcium seems dangerous, but when there is enough phosphate to anchor it in the bones or precipitate it in the faeces, the danger is eliminated.

This communication does not discuss human nutrition, except to point out that the addition of calcium to betel nut and coca and maize has not always been noted in the calculation of the calcium intake of primitive people. The boiling of maize in lime water also frees nicotinic acid from non-utilizable combination and prevents pellagra.

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### Role of the Rumen Ciliates in Bloat in Cattle

BLOAT (tympanites) in ruminants is characterized by distension of the forestomachs following interference with the normal elimination of gas produced by the microbial digestion of food in those organs. Bloat occurs in cattle usually when fresh legumes are fed, but it may also develop when high-grain rations are supplied in the feed. Many hypotheses have been put forward to explain the ailment, but all have been inadequate in view of its variable nature<sup>1</sup>. Not all animals bloat readily and not all legume pastures produce bloat in susceptible animals.

It appears from experiments carried out in this laboratory that the severity of legume bloat may be partly

determined by the influence of the rumen ciliates on the rate of gas production in the rumen. This work was initiated following the remark of Coleman<sup>2</sup> that Becker<sup>3</sup> and Usuelli<sup>4</sup> had observed that the ciliates of sheep rapidly ingest a large proportion of the starch grains in ground barley and convert them to glycogen. Defaunated animals on the same ration appeared to be bloated, and it was suggested that removal of starch by the protozoa reduced the amount or rate of gas production<sup>5</sup>.

Two pairs of identical twin cows with rumen fistulae were stall-fed on fresh red clover (*Trifolium pratense* L.) at 8.30 a.m. and 1.00 p.m. on weekdays. At night they were kept in a bare yard with free access to water. Before the morning feed the rumen contents were briefly stirred with a hand inserted through the fistula. About 250 ml. rumen liquor was removed, strained through gauze and quickly cooled to 4°. The ciliates were removed from 25 ml. of this sample by centrifuging with a hand centrifuge and washing with buffered solution<sup>5</sup>. The protozoa were finally washed through a 200-mesh sieve. The resulting suspension, which was almost free of plant material, was centrifuged, dried at 100° C and weighed.

The dry wt. protozoa/ml. was plotted against the bloat score as assessed by the method of Johns<sup>1</sup>. Fourteen samples were obtained from the four cows before feeding on six days on which bloat occurred after the first feed. The results for these fourteen samples are shown in Fig. 1.

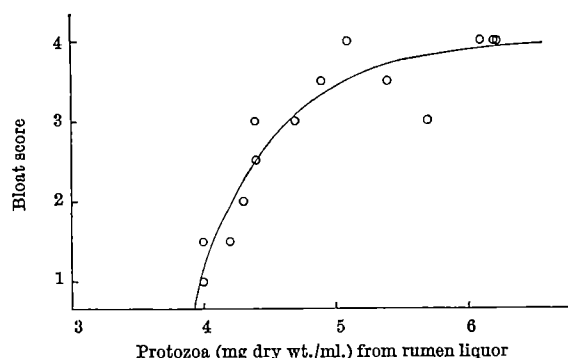


Fig. 1. Relation between bloat score and weight of protozoa in rumen liquor taken before feeding

A curve of the type  $B = 4.0 - A e^{-CP}$  was fitted, where  $B$  = bloat score (arbitrary scale),  $P$  = protozoa content (mg dry wt./ml.), and  $A$  and  $C$  are constants. The fitted curve was  $B = 4.0 - 1763.9 e^{-1.608P}$ .

It was assumed that the bloat score could not rise above 4.0 and that the scale was linear. The fact that there were discrete steps in the bloat score was ignored.

Fig. 1 shows a significant relationship between the weight of protozoa and the bloat score. The greater the dry wt. protozoa/ml. the more severe was the degree of bloat recorded.

The increase in the weight of protozoa probably resulted from a higher content of ingested food and storage material. Counts of the protozoa were not carried out, but since an earlier survey (unpublished) of the relation between protozoal numbers and bloat did not show an increase in numbers immediately prior to bloat, and since the volume of protozoa collected and weighed varied only between 1.3 and 1.6 ml., the increase in weight probably resulted from a higher content of ingested food and storage material. The presence of ciliates full of food and storage polysaccharide could allow maximum gas production by bacteria following feeding as suggested by the results of Becker<sup>3</sup>. Empty ciliates, on the other hand, could remove relatively large quantities of starch and soluble sugars and delay their fermentation to gas and acids. Reduced acid production

would lower the amount of gas released from the bicarbonate of rumen liquor and reduce the severity of bloat. Protozoa full of storage material could also contribute to the overall gas production by fermenting carbohydrate directly to gas and acid.

It is suggested, therefore, that the rumen ciliates may sometimes influence the severity of bloat, the amount of storage material and ingested food in the protozoa determining the amount of food material available for rapid fermentation to acid and gas. Other factors may also be involved, and work extending the present observations is being carried out.

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## ANATOMY

### Organ Size of Human Foetal Bone Marrow

FOR quantitative investigations of haemopoiesis<sup>1</sup>, it is essential to know how much active bone marrow there is in the whole body. Estimates of the 'organ size' of the bone marrow have already been made in animals<sup>2,3</sup>, and similar determinations in the human foetus are relevant to various aspects of foetal haemopoiesis which are being investigated in this department. There seems to have been no previous work on total marrow quantity in the human foetus, although an investigation on three newborn infants has been reported<sup>4</sup>. The following is a summary of my observations.

Material was obtained at routine post-mortem. Fifteen cadavers were examined, of which nine were female and six male. Their body-weights ranged from 1,315 g to 3,720 g, and their approximate maturity, as calculated from body length and from the weight of certain teeth, ranged from 29 weeks to full term. All the foetuses had therefore been 'viable'. No skeletal abnormalities were detected and there was no evidence of pathological states known to exert a specific effect on the haemopoietic system.

It was found possible to estimate the organ size of the bone marrow by a method essentially similar to that used in animals<sup>2,3</sup>. The principle was that of measuring the

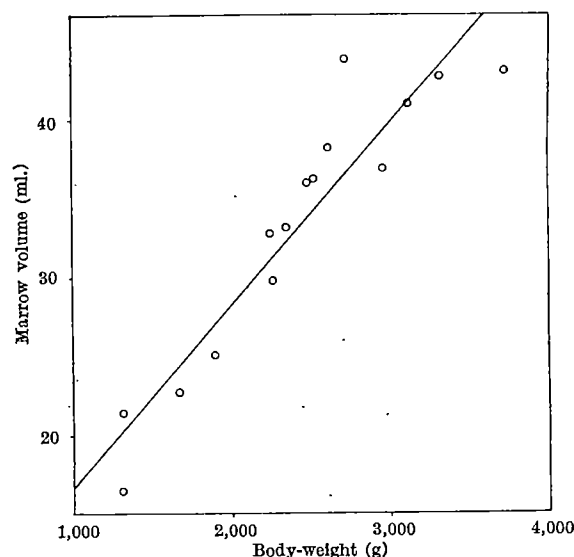


Fig. 1. Relationship between marrow volume and body-weight

volume of agar which, following agar-impregnation, occupied the spaces within the macerated skeleton. After correction for the volume of spaces which would not normally contain marrow, the result was taken to represent the total marrow volume. In the foetus, it may all be regarded as actively haemopoietic.

Fig. 1 shows the results plotted against the corresponding figures for body-weight. The data satisfied the requirements for the significance of the linear relationship, the regression coefficient being 0.012 and its standard error 0.0012. In haemopoietic investigations in similar subjects, one might make an approximate prediction of the volume of active bone marrow on the basis of the body-weight. (The amount of haemopoiesis in extra-medullary sites would also have to be considered.) Similar linear relationships were found between the figures for marrow volume and those for estimated maturity, for macerated skeleton weight and for total skeleton volume. The organ size of the bone marrow was, on average, approximately five times that of the spleen.

Of the total marrow, 29.6 per cent ( $\pm$  S.D. of 4.4 per cent) was found in the skull, 23.4 per cent ( $\pm$  2.6 per cent) in the vertebrae, ribs and sternum, and 47.0 per cent ( $\pm$  3.1 per cent) in the limb and girdle bones.

I thank Prof. J. M. Yoffey for advice and Dr. N. J. Brown for providing access to the post-mortem material.

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## IMMUNOLOGY

### Inheritance and Properties of the Antigen *MuB1* and its Relation to Haemolytic Complement

WE have previously reported the discovery of an antigen, *MuB1*, which occurs in the sera of certain mice and may be detected by double diffusion in agar of mouse sera against antibody of mouse origin<sup>1</sup>, the distribution of this antigen, *MuB1*, among inbred strains of mice<sup>1,2</sup> and the occurrence of a related antigen in other mammals. We have also given some evidence indicating that mice which lack the antigen *MuB1* do not possess the product of a gene allelic to *MuB1* (ref. 2). The present communication deals with the inheritance of this antigen, its connexion with complement and its properties.

The discovery of an inherited complement deficiency in mice, related to a complement factor *Hc* (ref. 3), has led us to examine the correlation between the antigen *MuB1* and the presence of complement. Tests for the presence of haemolytic complement in mice were made with sheep cells sensitized with very large amounts of antibody as suggested by Rosenberg and Tachibana<sup>4</sup>. By

this test, sera from some inbred strains of mice proved to have haemolytic complement, while sera from mice of other strains lacked it. Its presence or absence was found to be closely correlated with the presence or absence of the antigen *MuB1*. Thus mice of the following strains lacked haemolytic complement, and also lacked the antigen *MuB1*: *A/HeJ*, *AKR/J*, *CE/J*, *DBA/2J*, *RF/J*, *SWR/J*.

The following strains possessed haemolytic complement and the antigen *MuB1*: *BALB/cJ*, *BDP/J*, *BUB/Bn*, *CBA/J*, *C57BL/Ha*, *C57BL/6J*, *C57BR/cdJ*, *C57L/J*, *C58/J*, *MA/J*, *P/J*, *PL/J*, *SL/R1*, *SJL/J*, *SN1/J*, *T6* and *129J*. It is apparent, therefore, that good agreement exists between the presence of *MuB1* and the presence of haemolytic complement. To test this correlation further, hybrids between *MuB1*-positive mice possessing functional haemolytic complement (*C57BL/6J* or *C57L/J*) and *MuB1*-negative animals lacking functional haemolytic complement (*A/J*) were mated with animals of the same strain as the *MuB1*-negative parents (*A/J*). The offspring were tested for the presence of *MuB1* and of haemolytic complement.

Mice of strain *C57BL/6J* are *MuA2*- and *MuB1*-positive; mice of strain *A/J* lack the specificity *MuA2* as well as the antigen *MuB1*. Matings between hybrids *C57BL/6J*  $\times$  *A/J* and inbred mice of strain *A/J* are therefore back-crosses with respect to *MuA2* as well as to *MuB1*, and the offspring of such matings can be used to test for genetic correlation between *MuB1* and *MuA2* (refs. 5, 6). The results of these tests are summarized in Table 1. On the basis of these results it is concluded that the inheritance of *MuB1* is determined by a single dominant gene; that there is no correlation between the inheritance of *MuA2* and the inheritance of *MuB1*, but that there is a highly significant correlation between the presence of *MuB1* and of haemolytic complement.

We next investigated the relation between the concentration of the antigen *MuB1* and the sex and age of various inbred mice by two methods. The first of these was double diffusion in agar, using various dilutions of normal mouse sera placed in peripheral wells, and an antiserum placed in a central well. The reciprocal of the lowest dilution of a normal serum, giving a visible precipitin zone in agar, was taken as an index of the concentration of *MuB1*. Alternatively the quantity of the antigen was estimated in single diffusion, by means of the penetration-distance of the precipitin band into the gelled mixture of agar and antibody<sup>7</sup>. By these techniques it was shown that the content of antigen *MuB1* in sera from male animals is 1.3–2 times as great as that in sera from female animals. These sex-associated differences were found in mice of the following strains: *BALB/cJ*, *BSVS/Sr*, *BUB/Wi*, *C58/J*, *CBA/J*, *DBA/1J*, *MO/Ko*, *SJL/J*, *T6* and *WH/Ht*. The concentration of *MuB1* in mice of strain *DBA/1J* increased as the animals grew from 3 weeks of age to 6 months. This difference, due to age, was much greater in the serum of male than of female animals (Fig. 1). Sex-associated differences were also observed in the number of haemolytic units per ml. of serum from male and female animals. It should be pointed out, however, that the relative (male:female)

Table 1. DISTRIBUTION OF ANTIGEN *MuB1*, HAEMOLYTIC COMPLEMENT (*Hc*) AND SPECIFICITY *MuA2* AMONG THE OFFSPRING OF BACK-CROSSES (Offspring from matings between hybrids of *MuB1*-positive and *MuB1*-negative parents with inbred mice of the same *MuB1*-negative strain as one of the parents)

Tested for:	Class of animals				Total	<i>P</i> ( <i>df</i> = 1)	$\chi^2$
Inheritance of <i>MuB1</i>	<i>MuB1</i> +	226 (244.5)	<i>MuB1</i> -	263 (244.5)	489	0.1 > <i>P</i> > 0.05	2.800
Correlation between <i>MuB1</i> and haemolytic complement ( <i>Hc</i> )	<i>MuB1</i> + <i>Hc</i> + 75 (38.7)	<i>MuB1</i> - <i>Hc</i> - 0 (36.3)	<i>MuB1</i> - <i>Hc</i> + 7 (43.3)	<i>MuB1</i> - <i>Hc</i> - 77 (40.7)	159	<i>P</i> < 0.005	113.16
Correlation between <i>MuB1</i> and <i>MuA2</i>	<i>MuB1</i> - <i>MuA2</i> + 42 (44.4)	<i>MuB1</i> + <i>MuA2</i> - 43 (40.6)	<i>MuB1</i> - <i>MuA2</i> + 53 (50.6)	<i>MuB1</i> - <i>MuA2</i> - 44 (46.4)	182	0.5 > <i>P</i> > 0.25	0.56

Italic numbers in parentheses represent values expected on the assumption that there is no correlation between the two characters tested.

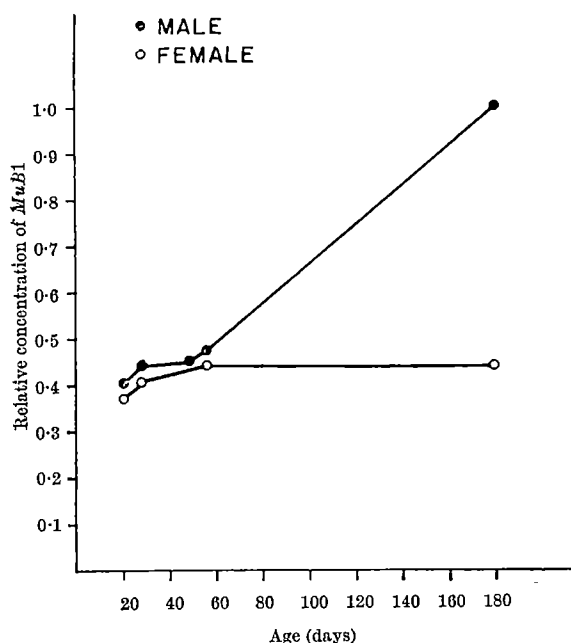


Fig. 1. Change in concentration of antigen *MuB1* as a function of age. Mice of strain *DBA/1J*; concentration of *MuB1* measured by single diffusion and expressed as a fraction of the concentration of *MuB1* in the serum of six-month-old male mice of strain *DBA/1J*.

haemolytic potency was not the same as the relative concentration of *MuB1*; in the serum of male mice of strain *BALB/cJ*, for instance, we found five times as many haemolytic units but only 1.4 as much antigen as in the serum of female mice of this strain.

Some properties of the mouse antigen *MuB1* have been investigated. Serum containing *MuB1*, when kept for 17 min at 56° C, loses 50 per cent of its reactivity with mouse antibody. The antigen is insoluble in distilled water and can be found by gel-filtration on Sephadex *G-200* (ref. 8) in a fraction containing molecules of molecular weight 150,000. Since we found that antibody to *MuB1* reacts with a molecule in guinea-pig and human serum<sup>2</sup>, the properties of the guinea-pig and human molecules corresponding to *MuB1* can be explored. These molecules are water-insoluble and are not affected in their reactivity with antibody by treatment with ammonia, hydrazine or zymosan. The guinea-pig antigen loses its reactivity with antibody when heated at 56° C for 1 h. The properties outlined are similar to those of the *C'5* ( $\beta_1F$ ; refs. 9, 10) component of complement and to those of the *C'3d* component of complement<sup>11</sup>.

Two recent papers have dealt with complement deficiency in mice and its correlation with the absence of an antigen<sup>12,13</sup>. Our reported findings<sup>1,2</sup> are in agreement with the strain distribution of the antigen (except for the strains *AKR* and *CBA*), and the mode of inheritance of *MuB1* and *Hc* (refs. 3, 13) is identical with the findings reported here. There is agreement also with the reported sex dependence of haemolytic efficiency<sup>12</sup>. The specificity of antibody to *MuB1* is identical with that of an antibody obtained by Erickson *et al.*<sup>13</sup>, as we have established by an exchange of immune sera with Drs. Herzenberg and Rosenberg.

It seems evident that there is a link between the possession of a functional complement system and the presence of the antigen *MuB1* (ref. 1), but the identity of a complement factor with *MuB1* has, so far, not been established by direct test.

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### Role of Complement in Passive Cutaneous Reactions of Mice

PASSIVE cutaneous anaphylaxis (PCA), an immediate type of skin reaction<sup>1,2</sup>, is initiated by combination between cell-fixed antibody<sup>3-7</sup> and antigen, and is characterized by increased permeability of the walls of minute blood vessels of the skin. Whether or not complement plays a part in this increase of permeability is, as yet, not definitively established, though it has been suggested that complement is involved in PCA of the rat<sup>8,9</sup> but not of mice<sup>10</sup> or guinea-pigs<sup>11</sup>.

Recently it has become evident that some strains of inbred mice have a deficient complement system (*Hc*<sup>9</sup>) (ref. 12). The discovery of an antigen *MuB1* (refs. 13-15), detected by a mouse antibody, has allowed the demonstration that the absence of this antigen is correlated with a non-functional complement system<sup>16-17</sup>. This has facilitated an extensive survey of inbred strains of mice for the presence or absence of a functional complement system (*Hc*) (ref. 12), and thus it has been possible to classify mice into *MuB1* positive and complement positive strains and *MuB1* negative and complement negative strains<sup>14-18</sup>.

In the following experiments we have investigated the part played by complement in PCA by comparing the reaction of *MuB1*-positive with that of *MuB1*-negative mice. The antigen chosen for this investigation was bovine pancreatic ribonuclease and the antibody was of rabbit origin<sup>18</sup>. Antibody was injected intracutaneously, and antigen, mixed with Evans blue, was injected intravenously. One hour after the intravenous injection the mice were killed. The skin was removed and the diameter of the blue area on the inner surface of the skin was recorded as the mean of two measurements at right angles. The size of the 'blued' area was investigated as a function of the interval between the intradermal injection of 3  $\mu$ g N antibody and the intravenous injection of 100  $\mu$ g N ribonuclease. In *MuB1*-positive non-inbred *Swiss* mice, the lesion was 5.7 mm when the reactants were injected simultaneously, and 4.8 mm when the antigen was given 180 min after the antibody. In the *MuB1*-negative mice of strain *AKR/J* the lesion was 6.0 mm if the reactants were injected simultaneously



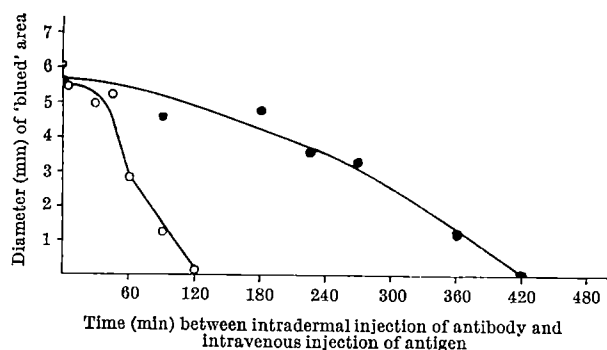


Fig. 1. Changes in the mean diameter of 'blued' areas observed in *MuB1*-positive Swiss mice and *MuB1*-negative mice of strain *AKR/J* as a function of the interval between the intradermal injection of  $3 \mu\text{g}$  *N* antibody and the intravenous injection of  $100 \mu\text{g}$  *N* antigen. Antigen, bovine pancreatic ribonuclease; antibody, raised in rabbits; mean diameter of 'blued' area observed in the skin of six-week-old, male animals. ●, Swiss mice (*MuB1*-positive); ○, mice of strain *AKR/J* (*MuB1*-negative).

but was not demonstrable if the interval between injections was longer than 2 h (Fig. 1). If the larger doses of antibody were injected the 'blued' area could be observed in *MuB1*-positive and *MuB1*-negative animals if the interval between the two injections was much longer, but the relative difference between the action of *AKR/J* and Swiss mice remained the same. On the basis of these experiments, mice from three additional strains of *MuB1*-positive animals (*BALB/cJ*, *BUB/Bn* and *SJL/J*) and mice from three additional strains of *MuB1*-negative animals (*AHe/J*, *RF/J*, *SWR/J*) were injected intradermally with  $3 \mu\text{g}$  *N* antibody and were given, 3 h later, an intravenous injection of ribonuclease ( $100 \mu\text{g}$  *N*). Whereas the *MuB1*-positive animals responded with blued areas 8.8–5.5 mm in diameter, the *MuB1*-negative animals did not give a significant response.

It is therefore clear that a permeability increase can be observed in *MuB1*-positive mice if an interval of 3 h is allowed between the intradermal injection of antibody and the subsequent intravenous injection of antigen, and that this reaction is not observed in *MuB1*-negative mice. We investigated, next, whether the difference in the response of the two groups of mice was attributable to a humoral factor. To this end, *MuB1*-negative mice of strain *AKR/J* were injected intradermally with antibody ( $3 \mu\text{g}$  *N*) and 3 h later with a mixture of ribonuclease, Evans blue and the serum from *MuB1*-positive animals (*BALB/cJ*, *CBA/J*, *C57BR/cdJ*, *C57L/J*, *C58/J*, *MA/J*, *PL/J*, *SL/R1*, *129/J*, *C57BL/6J*). Under these conditions 'blued' areas were observed. 'Blued' areas did not occur if sera from animals lacking *MuB1* (*AHe/J*, *CE/J*, *DBA/2DeJ*, *RF/J*, *SWR/J*) replaced the *MuB1*-positive sera in the aforementioned procedure.

It had thus been demonstrated that a humoral factor is involved in PCA and that this factor is present in the serum of *MuB1*-positive but not of *MuB1*-negative animals. It was also possible to supply the humoral factor, required for the permeability increase, by the injection of fresh, but not of heated ( $56^\circ$ , 1 h), guinea-pig serum. In fact, a unit volume of fresh guinea-pig serum was five times as effective as a unit volume of fresh serum from Swiss mice.

The permeability increase, caused by the simultaneous injection of antigen and antibody, is the same in *MuB1*-positive and *MuB1*-negative animals. On the other hand, the permeability increase, occurring when antigen is given some hours after the intradermal injection of antibody, depends on the presence of a humoral factor which is present in the serum of *MuB1*-positive but not of *MuB1*-negative mice. It would thus seem reasonable to conclude that two different reactions are involved in permeability increase. The first of these may involve antibody not fixed to cells or antibody which fixes to cells very rapidly.

The second reaction depends on relatively slow fixation of the antibody to cells and should therefore increase after prolonged residence of antibody in the skin. A humoral factor, most probably complete haemolytic complement, is involved in the second, but not in the first, reaction. However, the two reactions occur simultaneously, their relative importance depending on the quantity of antibody and the interval between the injections. Contradictory results in the literature may be attributable to the differences in the properties and concentration of different types of antibodies, but also to differences in the relative contribution of the two reactions as a function of the quantity of antibody and the interval between injections of the reactants.

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## RADIOBIOLOGY

### Dose Ratio of X-rays to Fast Neutrons in producing Dominant Lethals in Flour Beetles, *Tribolium castaneum*

GENETIC effects of radiations of varying relative biological effectiveness on germ cells in different stages of development and differentiation have been examined in mice<sup>1,2</sup> and *Drosophila*<sup>3,4</sup>. The authors found similar relative biological effectiveness values on the basis of radiation-induced dominant lethals (50 per cent) or recessive lethals. Earlier work<sup>5</sup> showed that the frequencies of fast neutron-induced chromosome aberrations (a measure of relative biological effectiveness) were similar for *Drosophila* and *Tradescantia*.

This communication reports the 'dose ratio'<sup>6</sup> of 250-kV X-rays and fast neutrons of mean energy 4.6 MeV for 50 per cent dominant lethals in flour beetles, *Tribolium castaneum*, observed during two weeks post-irradiation. Detailed examinations of X-ray and fast neutron radiation effects on productivity of this species of flour beetles will

be reported elsewhere'. 'Dose ratio' is a more appropriate term than 'relative biological effectiveness' in evaluating these findings, since dose-response curves (dominant lethals) were of the 'multi-hit' type curves for X-ray and 'one-hit' type for fast neutrons. Such curves cannot be compared for relative biological effectiveness calculations because of their different slopes.

Fast neutrons were 2-3 times more effective than X-rays in producing 50 per cent dominant lethality (Table 1). Although only a single fast neutron exposure (830 rads) was given, the dose-response curve (per cent dominant lethals) was extrapolated through it to the origin in order to obtain the radiation exposure which induced 50 per cent dominant lethals. This curve seemed justified for three reasons, namely: (1) the proximity of observed dominant lethal per cents in male- and female-exposed groups to 50 per cent; (2) the linear dose-response curves for fast neutrons from other works<sup>3-5,9</sup>; (3) similar dose ratio values were calculated by determining the X-ray dose which caused the same effect as 830 rads of fast neutrons (Table 1, last column).

Table 1. DOSE RATIOS FOR DOMINANT LETHALS INDUCED BY 250-KVP. X-RAYS AND 4.6-MEV FAST NEUTRONS IN FLOUR BEETLES, *Tribolium castaneum*, CULTURED AT DIFFERENT TEMPERATURES FOR TWO WEEKS

Temperature (C)	Sex exposed	Dose ratio for dominant lethals 50% effect (calculated)	830 rads effect* (observed)
25	♂	2.7	2.6
	♀	2.6	2.7
	♂♀	2.8	2.6
29	♂	2.5	2.5
	♀	2.3	2.4
	♂♀	2.1	2.2
32	♂	1.8	2.4
	♀	1.9	2.2
	♂♀	2.5	2.4

\* Rads of X-ray to obtain fast neutron effect  
Observed effect at 830 rads of fast neutrons

Dose ratio values were comparable regardless of sex exposed or temperature. The mean dose ratio of 2.4 which represents the response of all meiotic stages at the time of irradiation is similar to that of 3.4 for *Drosophila* calculated from the data of Alexander<sup>3</sup>; corresponding relatively biological effectivenesses for the latter were 1.6, 2.3, 3.2, 3.1 and 6.6 for meiotic through sperm stages. Ionizing radiations would be more hazardous to pre-meiotic or meiotic cells than to post-meiotic stages because the latter are of brief duration and therefore subjected to fewer chances for irradiation.

In conclusion, to obtain similar frequencies of dominant lethals, X-ray doses 2-3 times higher than those for fast neutrons exposure were required. The mean dose ratio of 2.4, which produced 50 per cent dominant lethals in *Tribolium*, corresponds with the value of 3.4 in *Drosophila*, for which relative biological effectivenesses (dose ratios) at particular spermatogenic stages were known.

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## Circulating Stem Cells: Variation with Duration of Partial Body X-irradiation

LEUCOCYTE depression is often produced by localized radiation therapy. This frequently appears inordinate for the amount of bone marrow within the radiated field. A possible explanation for this leucopenia is that during a course of radiation therapy (usually given in daily doses for a number of weeks) a large number of circulating stem cells<sup>1-6</sup> enter the radiated field, are destroyed, and thus a leucocyte depression is produced. I have attempted to test this hypothesis in previous experiments<sup>7,8</sup> by subjecting two groups of mice to partial body irradiation. Both groups received the same total dose; however, one group was radiated for 2-3 min while the other was exposed for 100-120 min. If the hypothesis is correct, then the mice exposed for the longer duration should develop a more profound and prolonged leucopenia. This is what was observed. The present experiment attempts, more directly, to observe the effects of the duration of partial body irradiation on the number of circulating stem cells to see whether these changes in the leucocyte levels reflect changes in stem cells.

Till, McCulloch *et al.*<sup>9-12</sup> have demonstrated that intravenously injected bone marrow cells will form discrete colonies in the spleens of mice previously receiving lethal doses of whole-body irradiation. These colonies contain various immature hematopoietic cells and appear to be derived from single cells<sup>10</sup>. The number of spleen colonies is thought to be proportional to and can be used to assay the number of stem cells in the injected bone marrow. Recently, it has been shown that peripheral blood contains cells capable of colonizing the spleen<sup>6</sup>.

In this experiment mice were exposed to 900 r. with the left lower extremity shielded. It seemed possible that stem cells from the unirradiated extremity would circulate, and colonize the spleen. The number of colonies could then be used to assay the number of stem cells coming from the unirradiated extremity. The duration of radiation exposure was varied. If the hypothesis is correct, then the more protracted irradiation would allow more stem cells to circulate from the shielded limb into the remainder of the body during the radiation period, be sterilized and, therefore, the number of spleen colonies should decrease with increasing duration of radiation.

Female 3-5 months old C3H/CGRL mice bred in this laboratory were used for this experiment. All the mice were anesthetized with 1 mg of sodium pentobarbital, 6 mg of tribromethanol and 3 mg of amylene hydrate intraperitoneally. They then received 900 r. with the left lower limb shielded by 3 × 5 cm sheet of lead 2.5 mm thick. The radiation factors were: 250 kV X-ray with 2 mm aluminium filtration, half-value layer 0.8 mm copper. The dose was measured in air at the radiation distance with a Philips (R) Universal dosimeter, and the output was monitored during radiation. The dose rate was changed by varying the distance from the radiation source (from 33-162 cm) and the current (7-19 m.amp). Surviving mice were killed 9 days following radiation, the spleen removed and, after fixation in Bouin's solution, macroscopically visible colonies were counted.

Control animals received the same dose of 900 r. with no limb shielding and spleen colonies were counted in a similar fashion. These animals (15 in each group) receiving 900 r. whole-body radiation without limb shielding demonstrated a mean spleen colony count of 0.46 (± 0.09) when the radiation duration was 1.4 min. When the duration was extended to 86 min, the mean spleen colony rose to 3.3 (± 1.6). This demonstrated the usual and expected dose rate effect, the high intensity short duration radiation being more effective<sup>13-17</sup>.

Fig. 1 shows the mean number of spleen colonies in mice receiving 900 r. with one limb shielded plotted against the duration of irradiation. With increasing duration the number of colonies decreased. The statistical

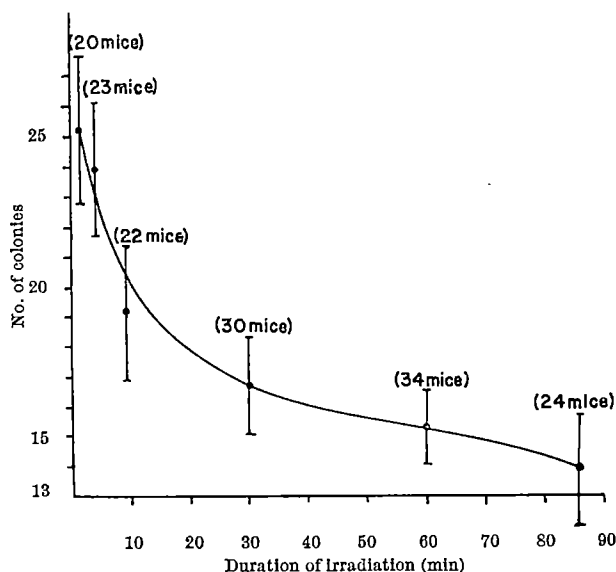


Fig. 1. The mean ( $\pm$  S.E. mean) number of spleen colonies in mice nine days after 900 r. with left lower limb shielded plotted against the duration of radiation. The number of mice in each group is in brackets

significance of these differences is shown in Table 1. These results are consistent with the hypothesis that stem cells do circulate from the unirradiated limb and colonize the spleen. The more extended radiation time allows a greater number to enter the field during the radiation period and be sterilized and, therefore, fewer stem cells remain available in the non-irradiated extremity to form spleen colonies later.

Table 1. MEAN NUMBER OF SPLEEN COLONIES OBSERVED IN ANIMALS EXPOSED TO 900 R WITH LEFT LOWER LIMB SHIELDED

Radiation duration (min)	Spleen colonies ( $\pm$ S.E. mean)	Diff. from 1.4 min	P value of diff.
1.4	25.2 $\pm$ 2.4	—	—
4.2	23.9 $\pm$ 2.2	1.3	—
9.0	19.2 $\pm$ 2.3	6.0	0.1-0.05
30.0	16.7 $\pm$ 1.6	8.5	0.01-0.001
60.0	15.3 $\pm$ 1.2	9.9	< 0.001
86.0	13.9 $\pm$ 1.7	11.3	< 0.001

If the rate of release of stem cells by the unirradiated limb is constant, then a linear function should be described in Fig. 1. This was observed by Hanks in a somewhat different experiment<sup>18</sup> using longer time-periods. It is of interest to speculate about a possible explanation for the observed deviation from a linear function. It seems reasonable to assume that the number of stem cells able to circulate and colonize the spleen from the unirradiated limb is limited. Since the remainder of the body is being irradiated, the number of viable cells entering this limb is then being depleted by egress without compensatory entry of viable stem cells. This would explain the prompt reduction in colonies which then gradually tapers as the pool becomes exhausted. This rapid reduction in size of pool would suggest extensive migration of stem cells from marrow sites into the peripheral blood. An alternative possibility is that in the normal steady state migration is not this extensive but rather that radiation in some way accelerates the release of available stem cells from unirradiated areas.

Complicating the interpretation are considerations of cell recovery from irradiation. With whole-body radiation more spleen colonies were found when the radiation was protracted. This is consistent with the generally observed decrease in effectiveness of a given dose of radiation as dose rate is reduced. When the limb is shielded, one would expect the same dose rate effects, and therefore the more protracted radiation would be less effective. This would tend to flatten the curve as the dose rate is decreased and counteract the initial rate of reduction of colony-forming units.

Further experiments are in progress to try to elucidate these problems. It seems clear, however, that cells able to form spleen colonies do circulate, and protracted localized irradiation allows time for a greater number to be destroyed.

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## BIOLOGY

### Productivity of the Bassin de Chasse in Ostend

IN view of the increased interest in the possibility of marine fish farming, the following observations of high primary production and high growth rate of molluscs in a semi-natural lagoon in Ostend suggest a simple approach to this problem.

It will be recalled that there have been two main lines of approach to the problem of fish farming. The early fisheries biologists saw that the fecundity of the food fishes was high, but that there was a very high mortality of eggs and young larvae. They thought that by reducing this mortality they could increase the numbers of fish reaching the adult fish population. They, therefore, sought to rear young fish through the critical period and to release them subsequently to the sea. To affect the stock in the sea the number released would have to be very high and the fish would have to be sufficiently robust to survive the transfer and to compete for food with the natural population. Expensive hatcheries were required for this work and great control of the food supply and the environment in the tanks was necessary. Only now has Shelbourne<sup>1</sup> succeeded in bringing such work to a stage when mass production of one-year-old plaice from a 'fish factory' becomes possible. Ideally, these fish should be released into a rich environment so that a growth rate higher than that in a wild population is achieved.

The work of Gross<sup>2</sup> and his collaborators was aimed at increasing the productivity of a restricted sea area so that the growth rates of the fish would be increased. To this end great quantities of phosphates and nitrates were poured into two sea lochs. In both cases a high zooplankton production was recorded. However, the main benefit from the increased production went to benthic animals and sub-littoral algae. Growth rates of plaice and flounders were increased about four-fold but, apparently because the growing fish migrated from the lochs, the

experiments did not indicate that such a scheme would be economic<sup>3</sup>.

A similar experiment by Buljan<sup>4</sup> in Yugoslavia was rather more successful. Here, 'fertilization' was aimed at increasing the growth rates of oysters and mussels. There were increases of some 20 per cent and 30 per cent, respectively, in the seasons following 'fertilization'. In the Bassin de Chasse in Ostend the natural growth rates of oysters and mussels are as high as those obtained after such procedure.

The Bassin de Chasse<sup>5</sup> is an artificial lagoon enclosed by a brick retaining wall and covering an area of some 86 hectares. To the west it opens by a main sluice into Ostend harbour and on the south side three small sluices communicate with a branch of the Bruges Canal. The average depth of water is between 1 and 1.5 m and its level can be regulated by opening or closing the sluices. The bottom is partly of soft black mud, but towards the east the proportion of fine sand increases until at the extreme eastern end there is pure sand in places.

Attempts at oyster cultivation<sup>6</sup> in the lagoon were made from 1933 to 1939. Successful breeding and growth of spat was obtained in 1938, but the war prevented marketing of these oysters. During the war a dam was built across the western end of the basin, sealing off the main sluice. It was then used as a sea-plane base. From 1942 to 1955 it remained out of communication with the sea. Attempts at growing oysters were not successful and in 1956 the dam was removed, enabling the basin to be fully drained.

Young Dutch oysters are cultivated by cementing them on to greenheart stakes which are suspended vertically from platforms. The oysters are put out in the basin in March or April and are harvested for sale, beginning in August and continuing into the early winter. At this time the water is released and the basin is allowed to remain dry during the winter. In the following March the basin is refilled by raising the sluice gate at high water. Leloup<sup>6</sup> has shown that in the harbour there is a tongue of high salinity water close to the bottom and it is this water which is used to refill the basin in spring, when the oysters are planted out.

The oysters show very high growth rates, as can be seen in Fig. 1(a). This high growth rate is not merely a linear shell shoot; the indices of quality are high. Mean indices of quality<sup>13</sup> for the 1960 and 1961 seasons were 113.24 and 114.88, respectively. For comparison, the growth<sup>4</sup> of Yugoslavian oysters before and after the 'fertilization' experiments is also shown. The oysters from the western end of the Bassin de Chasse have a higher growth rate than even the experimentally augmented Yugoslavian oysters.

Walne<sup>6</sup> investigated the effects of tidal exposure on the growth of oysters at Conway. He showed that a 50 per cent difference in growth increment occurred between oysters experiencing 1.5 per cent and 14 per cent exposure. In other words, the oysters in deeper water with less exposure to air had greater growth. Part of the explanation of the high growth rate in the oysters of the Bassin de Chasse may then lie in their lack of exposure.

Fig. 1(b) gives a comparison of the growth of *Mytilus edulis* from the Bassin de Chasse<sup>6</sup>, Vigo<sup>7</sup> and Menai Straits (Davies, personal communication). The three sets of data all refer to mussels grown under water. Those from Vigo are cultivated on ropes hung from floating platforms in deep water. Andreu stated that their growth rate was the highest recorded in Europe. The data from Menai Straits refer to some experiments with mussels grown on horizontal plastic mesh trays, 1-2 ft. below the surface of the water. The data from the Bassin de Chasse give the growth from the time of settlement of mussels on the oyster stakes.

From these two examples alone, it is evident that the Bassin de Chasse is an area of high productivity. Indeed, one of the major problems in oyster cultivation has been the enormous production of competitors such as

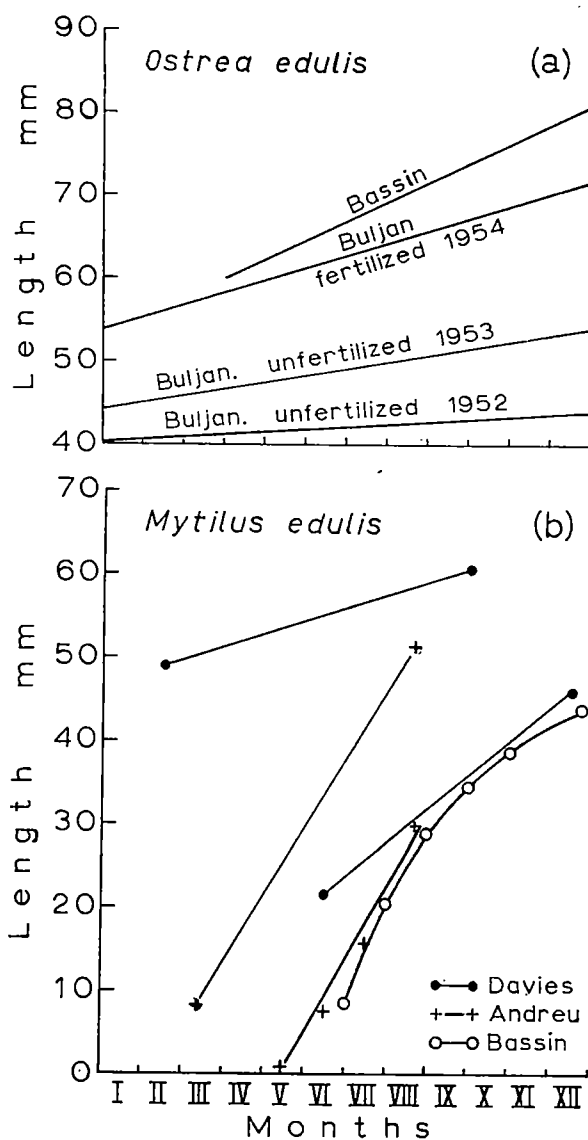


Fig. 1. (a) Growth rates of oysters from the Bassin de Chasse, Ostend, and of Yugoslavian oysters. (b) Growth rates of mussels from the Bassin de Chasse, from Spain and from North Wales

*Crepidula fornicata* and parasites such as *Polydora*. Polk<sup>10</sup> showed that in five months a length of 24.1 mm was recorded for *Crepidula* and that at four months some were already incubating eggs. Walne<sup>11</sup> reported, from data of *Crepidula* in the River Crouch, that a mean length of about 8 mm was reached by their first winter, and 14 mm at the end of their second winter. Females were spawning when about four years old. Again we see high growth rates in the basin. Noting that *Crepidula* larvae did not appear to survive outside the basin, a technique<sup>12</sup> by which the basin was emptied at peak *Crepidula* spawning was developed. In 1961 the basin water was changed four times during May and June. The observed settlement on 804 test plates was 17 individual *Crepidula*, compared with 15,746 on 828 plates in 1960. This technique might be further used in reducing the infection of *Polydora*.

The high growth rates of molluscs must be maintained by a high level of primary production. It is interesting to compare the quantities of copepods and phosphates in the basin compared with other areas. Leloup and his colleagues record a maximum number of copepods and nauplii of 685 per litre<sup>13</sup>. This compares with 600 per litre in the 'fertilized' sea loch and a maximum of six per litre in the 1948 plankton catches off Flamborough Head; however, these latter catches do not include nauplii. In the basin

and in the 'fertilized' loch it is interesting to note that the same species are involved, namely, *Temora longicornis* Müller, and *Centropagus hamatus* Lillj. In addition, *Acartia biflosa* was abundant in the basin.

The 1960 maximum phosphate level recorded in the basin was 1.197 mg/l. compared with a maximum of 0.1302 mg/l. obtained in January 1962 from a station in the North Sea near Ostend (P. G. W. Jones, private communication). In August 1954, a value of 1.535 mg/l. was given by Leloup. It is evident that there is a high phosphate reserve in the basin, as at that time there had been no direct communication with the harbour for 12 years.

The mechanism by which such high productivity is maintained has not been fully investigated. Under the conditions where the water is held in the basin from March until winter, death and decomposition of the annual plankton production returns the nutrients to the basin. But, unlike the sea loch experiments, where some nutrients remain permanently locked up in algae and benthic animals, many of these are killed by exposure to frost when the basin is drained in the winter. Perhaps the mud acts as a store of nutrients. In this connexion, it is interesting to note that Polk has observed that the growth rates of oysters in the western portion of the basin, where they lie over mud, are higher than those in the east where sand is present.

The Bassin de Chasse, with its sluices and shallow water, would seem to afford an ideal situation for a full study in basic productivity. As well as providing rich fattening grounds for oysters, where parasites and commensals are controllable, such areas might also be adapted for fish farming.

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### Blue Pigment of a Surface-living Oceanic Copepod

THE development of special nets has made it possible to sample specifically the plankton living in the uppermost layers of the sea. Routine sampling with such a net, designed by P. M. David at the National Institute of Oceanography, to fish only in the top 4 in. of the water, has been carried out during the present cruise of the R.R.S. *Discovery* in the Indian Ocean. One of the most striking features of the living hauls is the predominance of blue pigmented organisms, and these have been seen in a wide diversity of groups, among them copepods, mysids, decapods, stomatopod larvae, siphonophores, chaetognaths, salps, doliolids and appendicularians. The most typical members of the catch are pontellid copepods, and these show a blue colour more intense than most other groups. Since blue is a colour almost completely absent in deeper-living plankton, it was considered to be of interest to make a closer examination of this typical feature of the tropical surface plankton. Using the surface net it was possible to capture very large numbers of *Pontella fera* Dana and carry out a simple analysis of its pigment.

Three surface hauls were made in different regions and a few hundred *P. fera* were either picked out individually or, on one occasion when several thousands were caught, the other components of the catch were removed. Having separated animals of both sexes, excess water was removed on filter paper, and the animals were ground up with a little distilled water until a satisfactory homogenate was obtained. Further distilled water was added, and the suspension was centrifuged for 10 min at approximately 4,000 r.p.m. This caused a layer of solid carapace pieces to separate out at the bottom, above it a fatty layer, and above this an opaque blue liquid. This supernatant liquid was pipetted off and filtered through a Whatman No. 42 filter paper, the solids being discarded. The filtered liquid was a clear blue solution which was very stable on one occasion but rather more unstable on the two subsequent occasions, tending to precipitate out after some time. This is probably due to changes of pH occurring in the solution. The blue solution was examined in a spectrophotometer between 400 and 1,000 mμ using a 1-cm cuvette—the high organic content of the solution ruling out any useful examination in the ultra-violet region. The result is given in Fig. 1A, the curves being almost identical on each occasion, and showing a broad absorption band with a peak at about 640 mμ.

The blue pigment is insoluble in ether, benzene and carbon tetrachloride, and treatment with these agents accentuates the peak but does not alter its position. Treatment of the blue pigment with alkali, organic or mineral acids, alcohol or acetone permanently decolorizes it, giving a reddish-yellow solution, the characteristic absorption curve of which is shown in Fig. 1B. The blue colour is similarly lost on heating the solution slowly to about 65° C. After freezing the solution, precipitation occurs when it is subsequently thawed. Passage through activated charcoal completely removes the colour of the solution. When the pigment is allowed to decay by standing for a day or more there is a tendency for a transient greenish tinge to appear and the solution becomes cloudy.

It seems probable that the pigment is a chromoprotein complex of a carotenoid and a protein. This is suggested by its initial insolubility in fat solvents, the ease by which it is denatured, particularly when pH changes occur, and the fact that the peak absorption after denaturation (presumed to involve breakage of the carotenoid-protein link) is at 450-475 mμ, a region characteristic of the carotenoid pigments commonly found in planktonic animals. In the living animal the pigment appears to be uniformly distributed over the body, both in the exoskeleton and in the underlying epidermis. There are three main sites where it appears to be more concentrated, namely, the thickened

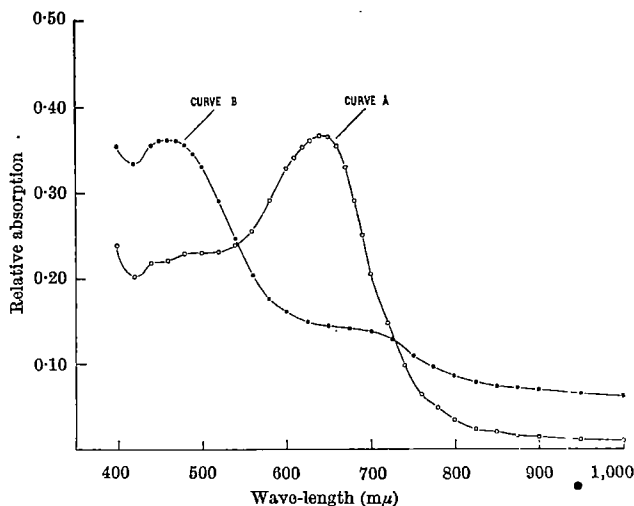


Fig. 1. Spectral absorption of the initial extract of blue pigment (curve A) and after treatment with citric acid (curve B).



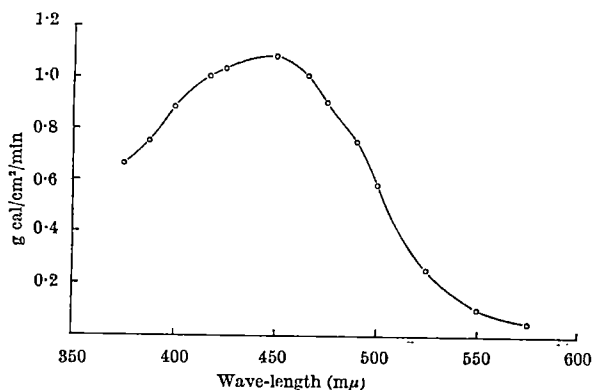


Fig. 2. Spectral energy curve of the upward illumination at the Swedish Deep Sea Expedition station 192 in the eastern Indian Ocean (after Jerlov)

grasping portion of the male right antenna, on the inside of the base of the first antennæ in both sexes, and over the ventral eye. The latter, particularly, appears deep blue, but dissection shows that a thickened layer of deep blue chitin overlies a red fat-soluble pigment spot.

The reason for the preponderance of blue in the tropical surface plankton is not immediately clear. The background colour of clear oceanic water is a deep blue, as can be seen from Fig. 2, showing the spectral energy of upward illumination at 2 m depth at the Albatross station 192 in the eastern Indian Ocean according to Jerlov<sup>1</sup>. It will be noted that the spectral transmission of the pigment is relatively high over the same region and only decreases at about 550 mμ, above which the background energy is exceedingly low. The pigment, therefore, transmits the ambient light with little wave-length change and the animal matches the background very well. This will only apply when it is viewed from above or from the side, but the main predators of surface-living copepods are probably flying-fish which are found at approximately the same horizontal level. Similarly, the blue of *Porpita* and other siphonophores may be a camouflage for the predator. This argument, however, would not seem to hold good for animals which are more usually transparent, for example, the chaetognaths, stomatopod larvæ and doliolids. In some species, such as the copepods, it may be that there is an obligatory accumulation of carotenoids, the basic red colour of which is masked by the linkage with a protein so that the animal becomes much less conspicuous.

The diffuse distribution of the pigment in almost every case, and its appearance in widely separate taxonomic groups, makes it seem more likely that it is, as Heinrich<sup>2</sup> has suggested, a protection against the strong solar radiation to which the tropical surface plankton is exposed. The ultra-violet wave-lengths are generally considered as the most biologically harmful, but it is not known whether the blue pigments do strongly absorb these wave-lengths. Though the penetration of the longer wave-lengths, particularly the infra-red, is very small, they may be an important factor in the top few inches of the surface of the sea, and the pigment with the peak absorption at 640 mμ would screen the organs from much of this energy. None of these explanations seems wholly satisfactory, however, and it is hoped that further work will make the reasons clearer. Preliminary work on another species of *Pontella* has given similar results and it is hoped to carry out further analysis of the pigment of this and other species of surface plankton.

I thank Mr. R. I. Currie and Mr. P. M. David for their interest and assistance in this work.

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\* From: R.R.S. *Discovery*; c/o G.P.O., London, E.C.1.

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## Variable Toxicity of Triazine Herbicides

WHEN applied to the soil surface several triazine herbicides have been shown to be toxic to gymnosperm seedlings, with older seedlings much less susceptible than young ones<sup>1,2</sup>. The method of herbicide application also greatly influences the toxicity of triazines. For example, no injury occurred to 2-year-old *Pinus resinosa* plants when simazine was applied to the soil surface at 4 or 8 lb./acre or when applied to the foliage only. However, incorporation in the soil of simazine applied to the soil surface caused severe injury and high seedling mortality<sup>3</sup>. The triazine herbicides apparently are less toxic when applied to the soil surface than when incorporated in the soil because they do not leach readily and, in the former case, roots of trees often are below the soil layers containing phytotoxic amounts of these chemicals. For these reasons differences in actual toxicity of different triazines may not be readily apparent when the herbicides are applied to the soil surface. Experiments have now been extended to evaluate the effects of several soil-incorporated triazine herbicides on development of young *Pinus resinosa* seedlings.

Herbicides tested included simazine, atrazine, propazine, prometryne and ipazine. Plainfield sand was placed in greenhouse flats to a 2-in. depth and the appropriate herbicide sprayed on the soil surface at 2, 4, 8 or 16 lb./acre. The soil and herbicide were then mixed in a Patterson-Kelly twin shell blender and distributed into cylindrical, one-pint paper cartons with holes in the bottom for drainage. One hundred *Pinus resinosa* seeds were planted in each of 10 cartons for each dosage of each herbicide. Ten cartons of mixed soil planted with seed but without added herbicide served as controls. The cartons were placed on a greenhouse bench and watered daily. Toxicity symptoms and survival counts were noted periodically. 110 days after planting the living seedlings were counted and their tops clipped off at the ground line for dry weight determinations.

Table 1. MEAN DRY WEIGHTS OF SHOOTS OF LIVE SEEDLINGS 110 DAYS AFTER HERBICIDE TREATMENT (PER CENT OF CONTROL)

Dosage † (lb./acre)	Atrazine	Simazine	Herbicide Prometryne	Propazine	Ipazine
2	0*	12.2*	19.8*	81.0	100.0
4	7.9*	8.5*	17.9*	81.7*	138.3
8	0.2*	5.3*	0*	12.8*	75.6
16	0.2*	0*	0*	0.2*	21.6*

\* Sig. diff. from control at 1 per cent level.

† Herbicide applied to soil surface at indicated rate and then incorporated into soil.

In general, toxicity to pine seedlings varied greatly among herbicides with atrazine the most toxic and ipazine the least. Within 10 days after germination variable herbicide toxicity was evident, with atrazine, simazine, and prometryne injury already apparent at the higher dosages. Neither ipazine nor propazine injury was observed at this early stage. Early toxicity symptoms included slight needle curling, chlorosis, and growth inhibition. The symptoms became more severe with time and later were apparent at low dosages of atrazine, prometryne and simazine. All, or virtually all, seedlings were killed within 110 days after planting by the two highest dosages of atrazine and prometryne and the highest dosages of simazine and propazine (Table 1). Atrazine was highly toxic at all dosages, while prometryne was far more toxic at the two highest dosages than at the two lowest ones. Propazine was only mildly toxic at low dosages but very toxic at the highest dosage. Ipazine was very toxic only at the highest dosage. Especially interesting was the accelerated late mortality under certain treatments. Ipazine, for example, caused no readily apparent damage at any dosage 80 days after planting. In the next 20 days, however, toxicity symptoms developed very rapidly in plants treated with ipazine at the highest dosage. This emphasizes the importance of investigating herbicide toxicity over relatively long

periods of time. This would seem to be especially important in the case of triazine compounds since they do not leach or degrade rapidly<sup>3,4</sup>.

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### Modifications of the Agar Film Technique for assaying Lengths of Mycelium in Soil

CRITICAL analysis of methods for assaying lengths of fungal mycelium present in unit weights or volumes of soil<sup>1</sup> has shown that the agar film technique<sup>2,3</sup> is least prone to experimental error. The technique has been used successfully in these laboratories for a number of years, and a number of slight modifications have proved useful in eliminating accidental errors and increasing the reliability of the technique. These modifications are incorporated in the following description of the procedure.

Variable amounts of soil (between 0.5 g and 4.0 g) are taken and the entire sample is ground in a mortar with 4 or 5 changes of sterile water (5 ml. water being used at each change). The time for the first grinding is 5 min and for the subsequent grindings 2 min each. Thus a total of 20–25 ml. of soil-water suspension is obtained.

All the soil particles are poured into the collecting container for the soil-water suspension, because observation of the larger soil particles left behind in the decantation procedure suggested in the original description of the technique<sup>2</sup> revealed the presence of fungal hyphae (a large proportion of which appeared to be free in the water retained by the soil, not directly associated with the larger soil particles).

Molten 1.5 per cent 'Oxoid No. 3' agar is then added to the soil-water suspension to give a known final dilution of the original soil sample (approximately 1 g soil in 50 ml.). The dilution prepared depends on the soil under investigation (in soils with a large amount of mycelium a higher dilution is used) and preliminary experiments are required to ascertain the optimum dilution for the soil under investigation. The final soil-water-agar suspension is maintained at 50°–60° C for the very short period between preparing the final suspension and the preparation of the agar films.

Immediately before preparation of the film the soil-water-agar suspension is thoroughly agitated and allowed to settle for 5–10 sec before small amounts of the suspension are pipetted from approximately 1 cm below the surface, placed on the platform of a haemocytometer slide (depth 0.1 mm). The well of the slide is immediately covered by a coverslip and the enclosed suspension is left to solidify. Care must be taken to ensure that the soil-water-agar suspension does not overflow from the haemocytometer platform as the depth of the film prepared will then exceed 0.1 mm.

Various possible sources of error have been checked. (i) It is conceivable, for example, that despite thorough stirring of the soil-water-agar suspension immediately before pipetting off small samples for the agar film preparation, there was an accumulation of mycelium floating on the surface of the suspension. This would lead to an overestimate of the amount of mycelium, since samples were always taken from just below the surface. A series of agar films was made from samples taken from three levels in a single soil-water-agar suspension (that is, top, middle and bottom). Four slides were made from

Table 1. TEST OF DISPERSION OF MYCELIUM IN THE SOIL-WATER-AGAR SUSPENSION: LENGTH OF MYCELIUM (M/G SOIL) BASED ON OBSERVATIONS OF AGAR FILMS PREPARED FROM 3 DEPTHS IN THE SOIL-WATER-AGAR SUSPENSION

Sampling position	1	2	Slide No. 3	4	5
Top	133.3	126.0	127.0	133.2	129.9
Middle	132.7	136.1	140.1	131.8	135.2
Bottom	141.0	138.3	127.2	139.9	135.6

Table 2. TEST OF PIPETTING PROCEDURE: LENGTH OF MYCELIUM (M/G SOIL) BASED ON OBSERVATION OF AGAR FILMS PREPARED FROM SUSPENSION FROM THREE POSITIONS IN THE PIPETTE

Sampling position in the pipette	1	2	Slide No. 3	4	5
Tip	93.2	99.6	90.1	95.6	94.0
Middle	100.5	96.2	94.0	91.8	95.6
Top	103.6	94.3	96.8	92.0	96.7

samples from each position; 100 observations were made per slide and the amount of mycelium at each depth was estimated (Table 1). There were no marked differences. (ii) The tip of the hand-drawn pipettes used was about 0.5 mm, and the possibility existed that in drawing up the suspension into the pipette there could be an accumulation of mycelium at the tip of the pipette, its narrow entrance acting as a filter. This would cause an overestimate of the amount of mycelium per unit amount of soil because only the first few drops delivered by the pipette were used in the preparation of an agar film. Quantitative assay of the mycelial lengths from preparations made from successive deliveries from the same pipette, however, indicated no filtering effect (Table 2). (iii) Because of the viscosity of the soil-water-agar suspension, the agar films produced could be thicker than 0.1 mm, therefore allowing an overestimate of mycelium lengths. This has been found to occur. To overcome this a small square weight (approximately 5 g) cemented to a coverslip can be placed over the suspension held in the well of the haemocytometer slide.

After the soil-water-agar suspension has solidified on the haemocytometer slide, the slide is immersed in distilled water and the coverslip removed. Surplus agar in the moats of the haemocytometer is cut away with a razor blade and the agar film is floated off with the aid of a fine camel-hair brush on to an ordinary microscope slide and allowed to dry at room temperature (acceleration of the drying process may cause cracking of the agar film). The dried agar film is then stained for 1 h using phenolic aniline blue, washed in 3 or 4 changes of 98 per cent alcohol and mounted in Euparal.

The slides are examined under the microscope ( $\times 200$ ) and lengths of mycelium in each of a number of fields on each slide are measured. The number of fields per slide and the number of slides examined per soil sample is experimentally and statistically determined. Measurement of hyphal lengths is made either by projecting the images of the fields examined, and measuring the hyphal fragments with a tachometer, or by means of a camera lucida drawing of the hyphal fragments in each field, the total amount of hyphae per field being measured by a tachometer. Thus lengths of mycelium in unit weight or volume of soil can be assessed by taking into account the area of the fields observed, the depth of the agar film, the number of fields viewed, the total volume of soil-water-agar suspension observed, and the degree of dilution of the original soil sample.

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## ENTOMOLOGY

## New Organ in Stenocephalidae (Hemiptera-Heteroptera)

INVESTIGATIONS have revealed the presence of a pair of hitherto undescribed organs of unknown function in the abdominal cavity of males of the genus *Dicranocephalus* Hahn (*D. agilis* the type species, *D. albipes*, *D. marginicollis*, *D. setulosus* and *D. lateralis* have been examined). These sac-like structures are situated principally in the seventh segment and appear to be formed by a prolongation of the posterior ventral margin of the eighth segment (Figs. 1 and 2) and are about 1 mm long. Distally the sacs are joined to a sclerotized plate from which arise about twenty-five to thirty semi-erect heavily sclerotized and pigmented tubes (Fig. 3), the entire structure being an invagination of the inter-segmental membrane of the eighth segment (Figs. 1 and 2).

The organs were first observed when entire abdomens were cleared (caustic potash, acetic acid and creosote): their surviving this treatment and their anatomical origin suggest the organs are exoskeletal. After clearing, the organs are hyaline and appear to be composed of a large number of plates overlapping rather like the tiles of a roof (Fig. 4), each being enlarged where it is exposed externally. Each plate has transparent spine-like processes scattered about in irregular groups (Fig. 5), the groups of processes increasing in number and density where the organs join the sclerotized plate. The sacs fill with air when they are heated in caustic potash. When the air is expelled by slight pressure it can be forced into the lumen of the sclerotized tubes and trapped there, although it disappears after several hours by diffusion. This suggests that the inter-segmental invagination must extend under the plate as an air-tight flattened sac.

The tubes are directed downwards towards the ventral surface of the abdomen and are of variable length; the longest on the margins, the shortest in the centre of the sclerotized plate. Each tube is densely clothed with semi-erect hairs, the hairs increasing in length and density distally (Fig. 6).

No similar structures have hitherto been described in Heteroptera, but an analogous organ has been found in the S. African pentatomid genus *Boerias* Kirkaldy<sup>1</sup>. In

*Boerias* there is a median sac lying within the ninth segment and opening into the extreme apex of the rectum; the duct is apparently closed by a pair of ligaments. Leston's structure is similar to that described here in being exoskeletal. All other ectodermal abdominal sacs described, for example, the large rectal diverticulum of *Belostomatidae*, are found in both sexes and in belostomatids are defensive in function.

It is possible that the sacs of *Dicranocephalus* collect a secretion from epidermal glands situated at the apices of the spine-like processes, and their function is almost certainly associated with mating.

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### Trans-4-oxohex-2-enal in the Odoriferous Secretion of *Sigarafalleni* (Fieb.) (Hemiptera-Heteroptera)

INTEGUMENTARY glands which produce odoriferous substances are of widespread occurrence in the Heteroptera. The glands are not confined to terrestrial forms but are found also in many aquatic species. Paired dorso-abdominal glands are present in the nymphs, but at the time of the adult moult these are replaced by glands in the metathorax which open to the exterior on the ventral surface.

Short-chain aliphatic aldehydes of variable chain length ( $C_6$ – $C_{10}$ ), both saturated and unsaturated, have been found in the secretions of coreids<sup>1,2</sup>, pentatomids<sup>2-5</sup> and the cimicid *Cimex lectularius* L.<sup>5</sup>. In addition to aldehydes the secretions of some pentatomids have been found to contain the paraffins dodecane and tridecane<sup>2,3</sup>. Unidentified constituents include dicarbonyls<sup>2,3</sup> and possibly furans and quinones<sup>6</sup>. *Trans*-hex-2-enyl-acetate has been identified in the secretion of males of the giant waterbug *Lethocerus indicus* (Lepeletier and Serville), and because it is sex-specific is thought possible to function as a sex attractant<sup>7</sup>. This would appear to be unusual, however, for there can be little doubt that the secretions

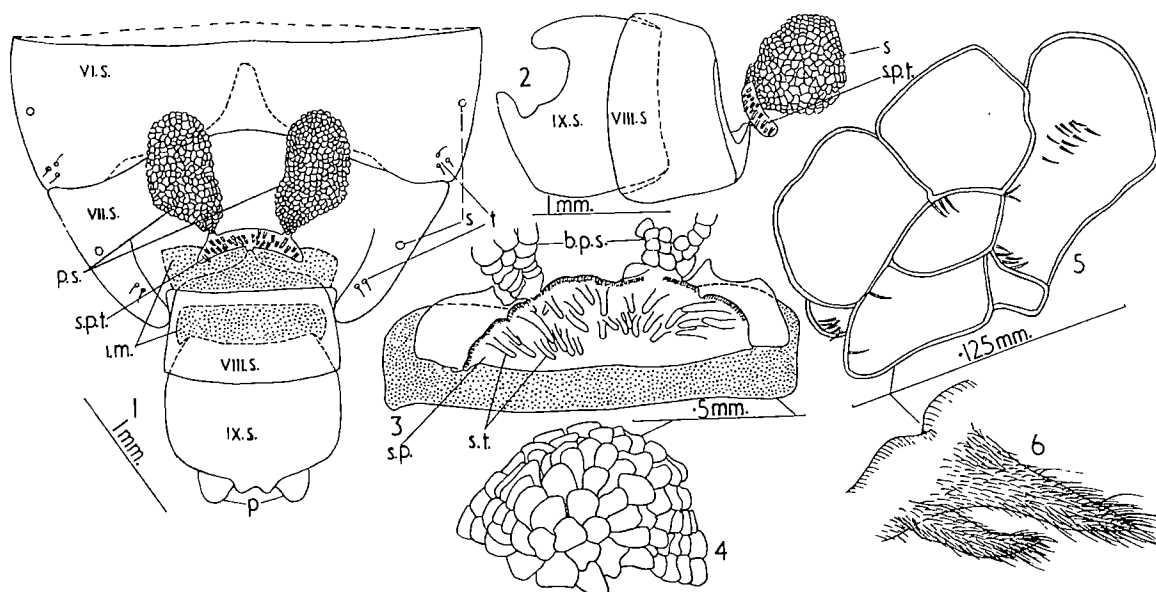


Fig. 1. Ventral view of abdomen of *D. albipes* (vi.s.; vii.s.; viii.s.; ix.s.) sixth to ninth abdominal segments; (p.s.) paired sacs; (t.m.) inter-segmental membrane; (t.) trichobothria; (s.) spiracles; (s.p.t.) sclerotized plates with tubes; (p.) parameres. Fig. 2. Side-view of eighth and ninth abdominal segments of *D. marginicollis*, parameres removed. Fig. 3. Sclerotized plate with tubes of *D. albipes* (b.p.s.) base of paired sacs; (s.p.) sclerotized plate; (s.t.) sclerotized tubes. Fig. 4. Proximal ventral view of sac of *D. marginicollis*. Fig. 5. Section of sac showing irregular grouping of transparent spine-like processes of *D. marginicollis*. Fig. 6. Tubes on sclerotized plate showing variation in length and density of hairs of *D. albipes*.

are widely used as a defence against predators, at least in the terrestrial Heteroptera<sup>8</sup>.

In members of the aquatic Corixidae the secretory apparatus of the adult comprises a pair of tubular glands and a median sac-like reservoir<sup>9</sup>: the external opening lies between the hind coxae and is provided with a closing mechanism. The secretion appears to be forced out by the elasticity of the distended reservoir and by an increase in body pressure. It passes into a cavity above the metasternal process to be conveyed along a pair of furrows in the cuticle, left and right, as far as a notch on the posterior edge of each mesothoracic epimeron which bears a tuft of slender bristles. The secretion exudes to form a pair of droplets which appear to be brushed off by the hind legs.

We have investigated the composition of the secretion of adults of *Sigara falleni* (Fieb.). The secretion was a pale yellow oil with a pleasant odour reminiscent of 'leaf aldehyde' (hex-2-enal). The main constituent was identified by its chemical and physical properties and by synthesis as *trans*-4-oxohex-2-enal (*trans*-3-propionyl acrylaldehyde), which appears to be new to the chemical literature. A minor constituent (less than 5 per cent) was not identified.

The secretions of male and female *S. falleni* were not distinguishable. Similarly, no difference could be found between the secretions of *S. falleni* and *Corixa dentipes* (Thoms.). It would thus appear that the secretion is neither sex- nor species-specific and may well be characteristic of the Corixidae.

The function of the secretion has not been investigated. There is some evidence to indicate that fish tend to avoid Corixidae<sup>10</sup>, but whether on account of the odoriferous secretion is not known. While the secretion may act as a defence against predators there is the possibility that it also confers some protection against micro-organisms by preventing them from settling on the body surface; some aldehydes have been shown to have both fungicidal and bactericidal properties<sup>6</sup>.

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## VIROLOGY

### Virus of Feline Panleucopænia

PROGRESS in understanding the immunological features of feline panleucopænia has, for many years, been impeded due to failure to isolate the causal agent of the disease. That this agent has the properties of a virus has been evident since the original observations of Verge and Christoforoni<sup>1</sup>, but although many viruses have been isolated from sick and healthy cats, none has been shown to be related to the feline panleucopænia syndrome<sup>2</sup>.

In February last year a virus, leopard virus (LV), was isolated in kitten kidney monolayers from the spleen of a leopard which died from a condition simulating feline panleucopænia<sup>3</sup>. The following evidence has since been accumulated that this virus is involved in the feline panleucopænia syndrome.

(1) The transient intranuclear inclusions seen in cells in tissue culture show stages of nuclear changes similar to those seen in gut epithelium cells of naturally affected kittens. Similarly, in organ cultures of cat spleen infected with third tissue culture passage LV, intranuclear inclusions appeared having the same morphology and distribution as those seen in organ cultures infected with extracts of feline panleucopænia tissues supplied by Wellcome Research Laboratories.

(2) Viruses showing the same characteristic transient cytopathic effect as LV have been isolated from four out of five field cases of feline panleucopænia which were diagnosed as such on clinical and histological features. A similar virus was also isolated from tissues supplied by Wellcome Research Laboratories. No such viruses have been isolated from the spleens of healthy cats, or cats dying from conditions other than feline panleucopænia.

(3) The properties of the virus in tissue culture fluids correspond closely to the properties of the virus of feline panleucopænia agent from infected tissues, in size of virus and the failure to infect other hosts *in vitro* and *in vivo*.

(4) The cytopathic effect of LV and the five other isolates is neutralized by high dilutions of hyperimmune feline panleucopænia sera, and similar neutralizing antibodies have been detected in a majority of normal cats and suckling kittens in this area, suggesting that infection with the virus is fairly common.

(5) Kittens showing low level antibodies to LV on being infected with fourth tissue-culture passage of LV developed panleucopænia and depression, similar in all respects to that shown by litter-mates infected with feline panleucopænia tissues.

(6) Kittens recovered from infection with LV resisted challenge with feline panleucopænia tissues, while litter-mates recovered from infection with feline panleucopænia tissues resisted challenge with passaged LV. In both instances control kittens developed typical panleucopænia.

(7) Following infection with feline panleucopænia tissues, kittens showed an antibody rise to LV similar to the antibody rise shown in litter-mates infected with feline panleucopænia tissues.

From the evidence accumulated, to be published in a more detailed form, it would appear that a virus has been isolated which is very closely related to the feline panleucopænia syndrome. It is considered highly probable that this virus is the causal agent of the disease.

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## GENETICS

### Isolation of Ploidy Mutants of Ehrlich Ascites Tumour of Mouse

To examine the effect of polyploidy on radiosensitivity in mammalian cells, it is desirable to induce a ploidy mutant from a clone derived from a single cell. Theoretically such a cell clone could be isolated by the tissue culture method developed by Puck *et al.*<sup>1</sup>. The following technique of injecting single cells is applicable also to cells which are difficult to culture *in vitro*.

A hyperdiploid line of Lettré's Ehrlich ascites tumour of mouse (ELD) served as the starting material. Adult as well as new-born mice were used as host animals. The

tumour was transplanted into the adult mice by injecting more than  $10^7$  cells. The new-born mice, which were not more than 48 h old, and which were previously irradiated with 300 r. of X-rays to stop the antigen-antibody reaction against the tumour, were injected intraperitoneally with a single tumour cell. Single cells were isolated from a dilute suspension of cells with the help of a micromanipulator. The cell was sucked from an airtight chamber into a thin capillary tube and was injected into the peritoneal cavity of the mouse with the finely drawn out point of the capillary tube. The dilution medium had the following composition: doubly distilled water 100.00; NaCl 0.95; foetal bovine serum (Microbiol. Assoc., Bethesda), 15.00; neomycin, 0.025; polymyxin, 0.0025. Native tumour ascites was diluted with the dilution medium to 1:125. The complete operation from the withdrawal of the tumour to the injection of single cells into the peritoneal cavity of the new-born mouse was performed in less than 1 h. The chamber, containing the suspension of tumour cells, was made by placing an ultra-violet-sterilized polymethacrylic acid ('Lucite' or 'Perspex') ring between two sterilized coverslips stuck on with a drop of liquid paraffin. A small hole in the ring permitted filling and micromanipulation, and the chamber was filled with a thin injection needle, avoiding air bubbles. The opening in the plexiglass ring was finally sealed with a drop of liquid paraffin. By inserting the capillary tube through this drop into the cell suspension (Fig. 1) it was possible to prevent a direct contact between cell suspension and air during the micromanipulation and thus to avoid drying by evaporation. The free sliding of the ring on the coverslips facilitated the manipulation and guidance of the capillary tube.

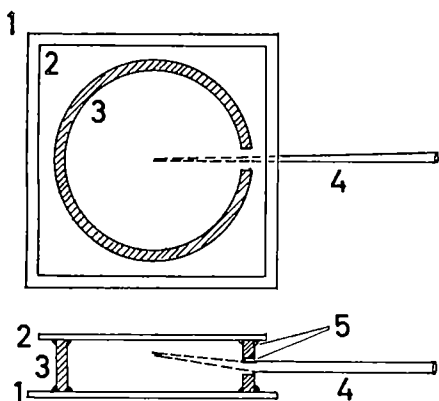


Fig. 1. 1 and 2, coverslip; 3, ring made from 'Perspex'; 4, capillary tube; 5, liquid paraffin

A certain number of new-born mice thus injected with a single cell each of the hyperdiploid line ELD developed tumour ascites about 3-4 weeks after injection which could easily be detected visually. All single-cell clones thus produced were analysed cytologically by the Feulgen method. To induce polyploidy by colchicine, two types of diploid clones were selected. 0.5 ml. of 2 per cent colchicine was injected intraperitoneally into the tumour ascites developed 8 days after transplantation of cells from a selected hyperdiploid clone. After about 2.5 h, the ascites was withdrawn, and, after washing, either single cells were isolated immediately and directly injected into new-born mice, or more than  $10^7$  cells were transplanted into adult mice. In this case single cells were isolated only after an intermediate tumour passage. The sub-clones thus produced were examined again cytologically.

Out of about 160 new-born mice, each injected with one single cell of line ELD, 40 single-cell clones were isolated. The chromosome number distribution of the stemline ELD and of the isolated clone ELDD<sub>1</sub> is given in Table 1.

Table 1

Chromosome No./cell	No. of cells		ELDD <sub>1</sub> + colchicine*
	ELD	ELDD <sub>1</sub>	
40	28	5	6
41	42	14	10
42	240	87	43
43	66	33	16
44	60	12	9
45	28	4	7
46	19	1	3
47	15	—	—
48	12	—	1
49	12	—	—
50	10	—	—
51-79	14	—	—
>80	16	5	14

\* Cells examined after one intermediate tumour passage.

In both cases the modal chromosome number was 42. However, the chromosome distribution spectrum of the clone ELDD<sub>1</sub> was narrower than in the stemline ELD. The proportion of hypertetraploid cells in the line ELD and in the clone ELDD<sub>1</sub> was about 3 per cent. After the colchicine treatment of the clone ELDD<sub>1</sub> and examination of the tumour after one intraperitoneal transfer, the proportion of hypertetraploid cells increased to about 14 per cent. From the clone ELDD<sub>1</sub>, treated with colchicine, sub-clones were isolated either with (group I) or without (group II) an intermediate passage and one such clone proved to be a hypertetraploid. Altogether, 20 sub-clones were isolated; 13 belonging to group I and 7 to group II, and 13 were examined in cytological detail. The hypertetraploid clone isolated belonged to group II, that is, the cell was injected immediately after colchicine treatment without any intermediate tumour passage.

Table 2

Chromosome No./cell	ELDT <sub>1</sub> No. of cells
80	—
81	4
82	3
83	16
84	38
85	20
86	12
87	3
88	2
89	—
90	—
>91	12

The chromosome number distribution of this isolated hypertetraploid (ELDT<sub>1</sub>) is given in Table 2. The modal chromosome number, 84, was exactly double that of the parent clone ELDD<sub>1</sub>. The characteristic median chromosome of ELDD<sub>1</sub> was represented twice in ELDT<sub>1</sub>. Thus it may be inferred that the chromosome number 42 of a cell of ELDD<sub>1</sub> was doubled and that certainly only one such cell was injected into a new-born mouse. Such hypertetraploid cells occur in the clone ELDD<sub>1</sub>, immediately after colchicine treatment, with a frequency of about 30 per cent. It thus follows that the isolated sub-clone ELDT<sub>1</sub> is induced by colchicine treatment with a probability ~ 0.9 and does not originate as a spontaneous mutant. Hornsey and Silini<sup>2</sup> and Révész *et al.*<sup>3,4</sup> have compared the relative radiosensitivity of a diploid line with a 'tetraploid' line of Ehrlich ascites tumour. They found that the 'tetraploid' line was more radio-resistant than the diploid line, under anaerobic conditions. Till<sup>5</sup>, on the other hand, failed to find any relationship between the chromosome number and radiosensitivity in lymphosarcoma cells. The hypertetraploid<sup>6</sup> Ehrlich tumour line investigated by us (Bhaskaran and Ditttrich) was found to be extremely radiosensitive. The conclusions drawn from the experiments conducted so far are not conclusive, because one cannot exclude the possibility that the lines compared, apart from ploidygrade, differ from each other genetically. Such a difficulty could be avoided by comparing a tetraploid sub-clone derived from a diploid clone, cultured from a single cell. The relative radiosensitivities of ELDD<sub>1</sub> and ELDT<sub>1</sub> are under investigation.



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### Computer Analysis of Concordance of Inherited Characteristics

IN anthropological surveys, investigations of the effects of mutagenic agents, and similar projects, the transmission of hereditary characters is frequently investigated in large populations. Where data are available it is advisable to ascertain whether the children's phenotypes agree with those of their presumptive parents. Lack of agreement indicates that the data were influenced by factors other than family relationships, for example, illegitimacy, errors of labelling, technique or recording, or the effects of mutation. The greater the number in the group, the more likely are the factors producing disagreement to be identified, but the more burdensome becomes the labour of correlation.

A digital computer programme had been evolved to analyse concordance, within families, of two unrelated characters (haptoglobin and transferrin) each determined by two frequently-occurring alleles, and to record variants due to a third allele, for use with data derived from processing of sera obtained in an anthropological survey of New Guinea natives. This programme, however, could not be used with data involving characters determined by more than two alleles, such as blood groups, haemoglobins, etc., so a general programme was evolved to enable the more complex data to be included.

Both programmes were written in the Fortran II autocode<sup>1</sup> for the I.B.M. '1401' computer although other computer languages such as Algol<sup>2</sup> could have been employed. The general programme is described here both because of its novel function and because it uses a digital computer in its algorithmic role to perform repetitive decisions.

An identifying number was allotted to each individual (NI) and to each family (NF). A family was defined as the two parents and their children. If an individual belonged to more than one family because of multiple marriages or because more than two generations were recorded he was allotted a card for each family and these cards differed from one another only in the NF number and in the parental status (NP) which was defined as mother (2), father (1) or child (0). Because each variable inherited (phenotypic) characteristic (M) was assumed to have been determined by the action of only two genes (S and T, derived one from each parent) it was encoded in two columns, defined as NS(M) and NT(M) of an 80-column Hollerith card. The characters expressed by alleles of genes S and T were represented in columns NS(M) and NT(M) respectively by numbers 1-9, a particular number having been assigned to the character expressed by the corresponding allele. An individual was encoded as a homozygote for the expressed allele when a character due to another allele was not observed because subsequent identification of 'misfits' by the computer in the related family allowed recognition of the individual as a possibly heterozygous parent. Each instruction to the computer contained in the programme is termed a

'statement' and may be identified by a reference number<sup>1</sup>. To commence the sequence the computer is instructed to read the first two cards and to index and store the values punched in them. The representation within the computer of all values punched in a card may be termed a 'card image'<sup>3</sup>. In a following statement the computer is instructed to compare the NF numbers of the two cards and, depending on the outcome, to decide which set of instructions it should follow. The general form of such a statement is:

$$\text{If } (M - N) \text{ } 1, 2, 3 \quad (1)$$

which can be translated "if  $M < N$  follow instruction 1; if  $M = 0$  follow instruction 2; and if  $M > N$  follow instruction 3", where M and N are real positive integers. Any two of these instructions may be identical as in the statement:

$$\text{If } (M - N) \text{ } 1, 2, 1 \quad (2)$$

which reduces the choice to one of two alternatives, and any one or two of these instructions may lead to the execution of a preceding instruction and thus establish a repetitive process which is continued until an alternative instruction can be executed or all cards have been read.

The specific form of the instruction to compare the NF numbers of two card images is:

$$\text{If } [NF(I) - NF(N)] \text{ } 1, 2, 3 \quad (3)$$

where I is the last and N the penultimate card image. If the cards are identical [ $NF(I) = NF(N)$ ] the next card is read and examined and so on until  $NF(I)$  exceeds  $NF(N)$  when the card images of the whole family have been stored and when another instruction is selected. The new instruction is to select the parents' card images (condition  $NP(I) \geq 1$ ):

$$\text{If } (NP(I) - 1) \text{ } -, 0, + \quad (4)$$

and to assign to the values of their inherited characteristics the subscripts<sup>1</sup> J and K (or J only if there is but one parent) in the form NS(J,M) and NT(J,M), etc.

The children's card images are also identified by statement (4) (condition  $NP(I) < 1$ ), their genetic characteristics are subscribed NS(L,M) and NT(L,M), etc., and comparison is then made with the characteristics first of one parent and then of the other:

$$\text{If } [NS(J,M) - NS(L,M)] \text{ } -, 0, + \quad (5)$$

As the computer directly compares the children's characters with those of the parents, an allelic array larger than 2 can be processed without altering the programme, and as the inherited characters are sequentially examined their number can be indefinitely extended by postponing only the last instruction, which is that the last card image stored (I), corresponding to the first card of the next family, should be indexed as the first, and that the reading for the following cards should commence.

If the genetic polymorphism of the characteristic is sufficiently large the programme can also be expanded to include the sequence for analysing a family consisting only of siblings. This sequence can be executed provided that there are at least three siblings, when the presence of two allelic homozygotes precludes there being more than two allelic genes in the sibship, when the presence of one homozygote allows no more than three alleles in the sibship, and when the absence of homozygotes allows no more than four alleles to be found (parents  $ST \times ST$ ,  $S^1T \times S^2T$  and  $S^1T^1 \times S^2T^2$  respectively). The decision as to which alleles 'belong' and which do not can only be made on statistical and not logical grounds, and the computer is therefore instructed to print the values of the whole family after determining whether the family obeys or does not obey the conditions of sibship.

I can supply copies of both programmes to which reference has been made. I thank Dr. C. C. Curtain of the Baker Medical Research Institute, Melbourne, for

advice. The work was done during my tenure of a research fellowship from Alfred Hospital, Melbourne.

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## STATISTICS

### All-or-none Traits and the Sensitivity of Experiments

THE choice of the strain of laboratory animal has been examined by Chai<sup>1</sup>, by Biggers, McLaren and Michie<sup>2</sup>, and by Becker<sup>3</sup>. Much of the discussion in these papers involved the choice of mice of inbred strains or those of the  $F_1$  generation for bioassay. In addition, Becker and Berg<sup>4-6</sup> have reported tests which were designed to determine which sex and strain of chickens produced the most sensitive nutritional experiments.

Many of the examples have used measurements of continuous or quantitative traits such as body-weights, observations on cell heights and mouse reflex times. The sensitivity of a specific experiment involving a quantitative trait can be measured by the ' $F$  value'. It represents the ratio of the among-treatments mean square to the within-treatments mean square, or that of the regression mean square to the mean square of deviations from linear regression in the analysis of variance. The theory and application of the use of the  $F$  value as an indication of the sensitivity of experiments have been set forth by Schumann and Bradley<sup>7,8</sup>.

An opportunity to investigate some aspects of the choice of animals and the sensitivity of experiments when an all-or-none trait was used was provided when four selection lines of White Leghorn chickens developed by Becker and Bearse<sup>9</sup> were each placed on two nutritional treatments in each of two years. The trait was the presence or absence of a blood spot in the egg as determined by observations of the broken-out egg within 72 h of oviposition. The treatments employed in this investigation were a ration adequate for egg production but lacking sufficient vitamin A, and one that was fortified with this vitamin. Diets below optimum requirements for egg production in vitamin A were shown to raise the blood spot percentage<sup>10</sup>.

Lines 1 and 2 had been selected for a low incidence of blood spots and Lines 3 and 4 for a high incidence. In both years (Exps. 1 and 2) hens' records were randomly removed so that there were equal numbers of hens within each sub-class in each analysis.

In Exp. 1,  $n = 68$  per sub-class, and the incidence of blood spots was determined for all eggs laid by each hen in a 10-day period. In Exp. 2,  $n = 69$  per sub-class, and eggs laid during a 20-day period were broken open and examined. The incidence of blood spots was calculated for each hen.

A one-way layout of the analysis of variance was computed for each of the lines in each of the two years and the  $F$  value (ratio of mean square, between dietary treatments, to mean square among hens within diets) was calculated. The arc sine transformation resulted in only slight changes in the  $F$  values. Therefore, data reported herein refer to the actual percentages only.

The estimates of the overall mean, the response of a line to the dietary changes (expressed as the difference between diets), the variance component attributable to the fact that diets were different, the error term and the  $F$  value of the one-way layout analysis for both experiments are given in Table 1. The results, in general,

Table 1. A COMPARISON OF FOUR BLOOD SPOT SELECTION LINES USED IN EXPERIMENTS DESIGNED TO DETERMINE DIFFERENCES IN BLOOD SPOT INCIDENCE FED DIETS EITHER ADEQUATE OR INADEQUATE IN VITAMIN A (VARIANCE  $\times 10^3$ )

Line	Blood spot incidence	Average over both diets $\bar{x}$ %	Adequate diet— inadequate diet %	Variance component	Error term	$F$ value
Experiment 1						
1	Low	11.4	7.6	280	2,824	7.7*
2	Low	17.5	11.5	584	4,980	9.0*
3	High	24.0	16.6	1,316	4,558	20.6†
4	High	39.8	28.9	3,651	8,141	30.4†
Experiment 2						
1	Low	12.0	7.7	260	2,142	9.4*
2	Low	12.7	5.2	64	4,118	2.1
3	High	23.9	13.5	858	4,367	14.6†
4	High	27.8	16.0	1,223	3,969	22.3†

\* Significant at the 1 per cent level.

† Significant at the 0.1 per cent level.

indicate that as the overall mean increased, the error term, response to diet, variance component and  $F$  value also increased.

Thus, the experiments involving an all-or-none trait were more sensitive, as determined by the  $F$  value, when the lines utilized had a genotypically higher incidence than when low incidence lines were used. In these experiments the data indicate that greater reliance could be placed on the results as the incidence of the all-or-none trait approached 40 per cent.

Table 2. EXPERIMENTS DESIGNED TO DETERMINE DIFFERENCES AMONG BLOOD SPOT SELECTION LINES ON DIETS ADEQUATE AND INADEQUATE IN VITAMIN A (VARIANCE  $\times 10^3$ )

Diet	Average $\bar{x}$ %	Variance component	Error term	$F$ value
Experiment 1				
Inadequate	31.0	260	673	27.3*
Adequate	15.3	594	3,515	12.5*
Experiment 2				
Inadequate	24.4	1,030	4,007	18.7**
Adequate	13.8	261	3,291	6.5*

\* Significant at the 0.1 per cent level.

It was also possible to analyse the data to determine the effect of the environment on the sensitivity of experiments when four lines were assumed to be the treatments. In Table 2 the overall mean, the variance component attributable to the fact that the lines were different, the error term and the  $F$  value (ratio of mean square among lines and among individuals within lines) are given. The experiments on the inadequate diet, which raised the incidence, had larger  $F$  values than the experiments determining differences among lines on the adequate diet.

It seems, therefore, that raising the incidence of an all-or-none trait, either by changing the environment when determining genotypic differences or by using genotypically high-level strains when ascertaining environmental differences, resulted in more sensitive experiments.

Whether or not the sensitivity of the experiments would react in the same manner for other all-or-none traits, other environments and other genotypes, would have to be determined by further experimentation.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, January 4

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. R. Knight and Mr. K. Hambleton: "The Present State of Gallium Arsenide Technology".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—Geographical Films.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. B. E. Moody: "Recent Developments in Container Glass".

## Tuesday, January 5

BRITISH INTERPLANETARY SOCIETY (in the Large Physics Theatre, University College, Gower Street, London, W.C.1), from 10 a.m. to 4.30 p.m.—Symposium on "The Engineering of Scientific Satellites".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Prof. S. Tolansky, F.R.S.: "The Curiosities of Sound" (Dr. Mann Juvenile Lecture).

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. Robert Mathieson and Mr. John Stebbings: "Design of a Modern Hydraulics Laboratory for Teaching and Research".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (joint meeting with the Science and General Division, at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. J. C. West, Dr. B. V. Jayawant and Mr. G. Williams: "Dynamic Performance of Induction Motors in Control Systems".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Water-Level Indicators for Pressure Vessels".

## Wednesday, January 6

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 3 p.m.—Meeting on "The Design of Ship's Bridges".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. J. Lawrenson and Mr. L. A. Agu: "Theory and Performance of Polyphase Reluctance Machines".

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fluid Dynamic Model Tests as a Basis for Engineering Design".

## Thursday, January 7

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Imperial College of Science and Technology, London, S.W.7), at 3 p.m.—Symposium on "Paint versus Corrosion".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 3 p.m.—Mr. R. Stanton Jones: "Ground Effect Vehicles" (Young People's Lecture).

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Is the Civil Engineer's Responsibility being Subordinated to Administration?" introduced by Mr. E. C. Beck and Mr. Oliver Dawson.

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Electrostatic Hazards in Factories".

## Friday, January 8

BRITISH MYCOLOGICAL SOCIETY (in the Department of Mechanical Engineering, City and Guilds College, Exhibition Road, London, S.W.7), at 10.45 a.m.—General Papers.

## Monday, January 11

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. S. Wilkie: "Modes of Causality in the Language of Biologists".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. Swafield: "Speech Compression".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Sir Vivian Fuchs: "Recent British Exploration in Antarctica".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT (with a good honours degree) IN THE DEPARTMENT OF AGRICULTURE (Crop Husbandry) to assist with experimental work on the improvement of grassland—The Registrar, University College of Wales, Aberystwyth (January 9).

RESEARCH BIOCHEMIST (with at least a good second-class honours degree) to assist Dr. R. M. C. Dawson in lipid metabolism studies—The Secretary, Institute of Animal Physiology, A.R.C., Babraham, Cambridge (January 9).

ASSISTANT LECTURER (with an honours degree in pharmaceutical chemistry or equivalent, and preferably some experience in industrial research or medicinal chemistry) IN PHARMACY—The Registrar, The University, Manchester, 13 (January 11).

POST-DOCTORAL RESEARCH FELLOW IN THE APPLIED NUCLEAR SCIENCE GROUP, DEPARTMENT OF PHYSICS, for work concerned with the problems of dental caries under the terms of a Medical Research Council award—The Administrative Officer, Department of Physics, The University of Birmingham, Birmingham, 15 (January 11).

CHAIR IN ORGANIC CHEMISTRY, in an integrated Department of Chemistry; CHAIR IN THE DEPARTMENT OF MATHEMATICS; and CHAIR IN THE DEPARTMENT OF PHYSICS—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (January 12).

GRADUATE CARTOGRAPHER (person who has included geography in their degree, with experience in the compilation and drawing of maps for publication) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, The University, Aberdeen (January 15).

LECTURER IN BACTERIOLOGY at the Western Infirmary—The Secretary of University Court, The University, Glasgow (January 16).

UNIVERSITY LECTURER IN THE STATISTICAL LABORATORY IN THE DEPARTMENT OF PURE MATHEMATICS AND MATHEMATICAL STATISTICS, University of Cambridge—The Secretary of the Appointments Committee, Faculty of Mathematics, Arts School, Bene't Street, Cambridge (January 16).

LECTURER IN ANTHROPOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (January 18).

CHAIR OF EDUCATION at King's College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 22).

LECTURER OR SENIOR LECTURER IN CLINICAL PATHOLOGY IN THE DEPARTMENT OF ANIMAL HEALTH, in the Faculty of Veterinary Science, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University (New Zealand and London, January 22).

SENIOR LECTURER OR LECTURER IN VETERINARY ANATOMY; and a SENIOR LECTURER OR LECTURER IN MICRO-ANATOMY IN THE DEPARTMENT OF VETERINARY BIOLOGY, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University (New Zealand and London, January 22).

PRINCIPAL (with a degree or honours degree in agriculture, experience of tropical agriculture, administrative ability, and preferably teaching experience) of the College of Agriculture (University of Malaya)—The Secretary, Council of the College of Agriculture, P.O. Box 203, Sungai Besi, Selangor, or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, January 23).

LECTURER OR SENIOR LECTURER IN MORBID ANATOMY IN THE DEPARTMENT OF PATHOLOGY, University of the West Indies, Kingston, Jamaica—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (January 25).

READER IN PURE MATHEMATICS at Queen Elizabeth College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 25).

ASSISTANT LECTURER IN GEOLOGY—The Registrar, Room (X), The University, Hull (January 26).

ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, Room (X), The University, Hull (January 28).

SENIOR LECTURER IN THEORETICAL PHYSICS; LECTURERS OR ASSISTANT LECTURERS IN THEORETICAL OR EXPERIMENTAL PHYSICS; and a DEMONSTRATOR IN THE DEPARTMENT OF PHYSICS—The Registrar, Room (X), The University, Hull (January 28).

ASSISTANT LECTURER OR LECTURER (or, in special cases, Senior Lecturer) IN ELECTRICAL ENGINEERING in the School of Applied Sciences—The Assistant Registrar (Establishment), University of Sussex, Stanner House, Stanner, Brighton, Sussex (January 29).

LECTURER (with a good honours degree in geography, and either a higher degree or actively engaged in research leading to a higher degree) IN GEOGRAPHY, at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 29).

READER, SENIOR LECTURER and a LECTURER IN MATHEMATICS at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 29).

LECTURER/ASSISTANT LECTURER (with a higher degree in biochemistry, some teaching and research experience, and preferably a special interest in physical biochemistry or enzyme biochemistry) IN THE DEPARTMENT OF BIOCHEMISTRY, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, January 30).

LECTURER OR ASSISTANT LECTURER IN INORGANIC CHEMISTRY; and a LECTURER OR ASSISTANT LECTURER IN PHYSICAL CHEMISTRY—The Secretary, The University, Aberdeen (January 30).

SUB-LIBRARIAN at the University of Ife, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 30).

ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, Room (X), The University, Hull (January 31).

HEAD OF THE BACTERIOLOGY DEPARTMENT—The Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh (January 31).

CHAIR IN AND HEADSHIP OF THE DEPARTMENT OF ELECTRICAL AND CONTROL ENGINEERING; CHAIR IN AND HEADSHIP OF THE DEPARTMENT OF MECHANICAL ENGINEERING; and CHAIR IN THE DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY—The Secretary, Battersea College of Technology, Battersea Park Road, London, S.W.11 (February 1).

LECTURER IN PSYCHOLOGY IN THE DEPARTMENT OF EDUCATION—The Registrar, The University, Hull (February 3).

LECTURER (graduate in medicine with experience or special interest in pharmacology) IN PHYSIOLOGY (Pharmacology) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (February 5).

CYTOTOLOGIST (with a first- or good second-class honours degree in biology or a medical qualification and preferably experience in human or mammalian cytogenetics)—The Director, Population Genetics Research Unit, M.R.C., Old Road, Headington, Oxford (February 15).

HAZEL CLIFFORD POSTGRADUATE STUDENT for research in medicine or on a scientific topic concerned with the advancement of medical knowledge—The Secretary to the Scholarships Committee, University of London, Senate House, London, W.C.1 (February 15).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with the degree of Ph.D. or with equivalent research experience) IN BIOCHEMISTRY, CHEMISTRY, ENGINEERING, PHARMACOLOGY, PHYSICS OR CHEMICAL ENGINEERING—The Secretary, The University, Edinburgh (February 15).

NEMATOLOGIST (with a degree in the biological sciences with experience in the identification of plant parasitic nematodes and an interest in taxonomy) at the Commonwealth Bureau of Helminthology, St. Albans, Herts—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (February 15).

ANIMAL HOUSE TECHNICIAN (man or woman with suitable experience and qualifications) to take charge of the Animal House—The Professor of Zoology, The University, Hull.

BIOCHEMIST (with previous experience) for research in hormonal metabolism—Dr. B. H. Billing, Medical Unit, Royal Free Hospital, London, W.C.1.  
CHAIR OF PHYSIOLOGY—The Registrar and Secretary, The University, Bristol.

ENTOMOLOGIST (Tsetse) (with an honours degree in natural science, entomology or zoology) in Zambia (Northern Rhodesia), to plan and assess tsetse control measures involving surveys of fly incidence and its relation to local ecological factors, to determine the most suitable methods of control, and to supervise tsetse control staff—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 217/132/01.

LECTURERS AND ASSISTANT LECTURERS IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Manchester, 13.

MATHEMATICIANS (specialized in any branch of mathematics and preferably with the degree of Ph.D.)—Head of the Department of Mathematics, Dalhousie University, Halifax, Nova Scotia, Canada.

PHYSICAL CHEMIST OR CHEMICAL ENGINEER (graduate under 30) for research in wool dyeing processes—Wool Industries Research Association, Torridon, Headingley Lane, Leeds, 6.

POST-DOCTORAL FELLOWS IN THE DEPARTMENT OF CHEMISTRY, to work in the field of gas kinetics using flash photolysis and shock wave techniques—Prof. G. Burns, Department of Chemistry, University of Toronto, Toronto 5, Ontario, Canada.

PROFESSOR (preferably with a Ph.D. and some industrial experience) OF MECHANICAL ENGINEERING to teach engineering design and to conduct research in stress analysis or related fields—Head of the Department of Mechanical Engineering, University of Windsor, Windsor, Ontario, Canada.

RESEARCH ASSISTANT (with a degree or diploma in chemical engineering) IN THE CHEMICAL ENGINEERING SECTION OF THE DEPARTMENT OF CHEMISTRY AND CHEMICAL TECHNOLOGY—The Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

RESEARCH ASSISTANT (graduate with training in bacteriology) IN THE DEPARTMENTS OF MEDICINE AND MICROBIOLOGY—Dr. N. Crowley, Microbiology Department, Royal Free Hospital, Gray's Inn Road, London, W.C.1.

RESEARCH BIOCHEMIST (young, well qualified biochemist with a Ph.D. degree and an interest in protein chemistry and enzymology)—Dr. P. J. Mulrow, Yale University Department of Medicine, 333 Cedar Street, New Haven, Conn., U.S.A.

RESEARCH LABORATORY TECHNICIAN (preferably with experience in electronics or optics) to assist in a laser project—The Secretary, Royal Holloway College, Englefield Green, Surrey.

SENIOR GRADE BIOCHEMIST to work under the Chemical Pathologist in a well equipped laboratory—The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

SENIOR TECHNICIAN to assist with the development and construction of research equipment—The Secretary, Royal Holloway College, Englefield Green, Surrey.

TECHNICIAN OR JUNIOR TECHNICIAN (preferably with some experience in histology) IN THE DEPARTMENT OF ANIMAL HUSBANDRY at the Royal Veterinary College's field station in Hertfordshire—The Assistant Secretary, Royal Veterinary College, Boltons Park, Hawkshead Lane, Potters Bar, Middlesex.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Forestry Commission. Booklet No. 11: Extraction of Conifer Thinnings. By R. E. Crowther. Pp. 74 + 15 plates. 5s. net. Booklet No. 12: Double Drum Winch Technique. By R. E. Crowther and S. Forrester. Pp. 38 + 8 plates. 3s. net. Leaflet No. 12: Income Tax and Estate Duty on Woodlands. Pp. 6. 1s. net. Leaflet No. 49: Resin-Top Disease of Scots Pine (*Peridermium pini*). By Dr. R. G. Pawsey. Pp. 8. 1s. 6d. net. Leaflet No. 50: Grey Mould in Forest Nurseries (*Botrytis cinerea*). By Dr. R. G. Pawsey. Pp. 7. 1s. 6d. net. Grants for Woodland Owners: Planting and Management Grants—Technical Advice. Pp. 13. Short Guide to the Queen Elizabeth Forest Park. Pp. 14 + 4 photographs and map. 1s. net. (London: H.M. Stationery Office, 1964.) [211]

General Register Office. The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, Quarter ended 30th June 1964. (No. 402, 2nd Quarter 1964.) Pp. 24. (London: H.M. Stationery Office, 1964.) [211]

Department of Scientific and Industrial Research: National Chemical Laboratory. Safety Measures in Chemical Laboratories. Third edition. Pp. iv + 35. (London: H.M. Stationery Office, 1964.) 3s. net. [211]

Royal Institute of Chemistry. Monographs for Teachers, No. 8: Principles of Atomic Orbitals. By Dr. N. N. Greenwood. Pp. 46. (London: Royal Institute of Chemistry, 1964.) 6s. [211]

North of Scotland College of Agriculture—School of Agriculture, Aberdeen. Report on Investigations and Research 1963 (With List of Current Projects). Pp. 47 + vii. Report on Field Trials and Observations, Summer 1963 and Winters 1962-63 and 1963-64. Pp. iii + 59. Calendar, 1964-65. Pp. 64. (Aberdeen: North of Scotland College of Agriculture, 1964.) [211]

The Ordnance Survey Annual Report 1963-64. Pp. 11 + 12 appendices + 9 plates. (London: H.M. Stationery Office, 1964.) 6s. net. [211]

University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1963-64. (Supplement to *Oxford University Gazette*, No. 3209.) Pp. 51-77. (Oxford: The University, 1964.) 2s. [211]

Memoirs of the Geological Survey of Great Britain. Palaeontology, Vol. 4, Part 3: Fossil Plants of the Carboniferous Rocks of Great Britain (Second Section). By Dr. Robert Crookall. Pp. vii-xii + 217-354 + plates 59-81. (London: H.M. Stationery Office, 1964.) 140s. net. [211]

The Institution of Chemical Engineers. Scheme for a Part-Time Course in Chemical Engineering. Pp. 20. (London: The Institution of Chemical Engineers, 1964.) [311]

### Other Countries

Smithsonian Institution, Washington. Proceedings of the United States National Museum. No. 3492, Vol. 115: The Jambell Culture of South Coastal Ecuador. By Emilio Estrada, Betty J. Meggers and Clifford Evans. Pp. 483-558 + 12 plates. No. 3493, Vol. 115: A Revision of the Carcharhinid Shark Genera *Scoriodon*, *Loxodon*, and *Rhizoprionodon*. By Victor G. Springer. Pp. 559-632 + 2 plates. No. 3497, Vol. 116: Neotropical Microlepidoptera, IV—a New Genus of Stenomitridae with Descriptions of Four New Species (Lepidoptera: Gelechioidea). By W. Donald Duckworth. Pp. 97-114. No. 3500, Vol. 116: Three New Species of Frogfishes from the Indian and Pacific Oceans, with Notes on other Species (Family Antennariidae). By

Leonard P. Schultz. Pp. 171-182 + 3 plates. (Washington, D.C.: Government Printing Office, 1964.) [311]

Annals of the New York Academy of Sciences. Vol. 117, Article 1: Photo-Neuro-Endocrine Effects in Circadian Systems, with particular reference to the Eye. By E. N. Hague and 64 other authors. Pp. 1-645. (New York: New York Academy of Sciences, 1964.) 9 dollars. [311]

Annual Report of the Trustees of the Museum of Applied Arts and Sciences, Sydney, for the year ended 31st December, 1963. Pp. 28. (Sydney: Museum of Applied Arts and Sciences, 1964.) [311]

Consell Permanent International pour l'Exploration de la Mer, Charlottenlund Slot, Danemark. Rapports et Procès-Verbaux des Réunions, Vol. 155: Contributions to Symposium 1963 on the Measurement of Abundance of Fish Stocks. Edited by J. A. Gulland. Pp. 223. (Copenhagen: Andr. Fred. Hest et Fils, 1964.) 60 kr. [311]

Koninklijk Nederlands Meteorologisch Instituut. Mededelingen en Verhandelingen. Nr. 80: The Influence of Topography and Orography on the Precipitation Patterns in the Netherlands. By H. Timmerman. Pp. 49. (De Bilt: Koninklijk Nederlands Meteorologisch Instituut, 1963.) 6.50 f. [311]

Smithsonian Institution. United States National Museum. Bulletin No. 231: Early American Scientific Instruments and Their Makers. By Silvio A. Bedini. Pp. xii + 184. (Washington, D.C.: Government Printing Office, 1964.) 1 dollar. [311]

The Danish Bag Record I: Studies in Game Geography Based on the Danish Bag Record for the years 1956-57 and 1957-58. By H. Strandgaard. (*Danish Review of Game Biology*, Vol. 4, Part 2.) Pp. 116. (Copenhagen: J. H. Schultz, Ltd., 1964.) [311]

United Nations Committee on the Peaceful Uses of Outer Space. Review of National and Co-operative International Space Activities. Pp. 114. (New York: United Nations, 1964.) [311]

New Zealand Forest Service: Forest Research Institute, Forestry Research Notes, No. 37: The Effect of Physiological Age of Scion on Growth of Grafts in *Pinus radiata*. By G. B. Sweet. Pp. 8. (Wellington: Government Printer, 1964.) [311]

United States Naval Observatory. Circular No. 101: Solar Eclipses, 1971-1975. By J. S. Duncombe. Pp. 11. Circular No. 102: Total Solar Eclipse of 30 May 1965. By J. S. Duncombe and B. L. Morrison. Pp. 21. (Washington, D.C.: U.S. Naval Observatory, 1964.) [311]

Comptes Rendus des Travaux du Laboratoire Carlsberg. Vol. 34, No. 8: Studies on Hexokinase from Yeast—a Reappraisal. By Paul Ottolenghi. Pp. 242-274. 6 kr. Vol. 34, Nos. 9-10: Studies on Rennin. VII: On the Amino Acid Composition of Prorennin, Rennin and of Peptides Liberated During the Activation of Prorennin. By B. Folmann. VIII: On the Molecular Weight of Prorennin and Rennin. By R. Djurtoft, B. Folmann and A. Johansen. Pp. 275-298. 4 kr. Vol. 34, No. 11: A Discussion of the pH Dependence of the Hydrogen-Deuterium Exchange of Proteins. By Aase Hvilt. Pp. 290-317. 3 kr. (Copenhagen: Danish Science Press, Ltd., 1964.) [311]

Organization for Economic Co-operation and Development: European Nuclear Energy Agency. Sixth Report on the Activities of the Agency. Pp. 192. (Paris: Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 1964.) [311]

Republic of South Africa. Department of Commerce and Industries: Division of Sea Fisheries. Investigational Report No. 49: Observations on the Ecology and Distribution of Copepoda in the Marine Plankton of South Africa. By A. De Decker. (Reprint from *Commerce and Industry*.) Pp. 33. (Sea Point, Cape Town: Director of Sea Fisheries, 1964.) [311]

Smithsonian Miscellaneous Collections. Vol. 148, No. 1: A New Theory Identifying the Locale of Columbus's Light, Landfall, and Landing. By Ruth G. Durlacher Wolper. (Publication No. 4534.) Pp. vii + 41. (Washington, D.C.: Smithsonian Institution, 1964.) [311]

National Academy of Sciences—National Research Council. Washington. Publication No. 1195: An Evaluation of Public Health Hazards from Microbiological Contamination of Foods. (Food Protection Committee of the Food and Nutrition Board.) Pp. vi + 64. (Washington, D.C.: National Academy of Sciences—National Research Council, 1964.) 2 dollars. [311]

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 34, Fascicule 5: Astronomical Observatories in the 17th and 18th Centuries. By M. C. Donnelly. Pp. 37 + 26 figures. 180 francs. Tome 34, Fascicule 6: La Géométrie Différentielle des Surfaces Considérées dans l'Espace Régulé. Par Lucien Godeaux. Pp. 83. 80 francs. Tome 34, Fascicule 7: Le Granite et les Déformations Mineures des Roches dans les Vosges Hercyniennes. Par Paul Fourmarier et Michel Ruhland. Pp. 41. 80 francs. (Bruxelles: Académie Royale de Belgique, 1964.) [311]

The Connecticut Agricultural Experiment Station. Bulletin 664: Stomata and Water Relations in Plants: Papers and Discussions given July 1 to 12, 1963, as part of the Advanced Science Seminar on the Physiology and Biochemistry of Leaf Stomata. Edited by Israel Zelitch. Pp. ix + 116. (New Haven, Conn.: The Connecticut Agricultural Experiment Station, 1963.) [311]

National Hydatids Council. Fourth Annual Report and Statement of Accounts, year ended 31st March, 1964. Pp. 32. Eradication Campaign Policy. Pp. 4. (Wellington, N.Z.: National Hydatids Council, 1964.) [311]

New Zealand. National Radiation Laboratory, Department of Health. Fallout in New Zealand—Quarterly Report, April-June 1964. Pp. 17. (Christchurch, N.Z.: National Radiation Laboratory, Department of Health, 1964.) [311]

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 46 (August, 1964): Increase in Survivorship Since 1840. Hospitalization and Surgical Care of Children: Congenital Conditions. Diabetes Today—a World Survey. Pp. 12. (New York: Metropolitan Life Insurance Company, 1964.) [311]

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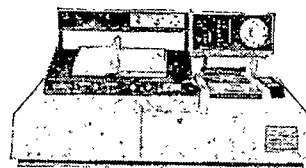
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## LAND USE AND RECLAMATION

FOLLOWING on Lord Molson's reference in the House of Lords on November 10 to the memorandum on the preservation of amenities (both within and without the national parks) which was being sent to the Government by the Council for the Preservation of Rural England, the question of electricity pylons in areas of natural beauty was raised in the House of Lords on November 17 by Lord Egremont, who was strongly supported by the Marquess of Salisbury, the Earl of Bessborough, the Earl of Dudley, Lord Chorley, Viscount Mersey and Lord Somers. Lord Egremont was particularly concerned about the position of a small area of outstanding beauty in West Sussex and challenged the commonly accepted figures for the cost of underground cables. Lord Salisbury also questioned these figures as the justification for pylons, while the Earl of Bessborough strongly urged the need for further research, and the Earl of Dudley directed attention to the lack of progress in insulating materials used for this purpose. Lord Chorley quoted a decision of the previous Minister against overhead lines from Derbyshire across South Yorkshire, and Viscount Mersey directed attention to costs of pylons which were often disregarded.

Lord Stonham, who replied for the Government, although promising that the Minister of Power would study the whole debate and note what had been said, gave little positive reassurance. He did not consider that decisions were taken without regard to amenity, and gave a figure of £54,000 per mile for heavy duty 400 kV overhead lines compared with £1,182,000 a mile underground. Moreover, he pointed out that even with underground lines considerable disturbance would be caused by the trenches and drums of cables, and heat-exchanger units would be required every few miles. A good deal of money had already been spent on research, but Lord Stonham did not meet the point that the Ministry of Power—and other departments—appears to be allowed to judge in its own cause.

This position is not new although the question of pylons or underground cables has been the subject of steady protest over the past twenty years. What is new is that while the Minister of Power, like those Ministers for Defence, Transport and Agriculture, is a member of the Cabinet, the Minister for Land and Natural Resources is not, and it is to him and not to the Minister of Housing and Local Government that the National Parks Commission is now responsible. Thus the Commission is now responsible to a Minister who is not of Cabinet rank, whereas the major threats to amenity, so far as Government is concerned, arise from departments the Ministers of which enjoy that status.

That position is disturbing and possibly the more so in that it is the reverse of what might have been expected. In view of the importance which has been attached by the Government itself to the use of land, one would have expected to find that any Minister concerned with that sphere would be in the Cabinet with status equal to—if

not greater than—that of Ministers from whom emanate competing and conflicting claims on that resource. Neither in the Machinery of Government Bill, nor in the debate when the Bill received its second reading in the House of Commons on November 19, was any attention directed to the scope or functions of the Ministry for Natural Resources.

The dangers of this situation are clear enough even in such a pamphlet as *The Right to Roam*\*, recently issued by the Ramblers' Association. Moreover, a study of industrial dereliction and how it may be redeemed, recently published by the Civic Trust†, makes nonsense of the whole structure. It is indefensible to anyone who is concerned with the wise use of land or other natural resources, let alone to those who value in the least degree Britain's heritage of natural beauty or her flora or fauna. The Ramblers' Association merely presents a reasonable survey of some of the pressing problems arising in regard to amenity, particularly in the national parks, including water supply, afforestation, Armed Service training areas, and transport. The Civic Trust, in publishing this survey, displays frankly a responsibility that lies fairly and squarely on the Government and which has implications which, if disregarded, would make part of the Government's programme hypocritical.

By and large, the proposals of the Ramblers' Association cover ground which is traversed by recommendations from the National Parks Commission itself and the Council for the Preservation of Rural England. The Association urges direct finance of the Commission by the Exchequer, including grants of 75 per cent for administrative costs, and removal of any restrictions which prevent the Park Planning Authorities undertaking work which could be done by other authorities under other Acts. All alternatives to reservoirs should be explored before any damage is done to the parks, and the Park Planning Boards should have control over the planting of trees, as well as an effective voice in road policy in their areas. There are other sensible suggestions about road policy appropriate in a national park, about 'areas of outstanding beauty', and a plea is made for early legislation embodying the basic recommendations of the Royal Commission on Common Land.

One of the most significant comments in this pamphlet is the observation that "perhaps the greatest failure of town and country planning is seen in the widespread changes in the character of rural landscapes brought about by isolated developments which contradict the existing and dominant colours, textures, topographical form and scale of the components of the landscape". The Association does not conceal its belief that the discontinuance of the title "Ministry of Town and Country Planning" for the Ministry set up in 1943 represents something more than a greater emphasis on housing.

\* The Ramblers' Association. *The Right to Roam: The Rambler and the Countryside*. Pp. 32. (London: The Ramblers' Association, 1964.) 2s.  
† Civic Trust. *Derelict Land: a Study of Industrial Dereliction and How it may be Redeemed*. Pp. 72. (London: Civic Trust, 1964.) 7s. 6d.

The Association is studiously moderate when it urges that the Ministry of Housing and Local Government's primary duty should be to ensure that in all developments full attention is directed to the maintenance of the existing excellence of Britain's landscape, and that industrial processes should be selected, or developed if not available, which will not disturb the beauty of the countryside. Even then there were grounds for suggesting that what was required was not the restoration of its original title to the present Ministry but the appointment of a real Minister of Town and Country Planning who would be overlord of those of agriculture, transport, housing and local government and perhaps power. Certainly, the protest might well have been much more vigorous had it been foreseen that on the contrary the responsibilities for the National Parks Commission, the Nature Conservancy, the Forestry Commission and Commons Land, as well as for water conservation, would be transferred to a Minister outside the Cabinet, while the Ministers responsible for the conflicting and major unco-ordinated demands on land would remain independent and in the Cabinet.

The gist of the problem is expressed in the first two chapters of the Civic Trust's study, and is admirably brought out in the later chapters, which review such special problems of after-treatment as the establishment of grass and afforestation, before dealing with the main industries concerned—coal in Lancashire; ironstone in Northamptonshire; sand and gravel in Hertfordshire; brick clay in Bedfordshire; limestone in Derbyshire; china clay and tin in Cornwall. Other problems are presented by the disposal of town waste and pulverized fuel ash. These sections are contributed by county planning officers with personal experience of the work. The bibliography, which attests the extent to which the problem has already received attention, bears silent witness to the most conspicuous omission—effective control and co-ordination at the top.

The study is not concerned simply with the 150,000 acres and more which lie derelict in England and Wales alone. That is the legacy of past neglect. Some 36,000 acres of this lies in urban areas where undeveloped land is scarce and expensive; about 24,000 acres of it consists of spoil heaps, abandoned buildings and miscellaneous dereliction and should be reclaimed for urban development as fast as the demand allows, instead of prematurely urbanizing what is left of the open country in the neighbourhood. In the meantime, much of this waste land could be made inoffensive to the eye, and attractive to developers, by restoration so far as possible, to agricultural use. Another 12,000 acres of tipping holes is likely to be needed in due course for town wastes, and it is conceivable that thereafter it could be economically developed as sites for housing or playing fields. Such areas should be kept in reserve for refuse disposal, which might otherwise spread over virgin farm land. The remaining 114,000 acres is in less populous areas where there is no foreseeable prospect of large-scale development, and it is these that could be redeemed by regrading and grassing, or afforestation, so as to merge into the surrounding countryside.

Beyond this, however, there is an annual net addition to Britain's derelict acreage. Of some 12,000 acres a year now being taken for mineral working and tipping-spoil and waste, about 8,500 are later restored to farming use by the operators in compliance with conditions attached to their planning consent. Thus there is a further 3,500 acres a year which requires to be harmoniously

blended back into the landscape while the process of extraction proceeds. The rate of present-day dereliction could be reduced somewhat if more were known about the distribution and industrial usefulness of workable deposits, and if more care were taken to co-ordinate the activities of the various agencies responsible for digging holes and the disposal of wastes. However, there is only limited scope for further abatement of the spread of dereliction by the exercise of planning control over individual mineral undertakings.

Here the study stresses the great and growing contribution which minerals make to the national wealth. They must be worked where they are found, and worked efficiently and economically. The importance of the work of the Geological Survey and of ensuring that it is adequately supported could not be better underlined; while this is now to be the responsibility of the National Environment Research Council under the Ministry of Education and Science, and not, as seems more logical, under the Ministry of Land and Natural Resources, some doubt may well be entertained as to whether the consultations which the Secretary of State for Education and Science promised on November 26 are likely to be anywhere near sufficient.

Land reclamation schemes vary widely in cost, but present experience suggests that if all the 36,000 acres in urban areas were brought back into some sort of use as soon as possible instead of waiting for the time when it was economically ripe for urban development, the gross outlay would average about £700 per acre. Assuming that the market value of the reclaimed land continued to average about £220 an acre, the net cost would be about £480 an acre. On these figures a total of £11.5 million is reached, to which temporary treatment of tipping holes reserved for refuse disposal might add £250,000. Treatment of the remaining 114,000 acres by afforestation or permanent grassing is estimated to cost on average about £55 per acre or £6.25 million in all. A comprehensive 20-year programme for renewing by one means or another all land left derelict by past industrial activities would thus involve an annual outlay of nearly £900,000, or about £1 million if land left derelict by present-day mineral working were included. This figure, moreover, would diminish if the cost of alternative sites on undeveloped land continued to rise, as is probable, and takes no account of the value to the national economy of the farmland saved from premature or unnecessary development.

This figure cannot be regarded as an excessive price to pay for the social and aesthetic benefits that would accrue from the renewal of all derelict land. It should rather be regarded as an essential and inescapable part of the price to be paid for the unavoidable and continuing creation of new eyesores, and should be one of the first responsibilities of any genuine Ministry of Land and Natural Resources. Furthermore, as the Civic Trust reminds us, in the past fifteen years potentialities of landscaping have been transformed by technical revolutions in the mechanisms of shifting waste materials, trees and soils, and especially in the science of soil-making. It is not easy to point to a field where technological advances have more fundamentally transformed the possibilities for improving our environment—or perhaps where their significance has so far been so little appreciated.

This neglect is challenged by this study, which insists that ugliness has an economic as well as a social cost. It is clear enough that to-day it is sheer hypocrisy to



talk of land policy and utilization or of the conservation of natural resources and neglect to deal with this problem of derelict land. Not merely is the cost of reclamation comparatively light; it can yield appreciable economic as well as social returns, while, to say the least, it is imprudent to place no value on amenity.

What is so far completely lacking is any evidence that the Government appreciates what is involved. So far, the present Government has not given any indication that it has any more real concern than its immediate predecessors had for the preservation of amenity or the conservation of natural resources. In a written statement on November 26, the Prime Minister said that the Minister of Land and Natural Planning will be generally responsible for the availability of natural resources to meet the needs of the community and it will be for him to consider the use made of natural resources, the development of new resources, and the better use of those natural resources which are being inadequately used at present. Land is the most important and urgent problem, and the new Minister will be responsible for the establishment of the Land Commission as soon as possible and for future policies relating to the availability of land. Overall responsibility for the national and regional development plans rests with the Secretary of State for Economic Affairs, although the Minister of Land and Natural Resources will participate in their formulation. Transferred to him also will be the functions of the Minister of Housing and Local Government in relation to the National Parks Commission, those of the Minister of Agriculture for the Forestry Commission and for Commons Land, and he will also have responsibilities for the conservation and supply of water (however, the precise allocation of statutory responsibilities for water is to be the subject of a detailed statement later).

It will be noted first that the Nature Conservancy, along with the Geological Survey and Museums and the Hydrology Research Unit, now falls under the Natural Environment Research Council, which is the responsibility of the Ministry of Education and Science. This would appear to place research activities in this sphere on the same basis as research activities in science, medicine and agriculture, though not technology, and at least it remains the responsibility of a Cabinet Minister. Nevertheless, there are strong grounds for holding that a Ministry of Land and Natural Resources should be adequately equipped to pursue its own investigations if not research—reasons at least as compelling as those which have led to the Road Research Laboratory, in the face of the view of its own Board and of some other informed opinion, being transferred to the Ministry of Transport.

The most disturbing aspect, however, is the invidious position in which the Minister of Land and Natural Resources is now placed in relation to those of Transport, of Power, of Housing and Local Government, of Defence, of Agriculture, and of Trade. With no seat in the Cabinet he does not appear to possess the resources for independent enquiry and there is no indication that he possesses the powers to compel the Cabinet Ministers who have conflicting claims on the use of land and other natural resources to consider alternatives before irreparable damage is done. There is not a reference in the Prime Minister's statement to the question of derelict land, for example, nor to this vital co-ordinating and, in the ultimate resort, overruling function which any Minister of Land and Natural Resources must possess if he is to be effective.

## BIOCHEMISTRY OF SPERMATOZOA AND SEMINAL PLASMA

The Biochemistry of Semen and of the Male Reproductive Tract

By Dr. Thaddeus Mann. Pp. xxiii+493+19 plates. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1964.) 105s. net.

MANN'S *Biochemistry of Semen*, published in 1954, was an outstanding synthesis of the information which then existed about the biochemistry of spermatozoa and of their medium, seminal plasma. It contained about 62,000 words and 990 references. The new edition, published in the autumn of 1964, is nearly three times as long and contains twice as many references. It, too, is an outstanding contribution to the subject; but it differs from its predecessor in that it also contains an expanded account of the role of the male sex hormones—testosterone, androstenedione and other steroids—in the production of semen.

The book starts with a general account of semen, that is, spermatozoa and seminal plasma, including one very useful table of the published proceedings of recent congresses devoted to the physiology and biochemistry of spermatozoa. Chapter 2 is concerned with the morphology of spermatozoa (including a superb electron micrograph, by Prof. D. W. Fawcett, of a bat spermatozoon), capacitation, the acrosomal reaction and a somewhat brief account of male- and female-producing spermatozoa. The third chapter describes the male accessory organs and their secretion, seminal plasma. It contains useful photographs and diagrams of the male reproductive tracts of some animals; but further examples could have been included with some advantage.

In Chapter 4 the chemical and physical properties of semen from different species are described. This is an important reference chapter. The fifth chapter is concerned with the enzyme adenosinetriphosphatase, the cytochromes, hyaluronidase and the glycosidases within spermatozoa; the sixth and seventh with sperm proteins and the composition of seminal plasma including the enzymes vesiculase, coagulase, the phosphatases, 5-nucleotidase and amino-peptidase. There is also a valuable discussion of sialic acid in seminal plasma.

Chapters 8, 9 and 10 contain accounts of the important substances spermine, ergothioneine, creatine, serotonin, plasmalogen, prostaglandin, fructose, sorbitol, inositol and citric acid in seminal plasma. The metabolism of spermatozoa, including fructolysis and lipid metabolism, is described in Chapter 11, which also contains an account of sperm energetics.

The influence of hormones, nutrition and other environmental factors on the male reproductive tract and semen *in vivo* is the subject of Chapter 12, while Chapter 13 contains a description of the effects on spermatozoa of variations in the external environment. Chapter 14 deals with sperm antigens and antibodies, sperm-egg interacting substances, chemotaxis and thigmotaxis. Chapter 15 has an applied flavour and contains an account of spermicidal substances.

It would be easy for a reviewer, according to his own preferences and interests, to say that Mann pays too much attention to certain subjects and not enough to others, for example the male sex hormones. But this is a matter of taste and inclination; and it is certain that no readable book in 1964 can satisfy all the specialists likely to read it. In general, Mann has struck an excellent balance and it is hard to imagine who, among those interested in the many biological, chemical, clinical and veterinary aspects of reproduction, will not want to have *The Biochemistry of Semen and of the Male Reproductive Tract* on their bookshelves.

ROTHSCHILD

## CELLULAR BIOLOGY FOR THE LAYMAN

### The Life of the Cell

Its Nature, Origin and Development. By Prof. J. A. V. Butler. Pp. 167+16 plates. (London: George Allen and Unwin, Ltd., 1964.) 30s. net.

AT the present time there is a strong movement towards unity in biology, stimulated by the dramatic advances of the past decade, which have led to the establishment of new unifying concepts, the most fundamental being the evidence for the universality of the genetic code. Several books have appeared recently in which attempts have been made to compile this recent information systematically. To the layman (for whom this volume is mainly intended) the cell probably provides the most tangible starting point and Prof. Butler has chosen this approach. The present book might be considered a second edition of his earlier one entitled *Inside the Living Cell*, except that so much has happened in the past five years that it has had to be very extensively re-written.

The range covered is enormous, extending from elementary chemistry to the working of the brain. The author first considers the structure of biological macromolecules (proteins and nucleic acids) and manages to do so without introducing many chemical formulae. For those who are interested in these he provides an appendix. He goes on to discuss in some detail the replication of DNA and the synthesis of proteins. There is then a logical development towards a discussion of the genetic code, mutagenesis and the genetics of micro-organisms. At this point the difficulties of developing a logical exposition of this subject become apparent. A chapter on photosynthesis becomes interposed between a chapter on the genetics of micro-organisms and one on the origin of life; this is followed by one on differentiation and another on antibiotics. Later chapters deal mainly with questions concerning multicellular organisms (especially mammals) rather than problems of the cell. They include a consideration of immunity, a discussion of cancer and chapters on the brain and sense organs, nerves and muscle. There is a final chapter dealing with the possibility of life being distributed widely throughout the universe. The book therefore covers a much wider range than its title suggests. Facts are generally reported with impeccable accuracy; the only statement to which I could take serious exception is the definition of leukaemia on p. 63.

Non-essentials have been trimmed away and the essentials of even rather abstruse concepts described in a manner which should certainly be comprehensible to anybody who has studied any aspect of science and even, I believe, to the intelligent and intellectually curious student of the humanities. Hence, besides being a good general introduction to present-day problems in biology, this is just the kind of book which might help to bridge the gulf between the two cultures. One hopes that some journals commonly read by students of the "other culture" will also undertake to review it.

J. PAUL

## LABORATORY PROCEDURES IN BOTANY

### Laboratory Techniques in Botany

By M. J. Purvis, D. C. Collier and D. Walls. Pp. viii+371. (London: Butterworth and Co. (Publishers), Ltd., 1964.) 57s. 6d.

THE great importance of the work of technicians in running a modern botanical laboratory is perhaps not always fully appreciated. It is so often taken for granted that technicians are always there and that they somehow or another seem to know how to deal with every

practical problem that arises in the research and teaching laboratory as well as on the demonstration bench. Nevertheless, in spite of the great knowledge that many botanical technicians possess, it is unusual to find them writing books that give practical directions for laboratory procedures. We are therefore greatly indebted to the three authors of the book under review, all of whom are laboratory technicians, for giving us in writing the benefit of their first-hand experiences of a very wide range of laboratory methods. The topics that are covered range from cleaning glassware to histological and cytological procedures including those in use for electron microscopy. Tests for the chemical nature of cell walls and cell contents are also given. But the book is by no means restricted to microscopical techniques for there are chapters dealing respectively with the preparation of material for museums and herbaria; the care of plants in aquaria; the raising of organisms and the growth of tissues in pure culture. There are also directions for setting up demonstration experiments dealing with growth, photosynthesis, and respiration, while topics such as manometry, chromatography, and distillation techniques are also covered and directions given for the analysis of soils, water and plant material. The book also includes a selection of questions from the Advanced Level Examination for the General Certificate of Education, and others from the City and Guilds examinations for laboratory technicians. The volume ends with an index.

Naturally it has not been possible to treat all these as well as other topics exhaustively in a book of 371 pages, but references are given to literature for further study. It is likewise almost inevitable, with a book of this kind, that specialists will feel that one or more of their own pet methods have been omitted or are inadequately treated. Others again may feel that they would have preferred slightly different methods. To me, for example, it seems that in the excellent treatment of histological procedures insufficient attention has been directed to the many purposes for which the sledge microtome can be used. This very versatile instrument can in fact be used not only to cut sections of timber and other hard material as the authors rightly suggest, but it is also equally satisfactory for herbaceous specimens, including leaves, held in the clamp between pieces of pith or cork. All this can be done without having to resort to lengthy embedding techniques. Another very useful addition would be a few paragraphs on the histological examination of dried material such as herbarium and archaeological specimens. Then again, in my own experience, very few technicians seem to have discovered that compressed material that has been preserved in wet anaerobic conditions can be examined more successfully by cutting sections and then 'reviving' them rather than by 'reviving' the material before the sections are cut.

In spite of inevitable minor omissions such as the examples just given, the book is a most valuable contribution to botanical literature and it will no doubt be widely welcomed, especially on account of its broad content.

C. R. METCALFE

## INSECT PHYSIOLOGY

### The Physiology of Insecta

Vol. 1. Edited by Morris Rockstein. Pp. xiv+640. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 157s.

THE publication of Wigglesworth's *Principles of Insect Physiology* twenty-five years ago has resulted in a flood of valuable works in this field of investigation. Volume 1 of *The Physiology of Insecta* is the first of three large books in which it is planned to cover the field again. The editor, Morris Rockstein of the University of Miami, has selected a team of authors more than half of whom

are from universities in the United States. There is no overlap in authorship, and so far almost none in content, with the first volume of *Advances in Insect Physiology* published by Academic Press, the authors of which are mainly from England and treat narrower fields, chosen mainly for their timeliness and general interest.

Rockstein's plan is to consider insect physiology in a way not hitherto attempted, in three main sections (which do not correspond with the three volumes). Section A deals with the development of the insect from that of the egg in the ovary through moulting and metamorphosis up to senescence; Section B with perception and reactions to the environment; Section C with the homeostatic processes which include nutrition, excretion, water balance and immunology. Volume 1 completes the consideration of Section A with an introduction and five chapters on reproduction, metamorphosis and ageing, with emphasis on their endocrine control. Half Section B is also included and this has chapters covering the effects of temperature and humidity, the production and detection of light and sound, and the structure and function of the chemoreceptors.

The chapters contain many new diagrams and tables and include many valuable graphs and photographs, the style of writing is generally clear, and the subject is treated in a very stimulating manner, like an uninhibited account of an incomplete exploration in which the goals are still only imperfectly comprehended. The book has been produced with remarkable speed so that some of the references are to papers of 1963. However, the bibliographies to some of the chapters suggest that the authors have limited access to periodicals in languages other than their own, and it is probably a symptom of hurried production that the proof reading has been careless. The index to scientific names has a number of entries out of place and more than 3 per cent of the entries are wrongly spelt.

Many readers will be surprised to find that long-accepted stories, like Exner's explanation of insect vision, are based on erroneous observations and are no longer acceptable. Perhaps the main impression this book leaves is of a vast amount of information digested and critically summarized. This information is so much more substantial than that available, say, to Roeder and his team eleven years ago that many new generalizations are now possible. If the two volumes to follow reach the same high standard, this book should be extremely valuable as a source of fact and speculation to research workers and teachers in the universities of the world. G. C. VARLEY

## MAMMALIAN COCHLEA

### The Organ of Corti

*Its Histophysiology and Histochemistry.* By Ya. A. Vinnikov and L. K. Titova. Authorized translation from the Russian by Basil Haigh. Pp. xix + 253. (New York: Consultants Bureau, 1964.) 13.50 dollars.

THE authors of *The Organ of Corti* have clearly taken as their objective a modern and comprehensive presentation of information relating to the anatomy and physiology of the mammalian cochlea. In many ways they have achieved success. Almost every aspect of the subject is surveyed in a competent and analytical manner, and the results are then used for the synthesis of a new cytochemical theory of hearing.

In Chapter 3 a particularly valuable description is given of the technical methods used for the dissection and preparation of the cochlea for its examination by modern methods, using phase-contrast and electron microscopy and also the various histochemical techniques. It should perhaps be mentioned that many of these methods of dissection are not new and might, in fact, be described

as the technical stock-in-trade of all, including Retzius, who have devoted themselves to the investigation of the microstructure of the cochlea. Nevertheless, the authors are right in emphasizing that no systematic description of their methods is available, and in providing it they undoubtedly do a great service. The chapter is particularly relevant in view of the great and growing importance of the new microscopical techniques for which the methods of preparation are essential.

As judged by their previous publications, the primary interest and expertise of Vinnikov and Titova lie in the difficult field of histochemistry, and it therefore seems natural that a large part of the book should be devoted to the description and evaluation of their own contributions. However, for a proper assessment of their impact on our concept of the cochlear mechanism, these must of necessity be considered in a balanced relationship to the multitudinous data which have now been put at our disposal through the allied investigations of anatomists, physicists and neurophysiologists.

The authors clearly appreciate the need for this kind of synthesis, and strive to achieve it at every stage. However, their success is not absolute, and Hallowell Davis, in the course of his valuable preface, directs attention to apparent anomalies in the physical conditions of certain experiments concerned with the histochemical changes in the cochlea following acoustic stimulation.

The book has a certain arresting vigour which it must owe in large part to the eminence, as investigators, of the authors themselves, and this is reflected by the way in which thought and argument seem to be coloured by their own observations. Bias of this kind is sometimes irritating; nevertheless, it has in this case undoubtedly lent interest and authority to the presentation of an important and complex subject, the value of which has been greatly enhanced by an exceptionally meritorious English translator.

All in all, the book can only be described as a quite remarkable achievement for which its authors and translator are to be warmly congratulated. It will be welcomed by and invaluable to all who are interested in the scientific investigation of the organ of hearing.

C. S. HALPKE

## "THAT OLD ENEMY THE GOUT"

### A Short History of the Gout and the Rheumatic Diseases

By W. S. C. Copeman. Pp. ix + 236 + 19 plates. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 6 dollars; 48s. net.

DR. COPEMAN has special qualifications for writing this book, which is partly an expansion of a lecture he gave to the University of California in 1962. As a physician he has had extensive experience of gout and the rheumatic diseases; in addition, he is well known for his studies and investigations in medical history. When experts investigate a branch of medicine which they have made pre-eminently their own, their writings for this very reason are the more authoritative and valuable.

The first seven chapters deal with the history of gout from the earliest times to the recent chemical discoveries in the present century, such as carinamide and probenecid, an improved and non-toxic form replacing atophan in 1951. Probenecid and later synthetic variants have the power of inducing in the body a negative urate balance, either by influencing the production of uric acid, or, as is more likely, by stimulating the kidneys to part with it more readily. This new knowledge challenges Sir Alfred Garrod's simple conception of uric acid as the main cause of the disease. As the author states (p. 117): "It is becoming increasingly evident, however, that gout is the

end product of a disordered body metabolism, which is often genetically acquired".

The remaining five chapters of the book deal briefly but succinctly with acute rheumatism and chorea, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and non-articular rheumatism or fibrositis. Here there is much information both clinical and historical within small compass. Most interesting is the account Dr. Copeman gives of notable sufferers from the arthritic diseases, and of the way in which their maladies have influenced the course of history.

As regards gout, the great Renaissance family of Medici exemplified the association between outstanding ability and the gouty diathesis. The Emperor Charles V's recurrent attacks of gout, fostered by his gluttonous and huge appetite, through consequent incapacity and delay, affected the course of State affairs and his conquests. This was also the case with his son, Philip II, who inherited the malady. Other victims were Martin Luther, John Calvin and William Cecil, Lord Burghley. Burghley's frequent attacks probably influenced his outlook and policy. His son, Robert Cecil, Earl of Salisbury, also suffered from gout. The unfortunate Earl of Strafford had attacks of both gout and stone. Additional gouty patients were Sydenham, Sir William Temple, Francis Bacon, William Harvey, John Milton, William Penn, Dr. Johnson, the elder and the younger Pitts, Edward Gibbon, Horace Walpole, Thomas Gray, Benjamin Franklin; and coming to modern times, we may add Joseph Chamberlain and his son, Neville Chamberlain. Rheumatoid arthritis claims such celebrated sufferers as Pope Pius II, Christopher Columbus, Mary Queen of Scots, President Madison of the United States, and Renoir, the French artist.

Dr. Copeman in this book has made an important contribution to medical history. The illustrations are excellent and accord well with the text.

## ADVANCES IN ORGANIC CHEMISTRY

### Advances in Organic Chemistry

Methods and Results, Vol. 3. Edited by Ralph A. Raphael, Edward C. Taylor and Hans Wynberg. Pp. vii+333. 105s.

Methods and Results, Vol. 4. Edited by Ralph A. Raphael, Edward C. Taylor and Hans Wynberg. Pp. vii+361. 110s.

(New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1963.)

**A**MONG the multiplicity of alerting media available to chemists to enable them to keep abreast of developments in their respective fields of interest, reviews are proving ever more popular by virtue of their time-saving nature. These critical digests of pertinent data and evaluations of present-day progress in selected fields provide valuable sources of up-to-date information and extensive bibliographies, and are now generally accepted as a basic unit in scientific communication. In addition to the review journals issued by the scientific societies, serial review volumes are now issued regularly by some of the publishing houses under advisory and editorial boards comprising people of eminent standing in their field. The volumes under review belong to one such series. Two volumes have appeared earlier and they have found wide acclaim. In these two additions to the series again there has been a judicious selection of topics and a happy choice of authors.

Four themes are discussed in Volume 3—mass spectrometry as a structural tool (R. I. Reed), phosphorylation (D. M. Brown), selectively removable amino protective

groups used in the synthesis of peptides (R. A. Boissonnas), and protective groups (J. F. W. McOmie). It is entirely fitting that the new tools of the organic chemist, which are responsible for so many advances in the science, should be featured. The availability of high resolution mass spectrometers has provided the organic chemist with a very powerful and sensitive structural and analytical tool. The many and varied uses of the mass spectrometer are reviewed and illustrated with some well-chosen examples. The cracking patterns observed on the fragmentation of molecules are correlated with known structural features in the molecule. Certain useful generalizations are made but some important exceptions are noted. The ambiguities resulting from the occurrence of rearrangement ions and the difficulties of deducing the nature of the neutral fragments are stressed; these call for caution in the interpretation of the spectra. Notwithstanding the complications, some striking progress has been made in the analysis of spectra for structural elucidation and this aspect is treated in detail with reference to some dozen representative molecular structures.

In the course of his synthetic experiments the organic chemist often has to resort to the use of protective groups. These are designed to protect certain reactive groups or radicals in the molecule while another part is made to undergo chemical reaction. The synthesis of some complex molecules requires the use of several different protective groups which must be capable of selective removal one after the other under mild conditions. A valuable survey of the methods for introducing and selectively removing groups which are needed to protect the amino group in peptide synthesis is presented. Due emphasis is placed on the stability and general resistance to racemization of these groups under the conditions usually employed for the formation of peptide bonds. There also appears, for the first time, a comprehensive review of the use of protective groups (other than those noted above) throughout the broad field of organic chemistry. This contribution will be much appreciated. As the author points out, there is no systematic way of scrutinizing the literature for protective groups, and although he modestly claims that inevitably some protective groups will have been missed the coverage is extremely wide and reflects a very catholic knowledge of organic chemistry. It is interesting to read that certain cockroaches also make use of protective groups for biosynthesis!

Phosphate esters are found in enormous variety in and truly dominate the chemistry of living systems. Generally polyfunctional and often labile, the synthesis of the more complex systems of biochemical interest also necessitates special protective measures. These are covered in the chapter on "Phosphorylation", which also includes a broad survey of phosphate ester chemistry and phosphorylation methods generally.

Volume 4 is devoted to three topics—enamines (J. Szmuszkovicz), synthetic methods in the carotenoid and vitamin A fields (O. Isler and P. Schudel), and the coupling of acetylenic compounds (G. Eglinton and W. McCrea). It is only in the past ten years that the full chemical potentiality of the enamines, the structural moiety represented by  $\alpha\beta$  unsaturated amines, has been realized. In a wide-ranging survey, the synthesis, reactions and physical properties of enamines and some dienamines are discussed and the applications of enamine chemistry to a variety of problems are strikingly portrayed. An appendix listing the titles of some 43 relevant papers which appeared between the writing of this chapter and the publication of the book illustrates the *tempo* of present-day research activity in this field.

The chemistry and stereochemistry of vitamins A and A<sub>2</sub> and the related carotenoids are now firmly established and total syntheses have been achieved. The main methods used in the synthesis of these complex systems and the key intermediates are presented in a novel and systematic way by two members of a school which has

been conspicuously associated with these outstanding achievements. Various chain-lengthening methods, which have played an important part in the synthetic investigations, are prominently featured and stereochemical considerations receive due attention.

The last chapter deals with the oxidative coupling of acetylenes through the intermediacy of the cuprous derivatives. Discovered by Glaser some 90 years ago and applied by Baeyer in 1882 in his historic synthesis of indigo, this reaction, in its various modifications, has proved of great utility. It has figured in the synthesis of naturally occurring polyacetylenes and has been elegantly applied for the synthesis of a whole range of novel, unsaturated macrocycles including the first all-planar, cyclic homologue of benzene, [18]-annulene. The full scope and versatility of this reaction are clearly demonstrated in this review. However, one is left with the impression that much remains to be learned concerning its mechanistic aspects. While it has been brilliantly exploited by the organic chemists for preparative purposes it has been very much neglected by physical chemists and co-ordination chemists. Maybe this critical appraisal of the present state of knowledge will inspire fresh endeavours to shed more light on the course of these acetylene coupling reactions and the not unrelated oxidative coupling of phenols and aromatic amines by the same reagents.

The declared motto of one of the reviews is "to stimulate rather than to satisfy". Every single contribution will certainly not fail to stimulate and most, if not all, organic chemists will feel more than satisfied with the wealth of well-documented and systematically presented information which has been crystallized within these pages.

J. IDRIS JONES

## MÖSSBAUER SPECTRA AND CHEMICAL STRUCTURE

The Mössbauer Effect and Its Applications in Chemistry  
By V. I. Gol'danskii. Authorized translation from the Russian. Pp. vii+119. (New York: Consultants Bureau, 1964.) 12.50 dollars.

THE effect of recoil-less emission and resonant reabsorption of  $\gamma$ -rays was first reported by Rudolf Mössbauer in 1958 and it has already been used for a wide variety of elegant experiments in basic physics. During the past three years it has been increasingly recognized that the Mössbauer effect can also give valuable information on chemical problems, and one of the pioneers in this field, Prof. V. I. Gol'danskii, has now written the first monograph specifically aimed at describing these chemical applications. The treatment throughout is non-mathematical and eminently readable.

The account begins with a brief discussion of the energies and widths of resonance transitions. When an excited atom is bound in a crystal the  $\gamma$ -ray may be emitted without recoil or thermal broadening and the precision with which its energy is then defined depends only on the Heisenberg uncertainty relation. Energy quanta of unprecedented precision are therefore available and these have been used to probe the minute changes in the separation of the nuclear energy-levels which result from differing chemical environments. These ideas are developed in the second chapter, which also mentions briefly the appropriate experimental techniques.

In any spectroscopic technique, information can be derived from the number of lines observed, from their position, shape and intensity. In Mössbauer spectroscopy these parameters give information about chemical shifts, quadrupole coupling constants and hyperfine magnetic fields. The origin of these effects is traced in Chapters 3 and 4.

The Mössbauer effect has been detected in a large number of elements with atomic numbers greater than 26 and these elements are spread widely throughout the Periodic Table. The most extensively investigated nuclide is  $^{57}\text{Fe}$  and results on the compounds of iron are reviewed in Chapter 5. Alloys, mixed oxides, salts, co-ordination complexes and organometallic compounds are mentioned.

Compounds of tin have also been widely investigated and the results are discussed in Chapter 6. This is followed by an assessment of various other problems which might be tackled by Mössbauer spectroscopy. There is an appendix listing the relevant properties of some eighty nuclides of the forty-five elements for which resonance has been observed or is predicted, and the review ends with a supplement outlining results published during 1963.

Prof. Gol'danskii has provided us with a persuasively written and exciting account of this new technique and it is unfortunate that his efforts have not been matched by those of the publishers. The translation, though generally good, is marred by occasional oddities like "gamma transactions", "very weakly pronounced" and "defectoscropy". The typesetting is also variable in quality and it is difficult in places to distinguish between  $\Gamma$ ,  $\gamma$ , and  $r$ . The binding is inadequate and the price very high. The original Russian publication cost about 30 cents and an English translation is already available from the International Atomic Energy Agency, in Vienna. There is a further disturbing feature: this is a 65-page review which is being made by the publishers to masquerade as a 120-page book. There is no indication from the title on the cover, from the fly-leaf, from the title page itself, or from the author's preface that half the book has nothing to do with the Mössbauer effect but is a translation of a four-year-old paper on another subject by other authors, whose names do not even appear in the table of contents.

N. N. GREENWOOD

## WIDENING FIELD OF QUANTUM ELECTRONICS

### Quantum Electronics

Proceedings of the Third International Congress. Paris. Edited by Prof. P. Grivet and Prof. N. Bloembergen. Vol. 1: pp. xxix+1-966. Vol. 2: pp. 967-1923. (Paris: Dunod, New York and London: Columbia University Press, 1964.) 252s. the two volumes.

QUANTUM electronics is a term which was originally coined to cover the investigation of resonant phenomena associated with the interaction of radiation and matter. With the growth of new techniques has come an inevitable spreading of the boundaries of the subject. Some of the topics are new, such as non-linear optical effects and the stimulated Raman effect; in others interest has been revived. The development of the maser is responsible for a great part of this growth, particularly in its optical form, the laser.

The third international conference on "Quantum Electronics" was held in Paris in February 1963. (The term 'Congress' is used only on the title page of the *Proceedings*.) There were some 1,100 participants, compared with 160 at the first conference in September 1959, and 450 at the second in March 1961, ample evidence of the growth of interest in the subject. In all probability this will be the last conference of this nature; no suitable accommodation is available for the numbers that are likely to wish to attend in future.

There has been a long delay in the publication of the *Proceedings* of the conference, but the result has been well worth the wait. Two hundred of the papers presented at the conference are published, thirty-two of them in French, the rest in English. Among the new topics that



are treated in detail are the theory of the coherence properties of light, laser modes and cavity design, semiconductor and phonon lasers, and non-linear optics. The rest of the papers are grouped in the sections on optical pumping and magnetometers, molecular beam masers, gas lasers, the spectroscopy of solid-state materials, solid-state masers and solid-state lasers. It is clear that the laser is responsible, directly or indirectly, for about half the papers.

From such a wealth of material it is scarcely possible to choose a few papers for special mention. It is adequate to say that the papers in each of the ten sections make each an invaluable survey of its particular field. The conference attracted leading experts, many of whom gave survey papers covering their own subjects, among them E. Wolf, J. Brosel, N. F. Ramsey, W. R. Bennett, jun., C. G. B. Garret and P. A. Franken. Particularly valuable is a record of the discussion which followed each paper, and the editors are to be congratulated on their success in persuading the authors to present their published papers in full, and not merely in skeleton form. A useful luxury is appended to a few of the papers in the form of a summary, sometimes in the language of the paper and sometimes in the second language of the conference. The value of the *Proceedings* would have been considerably enhanced if short summaries of all the papers could have been given in both English and French, but one can appreciate the magnitude of the editors' task in achieving this ideal. Inevitably the price of the two volumes is formidable, but entirely commensurate with their value.

J. H. SANDERS

## DEEP-SEA SANDS, GREYWACKES AND FLYSCH

### Turbidites

Edited by A. H. Bouma and A. Brouwer. (Developments in Sedimentology, Vol. 3.) Pp. 264. (Amsterdam, London and New York: Elsevier Publishing Company, 1964.) 90s.

WITH an ever-increasing number of sedimentologists working in flysch and greywacke sequences, a book on turbidites will inevitably arouse interest. This volume consists of fourteen articles together with an introduction and a final summary. In the first article, Prof. Kuenen, who introduced the term 'turbidite' into geological literature and who is largely responsible for the present popularity of the turbidity-current hypothesis, considers the evidence for the origin of deep-sea sands and their possible ancient equivalents. Two further articles are concerned with modern sands in the Adriatic and the Mediterranean; another two pay some attention to methodology. Regional accounts of geosynclinal successions with flysch and greywackes are given from the Apennines, Britain, the Central Carpathians, Germany, the Maritime Alps and the United States. Points of sedimentary detail are described in another two articles and a comprehensive bibliography of turbidite literature has been compiled.

Kuenen presents a powerful restatement of the turbidity-current hypothesis as applied to the interpretation of deep-sea sands, flysch sandstones and greywackes. He also includes some penetrating criticism of alternative suggestions which have been made for the origin of these sediments.

Stanley and Bouma advocate the systematic examination of sedimentary features and the recording of data graphically and on punch cards preparatory to analysis using the digital computer. They go on to give an account of a palaeogeographical reconstruction using the 'multiple parameter' method which they have described. The reconstruction, however, differs in no obvious ways from others prepared over the past decade (for example, the

excellent palaeogeographical atlas of the Polish Carpathians) which do not claim to involve anything more than traditional methods. Or is it that sedimentologists have been using the 'multiple parameter' method all this time without realizing it?

The regional accounts vary from detailed analyses to short articles which are little more than bibliographical lists. The palaeogeographical reconstructions from the Maritime Alps (Stanley and Bouma) and the Central Carpathians (Marschalko) are very valuable examples. Meischner presents a detailed investigation of calcareous turbidites (called 'alldapic limestones') in which the sedimentary features are interpreted in terms of a model of sedimentation similar to schemes already suggested. Among other data from Britain, Kelling makes special mention of the frequent divergence in the current directions of sole markings and, a little higher in each bed, transverse ripples. In addition to recognizing the possibility of the reworking of turbidite deposits by bottom currents Kelling suggests that surge waves resulting from the passage of the turbidity current may have been responsible for the transverse ripples. With gradually accumulating evidence for the operation of bottom currents in present-day deep-waters, this problem of ripple-mark versus sole mark direction is likely to provide the basis for much future discussion. Spotts and Weser find that there may also be a discrepancy between grain-orientation and sole markings. Is this an aspect of the same problem—an interaction of turbidity-current flow and bottom currents or the effect of reflected surge waves?

Granted the operation of both types of current, how are the turbidites to be distinguished from those sands deposited from bottom currents? Massive graded beds without large-scale current bedding would appear to be typical of turbidites especially if, as Meischner points out in his investigation of the alldapic limestones, current sole markings, bed thickness and grain-size show some intercorrelation. But the distinction is very difficult when laminated and rippled fine-grained sandstones are considered. Van Straaten, following Bouma, maintains that the lamination sequence, parallel-ripple and convolute-parallel, is diagnostic of turbidites, but this would be regarded with some scepticism by other workers. Rizzini and Passaga contend that grain size analyses using the CM method developed by Passaga can be used to separate turbidite from traction deposits. This method has, however, the disadvantage that it can only be applied to sequences of sandstones.

The foregoing remarks will show that there is much of value in *Turbidites* and the specialist will find a number of papers well worth reading. There are, unfortunately, a number of features to be deprecated. The quality of some of the plates is deplorable and typographical errors can be found throughout the volume. Many readers will welcome the fact that some of the foreign authors have presented their work in English. It is clear, however, that some of these writers needed to rely heavily on editorial help in clarifying and correcting their work; this help was apparently not provided and some of the text is ambiguous or obscure.

There is, too, a vast unevenness of treatment. Does, for example, a paper describing an outcrop of one bedding plane, 10 square yards in extent and describing one small feature of sedimentation, merit 30 pages when the turbidites from the rest of the United States are treated in 12? Again, ten Haaf's provocative little paper on the Apennines raises a number of complex questions which I at least would have liked to have seen developed.

Perhaps the clue to the unevenness lies in the fact that the papers were initially gathered together to form a special issue of *Sedimentology*. The collection remains a series of papers for a journal and, however valuable some of them are individually, they do not form a satisfactory book—not at the price of £4 10s.

E. K. WALTON

## *Homo 'habilis'* AND THE AUSTRALOPITHECINES

By PROF. J. T. ROBINSON

Departments of Anthropology and Zoology, University of Wisconsin, Madison 6, Wisconsin

LEAKEY, Tobias and Napier have given a preliminary account of specimens which in their opinion belong to a hitherto unknown species of the genus *Homo*<sup>1</sup>. They re-defined the latter genus and proposed the species name *H. habilis*, with definition, for the new taxon which includes specimens discovered between late 1960 and late 1963.

### Diagnosis of the New Taxon

Two critically important functions of the original description of a new taxon are: (a) to demonstrate that the population sample under discussion represents a taxon which is different from all recognized taxa and is therefore indeed new and in need of a name; (b) to place on record the new name selected for the taxon.

In order that the first of these functions be satisfied a differential diagnosis is necessary. This brings out the points of difference between the specimens comprising the new taxon and all closely related specimens which have been referred to already existing taxa. From this point of view the original description of *H. 'habilis'* by Leakey *et al.* is unsatisfactory. Doubtless the authors intend to present further evidence elsewhere at a later date, but clearly the appropriate place is in the original description (in the technical taxonomic sense) so that others may judge the validity of the proposed taxon.

The proposed new definition of the genus *Homo* depends in part on the validity of the new species proposed for it. The new species is defined briefly as though proof had previously been presented that it is a new taxon—but in fact there is no discussion of 'evidence' which clearly establishes that this is the case. The definition does include a few very broad diagnostic comparisons, for example, "... premolars which are narrower (in bucco-lingual breadth) than those of *Australopithecus* . . .", but no evidence is presented to support them.

Furthermore, although reference is made to some previously known specimens with which the authors think their new species may be conspecific, no comparisons with the new material are actually included in their original description. In fact the following very curious statement is made: "The specimens originally described by Broom and Robinson as *Telanthropus capensis* and which were later transferred by Robinson to *Homo erectus* may well prove, on closer comparative investigation, to belong to *Homo habilis*". This statement clearly implies that the authors described a new species even though they believe that proper comparative investigation may well show that it is conspecific with specimens of which they are aware, the originals of which two of the authors have seen more than once and of which detailed descriptions exist in the literature. It is of interest also to note that the authors think that if the conspecificity be established in this case, then the material which was described more than a decade ago would belong to their new species rather than the other way about—as required by the *International Code of Zoological Nomenclature*.

One may thus conclude that: (a) the authors have not demonstrated that their proposed new species is in fact new; (b) the authors themselves are in doubt whether it is new; (c) since the validity of the new species has not been established, that of the new definition of *Homo* has not been established either. (In this general connexion see also the recent comment by Oakley and Campbell<sup>2</sup>.)

### Validity of the proposed New Species

Although the authors do not present evidence in support of the few suggested differentially diagnostic features separating the proposed new species from the australopithecines, it is worth enquiring into the validity of these features.

Evidently a character which has impressed the authors as significant<sup>1,3</sup> is "a marked tendency toward the buccolingual narrowing and mesiodistal elongation of all the teeth, which is specially evident in the lower premolars . . . and in the lower molars . . .". In particular it is held that the lower premolars are narrower than those of *Australopithecus* but fall within the range of *Homo erectus*. Through the courtesy of Dr. and Mrs. Leakey I was able to make a fairly detailed investigation, in 1961, of the mandible which has now been designated the holotype of *H. 'habilis'*. My measurements of  $P_3$ , made in the same manner as those on the South African australopithecines used for comparison, gave the following mesiodistal lengths and buccolingual breadths in millimetres: left,  $9.6 \times 9.4$  and right,  $9.6 \times 9.9$ . These figures yield length/breadth indices ( $L \times 100/B$ ) of 102.2 and 98.0 respectively. Leakey<sup>3</sup> lists these dimensions as  $11.0 \times 9.5$  in both cases and the index as 115.8. However, as comparison is being made with measurements made by me on the South African australopithecine material, my measurements on the Olduvai specimen should yield a better comparison since all the measurements involved were made by the same person using the same technique.

The table compares the mean length/breadth index and the observed range of the index for  $P_3$ ,  $M_1$  and  $M_2$  of *Paranthropus*, *Australopithecus* and Pekin Man (*Homo erectus*) with the corresponding values for the type mandible of *H. 'habilis'*.  $P_3$  of the latter falls within the observed range of that for *Paranthropus* and not far outside that for Pekin Man. The fit is slightly less close with *Australopithecus*; but the sample for this form is made up of only four specimens representing three individuals. The highest value listed for *Paranthropus* belongs to a tooth which had not yet begun to erupt and the final dimensions would possibly have been slightly higher than at present, although the length dimension already is very close to the upper limit for the sample of 12 specimens. However, since we are here concerned with proportions it is legitimate to include the value for this specimen as the crown is intact and undamaged and it is highly improbable that any slight increase in the crown dimensions that may have occurred before eruption would alter the shape so disproportionately as to change the shape index significantly.

In the case of  $M_1$ , the *H. 'habilis'* value falls slightly outside the ranges for *Paranthropus* and Pekin Man, but barely outside that for *Australopithecus*. Leakey<sup>3</sup> mistakenly quotes 114.6 as the highest observed and published value for *Australopithecus* (*sensu stricto*) and concludes that his value for the type mandible (117.0) falls outside that for *Australopithecus*. In the case of  $M_2$  the *H. 'habilis'* value falls within the observed ranges for both *Paranthropus* and Pekin Man and very slightly outside that for *Australopithecus*. In both cases the *Australopithecus* sample size is small.

In one section of the original description Tobias refers to a lower molar, of Hominid 4, which he believes to be either  $M_2$  or  $M_3$ , and writes: "The molar is 15.1 mm in

Table 1. LENGTH/BREADTH INDICES

	$P_3$			$M_1$			$M_2$		
	$N$	Mean	Range	$N$	Mean	Range	$N$	Mean	Range
<i>Australopithecus</i>	4	83.0	77.0-88.5	8	107.8	100.8-117.0	9	107.7	98.0-111.3
<i>Paranthropus</i>	14	85.2	76.0-112.3	19	106.2	100.7-110.8	12	108.4	101.4-117.3
<i>H. 'habilis'</i> type	2	100.1	98.0-102.2	2	117.2	117.2	2	114.3	114.3
Pekin Man	13	86.3	75.0-96.8	11	100.1	96.8-112.0	7	104.4	98.3-115.4

mesiodistal length and 13.0 mm in buccolingual breadth; it is thus a small and narrow tooth by australopithecine standards . . .". The length/breadth index of this tooth is 116.2; the observed range for *Paranthropus* is 101.4-117.3 for  $M_2$ , and 106.6-124.1 for  $M_3$ —indeed, the 'mean' index for the sample of 13 specimens for the latter tooth is 116.3. The ranges for this index for  $M_2$  and  $M_3$  of *Australopithecus* are 98.0-111.3 and 100.0-116.0 ( $N = 10$ ) respectively.

The size can best be compared by using the module so that single values can be compared instead of pairs. The module for the aforementioned Olduvai tooth is 14.05. This certainly is smaller than are either  $M_2$  or  $M_3$  of *Paranthropus*. However, the lowest modules for the small collection of these two teeth of *Australopithecus* are, respectively, 13.75 and 13.20. These figures thus show that the Olduvai hominid 4 tooth is neither exceptionally small nor exceptionally narrow by australopithecine standards.

This evidence, along with that from the type specimen, therefore does not support the contention that the shape characteristics of the *H. 'habilis'* mandibular teeth are recognizably different from those of the australopithecines. The *H. 'habilis'* specimens here considered have values which fall mostly toward the upper end of the ranges of variation at present available for the australopithecines. But it is very clear that in no case are adequate samples available: the ranges of variation are certainly smaller than they should be and insufficient is known about *H. 'habilis'* to know how representative the few known specimens are of the populations from which they came.

However, a far more trenchant criticism of the use made of the dental length/breadth index by Leakey *et al.* is that analysis of the index shows that it and the features on which it is based have extremely low phyletic valence so far as hominids are concerned. This is readily apparent from the extremely wide overlap of the ranges for this index for *Australopithecus*, *Paranthropus*, *Homo erectus* and *Homo sapiens*. It is not possible to distinguish taxonomically between these groups by means of this index if anything like adequate sample sizes are used. As may be seen from Table 1 the means for *Paranthropus* and Pekin Man are 85.2 and 86.3 respectively for  $P_3$  and (not shown in Table 1) values for samples of modern Bantu and aboriginal Australians differ from that of Pekin Man by a few tenths of a unit. Thus, populations covering so great a span of the hominids have means for this index which are very closely similar. On the other hand, various local populations of modern man have mean values for this index which actually show greater variation than the foregoing. That is to say, the intra-species variation in the mean in modern man is actually greater than the intergeneric differences in the mean for three populations of *Australopithecus*, *Paranthropus* and *Homo erectus*. Furthermore, these differences between the means are small compared with the range of variation observed within any one of the populations concerned.

The endocranial capacity of *H. 'habilis'* appears, on the scanty and indirect evidence available<sup>8,1</sup>, to have differed little from that of the australopithecines with a range overlapping that of the latter substantially.

The conclusion that the foot of the new form has a fairly advanced and *Homo*-like structure while the hands appear to have been relatively more primitive does not

help a great deal at present since not much evidence of these parts is available for either australopithecines or *H. erectus*. However, neither conclusion should occasion astonishment. If the australopithecines were erectly bipedal in posture and locomotor habit, as much evidence suggests, then the foot was being used in an essentially human fashion and is likely to have achieved a relatively advanced structure soon after the new locomotor habit was achieved. On the other hand, neither the australopithecines, as apparent tool-users, nor *H. 'habilis'* as an apparent primitive tool-maker, were culturally advanced and therefore one might expect that the moulding of the hand under the influence of manipulative activity of the human sort was not far advanced. Thus one might expect the foot to have had a more modern-looking structure than the hand in both the australopithecine stage and the early hominine stage. The apparently fairly advanced foot of *H. 'habilis'* does not therefore necessarily indicate affinity with *Homo* rather than *Australopithecus*—indeed, the fact that a striding gait appears to be inconsistent with the morphology of the *H. 'habilis'* foot<sup>9</sup> suggests that it is unlikely to have been significantly more advanced than that of *Australopithecus*.

In view of the foregoing it seems to me that Leakey *et al.*, in their original description of *H. 'habilis'*, have by no means provided a reasonable case for establishing a new species of *Homo* to accept the recently discovered Olduvai specimens. Furthermore, some of the distinguishing criteria used do not appear to be valid for the purpose in the light of available knowledge of early hominids.

#### Affinities of *H. 'habilis'*

In assessing the material attributed to *H. 'habilis'* it must be remembered that two groups of specimens are involved: one from Bed I and the other from Bed II. On the available dating evidence these two groups are separated by a significant time gap.

The morphological characteristics of the two groups are not the same. This is well shown, for example, by comparing the type mandible from FLKNN I with the mandible from MNK II (see Leakey and Leakey<sup>5</sup>, Fig. 3). I have elsewhere<sup>6,7</sup> discussed the shape of the internal mandibular contour, seen in occlusal view, and its narrow V shape in both types of australopithecine but its relatively wide U shape in *Telanthropus* and all other forms of *Homo*. The type *H. 'habilis'* mandible is damaged near the symphyseal region so that the partial right side has been displaced toward the left. However, the midline can be determined within very narrow limits and the internal contour of the left half is intact and undisturbed from very near the symphysis to a point behind  $M_2$ . It is therefore simple to reflect this contour on to the right side in a graphic reconstruction in order to determine the correct original position of the displaced right half. Carrying out this procedure shows that the type mandible had a typically australopithecine internal mandibular contour with the corpus thickness in the premolar region greater than the distance between the two halves of the mandible in that region. This is actually readily apparent on visual inspection since the corpus mandibulae is relatively thick compared with the breadth of the crowns of the teeth and much of this breadth is mesial to the teeth in the premolar region. In contrast the mandible from MNK II does not have this narrow V-shaped contour but

has the wide U-shaped contour and relatively thin corpus of the sort normal for *Homo*. The more recent, Bed II mandible thus agrees with *Homo erectus*, including 'Telanthropus', in this feature as well as its generally greater gracility, while the older Bed I mandible falls within the observed range of australopithecines in both respects. It is therefore by no means clear that the Bed I and Bed II groups of specimens necessarily belong to the same species.

The teeth of the type mandible show that the australopithecine affinities of this mandible are very clearly with *Australopithecus* and not with *Paranthropus*. The latter is characterized among other things, as witnessed by the South African, East African and Indonesian evidence, by a small canine as compared with the size of the premolars, especially in the mandible. Indeed, the lower canines of this form are so small that they fall within the observed size range for modern living man while the premolars are the largest known among fossil and modern hominids. *Australopithecus*, on the other hand, has relatively large lower canines, *Sts 3* from Sterkfontein being one of the largest hominid mandibular canines known, but the premolars are smaller than those of *Paranthropus*. Thus the relative size of canine to  $P_3$  in the mandible is very different in the two australopithecines and any individual mandible can be assigned to the correct genus without hesitation on visual inspection if these teeth are present. The *H. 'habilis'* canine to  $P_3$  ratio is like that of *Australopithecus* and quite unlike that of *Paranthropus*. This fact is further supported by greater morphological resemblance in general between the teeth of the Olduvai specimen and those of *Australopithecus* as compared with *Paranthropus*.

On the other hand, the Bed II mandible shows much greater resemblance to the 'Telanthropus' mandibles, but the latter can easily be distinguished from the Bed I type mandible.

In terms of the available evidence it would seem that there is more reason for associating the Bed I group of specimens with *Australopithecus* and the Bed II group with *Homo erectus* than there is for associating the Bed I and II groups with each other. This would therefore seem a perfectly reasonable course to adopt: placing the Bed I material as advanced representatives of *Australopithecus africanus* and the Bed II group as somewhat early members of *Homo erectus*.

However, the Bed I and II groups of specimens occurred in the same geographical area, both appear to have been tool-makers and there seems no obvious morphological reasons why the earlier group could not have been ancestral to the later group. Furthermore, it seems unlikely in terms of ecology and behaviour that two morphologically similar groups, both adapting at least to a significant extent by cultural means, would develop in the same general geographic area. Clearly at least two hominid lines did in fact exist simultaneously in this region: the material under discussion and *Paranthropus* (= 'Zinjanthropus'). But in this case the *Paranthropus* line consisted of forms which differed markedly in morphology and evidently also in ecology and behaviour not only from the *H. 'habilis'* material but also all other known hominids. If the Bed I and Bed II groups represented two different lines, this would indicate not only that they would be adaptively similar but also that three different hominid lineages existed in the same area. For these reasons it seems probable that they actually do represent the same lineage at two different time levels. If this is so, then it is reasonable to place them in the same species. On the other hand, as already seen, morphological considerations favour their being placed in two different taxa which already exist, in which case they belong to two different genera. The evidence in favour of the latter course is actually the stronger.

The two interpretations do not have to be mutually exclusive: the Bed I material may represent an advanced

form of *Australopithecus* and Bed II specimens an early *H. erectus* and at the same time the latter may be a lineal descendant of the former. This seems to me to be by far the most probable interpretation. According to this hypothesis the Bed I specimens represent a transitional stage between *Australopithecus* and *H. erectus* just at that stage where the essentially tool-using stage of the former was giving way to the primarily tool-making condition of the latter. The widely held belief of recent years that *Paranthropus* at Olduvai was responsible for the stone implements found associated with it never did seem probable to me in the light of the available evidence from the Sterkfontein Valley. However, it seems far more probable that the *H. 'habilis'* material from Bed I represents the remains of the maker of the stone industry from that level.

If the interpretation suggested here is correct, then clearly no new species name is needed: the situation is simply one common in palaeontology when specimens are found which link two already existing taxa. Creating a new taxon here is no solution; the two taxa between which the new one falls are already so similar that insufficient morphological distance exists between them to justify the insertion of another species. As is well recognized, conventional Linnean taxonomy is not suited to dealing with a problem such as this and, if the hypothesis is correct, whatever solution is adopted must be a compromise of some sort. The more conservative approach would be simply to place the Bed I material in the species *Australopithecus africanus* and the Bed II specimens in *H. erectus*. However, if it is a fact that the Bed I specimens were already primitive tool-makers and were ancestors of the Bed II material, the implication that the ends of a transitional sequence, which involved relatively little morphological and ecological change and did not occupy a geologically long period of time, should be in different genera seems very unsatisfactory, especially as the transition was gradual and did not involve a threshold followed by rapid re-adaptation.

A reasonable way of overcoming this difficulty would be to extend the genus *Homo* to include not only the new Olduvai material but also that at present in the genus *Australopithecus* (as distinct from *Paranthropus*). This genus would then include the whole sequence from the point where a shift to an omnivorous diet (by the inclusion of a substantial degree of carnivorousness) caused a new set of selection pressures to come into play favouring the whole complex of culture as a means of adaptation and thus caused the emergence of culture-bearing man. This suggestion comes very close to one made a long time ago by Mayr<sup>10</sup>, but differs from it in not including *Paranthropus* since there is good evidence to indicate that the basic adaptation of the latter was quite different from that of the *Homo* lineage as defined here, a lineage which was separate from that of *Paranthropus* from at least early Pleistocene times as the evidence now clearly indicates. This difference is most conveniently indicated by a generic distinction. The known hominids would thus fall into the two genera *Paranthropus* and *Homo*.

If this were done, then it would seem to be useful to modify the species division at the same time so that the re-defined genus *Homo* includes only two species. Since these would belong to the same lineage they obviously could not be sharply defined. The first could be *H. transvaalensis* and would include the tool-using phase of the lineage involving small-brained forms which were primarily tool-using, had relatively poor communication and comparatively simple social structure. The second could be *H. sapiens*, including larger-brained tool-makers who possessed greatly improved means of communication and comparatively complex social structure. These two species would clearly intergrade but by the very nature of the situation in a single lineage it is not possible to have satisfactory division points for taxonomic purposes, once enough material is available, since there is genetic con-

tinuity between successive time levels throughout the sequence. In this event the whole lineage in which culture is a very important adaptive mechanism is included in a single genus and the two species defined in terms of two major stages of cultural development.

- <sup>1</sup> Leakey, L. S. B., Tobias, P. V., and Napier, J. R., *Nature*, 202, 7 (1964).  
<sup>2</sup> Oakley, K. P., and Campbell, B. G., *Nature*, 202, 732 (1964).

- <sup>3</sup> Leakey, L. S. B., *Nature*, 191, 417 (1961).  
<sup>4</sup> Robinson, J. T., *Transvaal Mus. Mem.*, No. 9 (Pretoria, 1956).  
<sup>5</sup> Leakey, L. S. B., and Leakey, M. D., *Nature*, 202, 5 (1964).  
<sup>6</sup> Robinson, J. T., *Amer. J. Phys. Anthropol.*, 11, 445 (1953).  
<sup>7</sup> Robinson, J. T., *S. Afr. J. Sci.*, 57, 3 (1961).  
<sup>8</sup> Tobias, P. V., *Nature*, 202, 3 (1964).  
<sup>9</sup> Day, M. H., and Napier, J. R., *Nature*, 201, 969 (1964).  
<sup>10</sup> Mayr, E., *Cold Spring Harbor Symp. Quant. Biol.*, 15, 109 (1950).

## PROGRESS OF CANCER RESEARCH

THE British Empire Cancer Campaign for Research in 1963 spent more than £1 million in support of cancer centres (40 in number) and individual research workers. The investigation described in the 1963 report of the Campaign\* can be divided into three main groups dealing with (1) biology of cancer (I. H.); (2) radiotherapy and the physics and physical chemistry of radiations (P. A.); (3) clinico-pathology of cancer (A. L. L.). Part I can be subdivided into five classes of investigation, namely, carcinogens and carcinogenesis, immunity in cancer, characteristics of tumours and tumour cells, cytogenetics and cancer viruses; and the two first-mentioned groups, carcinogenesis and immunology, by far attract most work in this field of research.

### Biology of Cancer

Passey and Elson at the Chester Beatty Institute find that the result of cigarette smoke is a deleterious effect on the weight at birth and growth rate of young rats the mothers of which had been subjected to daily breathing of cigarette smoke in a smoking cabinet before being mated (quite apart from the fact that the cigarette is the cause of four-fifths of all human lung cancer, which now kills 25,000 a year in Britain).

Brookes and Lawley, also at the Chester Beatty, have investigated the binding of <sup>14</sup>C- and <sup>3</sup>H-labelled hydrocarbons (four carcinogenic and two non-carcinogenic) to the nucleic acids of mouse skin and conclude that "these results suggest that reaction with DNA is the significant reaction required for carcinogenic activity, and are consistent with the mutation theory of carcinogenesis". (Brookes and Lawley do not go into such questions as—how to fit into this scheme carcinogens like plastic films (but not plastic powders), metal foils, cholesterol or radiations; why some chemical compounds are potent carcinogens for some species of animal and not for others, or even for some tissues of a particular experimental animal and not its other tissues.)

Case *et al.* have examined the incidence of occupational bladder tumours in industries such as rubber and cable making where the operatives may be exposed to the naphthylamines, benzidine or related compounds, carcinogenic to the bladder. Case states: "The rubber industry has never been the subject of an efficient epidemiological survey of bladder tumours because it has been generally assumed that adequate grounds exist for believing that all the dangerous compounds had been brought to light. This belief was strongly contested by the authors of the report (Case and Hosker, 1954). The only means of assessing whether or not any danger still persists is a rather indirect one based on death certificates and employment tables, with perhaps a little help from the number of new cases of bladder tumour coming to light within the industry itself". Case quotes the number of death certificates where tumour of the bladder was mentioned relating to workers in rubber occupations: the numbers were 20 and

27 for 1952–56 and 1957–61 compared with 9.8 and 11 to be expected if the incidence were the same as in England and Wales generally. He concludes that "a detailed epidemiological study of the situation with the full co-operation of the rubber industry and the trade unions concerned, would therefore seem to be eminently desirable".

Powell and Wright have been investigating chemical carcinogenesis *in vitro* using  $\beta$ -propiolactone on cultures of lung tissue from 15-day-old August rat embryos; after a somewhat lengthy description of the experiments it is concluded that the treated cells were "histologically indistinguishable from malignant epithelial cells *in vitro*", but Powell says nothing about assaying their malignancy by inoculating the cultures back into August rats. At the same laboratory (Mount Vernon Hospital) interesting investigations have been made of changes in the vitality of CBA mice; this strain is now showing a high neonatal mortality and the same change is reported from four other centres; some kind of infection to which the CBA are particularly susceptible might be implicated, but such an explanation would not account for the same change occurring in other laboratories.

At the Royal College of Surgeons the carcinogenic process in mouse skin is being investigated by an examination of the lysosomes, which are acid phosphatase organelles, in the cells of normal skin and in skin during exposure to carcinogenic agents.

At Cambridge, Glucksmann and Cherry have been working on microcarcinoma of the uterine cervix: writing on the malignant process in the cervix, they state: "It is now fairly widely accepted that most carcinomas of the uterine cervix pass through an *in situ* stage in which the cells have the morphological characteristics of malignancy but move to and are shed from the surface rather than invade the stroma through a defect in the basement membrane. The stage of microinvasion or microcarcinoma occurs between that of the *in situ* lesion and that of frank malignancy, and is characterized by the change in the direction of cellular movement towards and through the basement membrane".

"In microcarcinomas, tumour cells pass through the basement membrane into the stroma but undergo a process of differentiation in the form of either keratinization or mucification and are incapable of sustained growth." From experiments on the interaction of radiation and chemical carcinogen (dimethylbenzanthracene, DMBA) in the induction of cancer of the genital tract in castrate and normal female rats, these workers conclude that the action of radiation ( $6 \times 400$  r.) in the process of chemical carcinogenesis is of a hormonal rather than an immunological or mutagenic nature. Heath finds that some pure powdered metals suspended in serum are carcinogenic when injected into the muscles of rats (cobalt, cadmium, nickel): others were not (arsenic, zinc, tungsten, iron, tantalum, copper, beryllium and manganese). The process whereby tumours are induced consists first in a breakdown of the muscle fibres to free mononucleate myoblasts and, secondly, the transformation of the free myoblasts into malignant variants. Cobalt powder injected into mice caused as much necrosis as in rats but the muscle fibres regenerate in 6 weeks and tumours do not develop.

\* British Empire Cancer Campaign for Research. Forty-first Annual Report covering the year 1963. Part 1: The Chairman's Statement and the Accounts of the Central Organization. Pp. i–xxv; The Autonomous Councils of the Campaign, Pp. xxvii–xl. Part 2: The Scientific Report of the Researches undertaken by the Central Organization and its Autonomous Councils in the United Kingdom and by some of its Affiliated Organizations Overseas. Pp. 1–707. (London: British Empire Cancer Campaign for Research, 1963.)



Daniel has observed malignant transformation of fibroblasts from the foetal rat of a hooded strain which had been in culture for 4 years; these malignant fibroblasts give tumours when inoculated into hooded rats but are strain specific, for they will not grow in albino rats. The cultures show a wide range of degree of malignancy: some clones have given no tumours in 20 weeks on inoculation into rats, another gave 6 tumours in 8 animals in less than 4 weeks; other variants are sensitive to the presence of glucose in the nutrient. Daniel reports that continuous lines of mouse cells, unlike some lines of dog and hamster cells, lose Forssman antigens; by using antisera to the histocompatibility antigens A and K, some lines of cells in culture were shown (by being destroyed by antibody) to retain both antigens, but in another line 2-4 per cent of the cells had lost antigens and by culturing survivors the percentage rose to 18, and these remained viable after exposure to the antiserum-complement mixture.

Baldwin and colleagues, using the carcinogen *N*-hydroxy-4-acetyl-aminostilbene, find that it induces carcinoma of the ear duct in 85 per cent of rats tested; why this compound should select ear ducts for its site of action remains to be explained.

Baldwin has confirmed that "seven normal liver cell sap antigens and at least six microsomal antigens are deleted from DMAB-induced (aminoazo-dye) rat liver tumour" and that: "Further studies on the antigenic composition of the cell sap fraction from DMAB-induced rat liver tumour, utilizing rabbit antisera prepared against this fraction, have indicated the presence of a number of abnormal antigens which do not cross-react with normal liver cell sap. Moreover, these antigens do not cross-react with components in cell sap fractions from apparently healthy liver taken from tumour-bearing rats, indicating that they arose during tumour induction rather than as a non-specific response to carcinogen feeding. Precise identification of the number and nature of abnormal cell sap antigens in tumour is still incomplete but immunoelectrophoretic studies have demonstrated three major and four minor components.

"Comparison of the antigenic composition of liver cell sap fractions from rats after varying periods of DMAB feeding, utilizing anti-normal liver cell sap antiserum, has demonstrated that one of the normal liver cell sap antigens which did not cross-react with tumour was deleted as early as 30 days after carcinogen treatment. Similarly a normal liver microsomal antigen which was deleted from tumour could not be detected after 30 days DMAB feeding. In view of the finding that the initial 4-6 weeks of carcinogen feeding is a critical period in carcinogenesis (Arcos *et al.*, 1960) a more detailed assessment is being made of cell antigen changes during this time.

"Sera from rats during the earlier stages of carcinogenesis are now being examined for circulating tissue antigens. Although not yet completed, these studies have shown that tumour cell sap antigen was detectable in serum as early as 90 days after DMAB feeding. Clearly, therefore, the abnormal antigens detected in DMAB-induced tumour will become available during carcinogenesis for the induction of an immune response in the host. Whether an immune response is induced and its possible significance in carcinogenesis have still to be evaluated."

Orr *et al.* have investigated the effect of injecting small quantities of DMBA subcutaneously into new-born rats. Nearly half the animals developed tumours after one year; these were chiefly haemangiomas, some of them malignant, in the subcutaneous tissues, muscle, spleen and uterine horns. Bonser at Leeds has worked for many years on some compounds used in the dye and rubber industries where they are liable to induce bladder cancer in operatives: 2-naphthylamine when tested on dogs induced bladder tumours in every dog surviving administration of the compound for two or more years, the latent period being about five years. Peacock in Glasgow has

tested the anti-tuberculosis drug isoniazid on the lung tissue of rats, hamsters and desert rats: this compound, like urethane, is a potent carcinogen for the lung of the mouse; he finds that only the mouse and not the other three species develop lung tumours. Mice of the *C3Hf* strain have an incidence of more than 20 per cent of lung adenoma even when kept in a soot-free atmosphere; although many adenomas are found in lungs that also show chronic inflammatory lesions, tumours of about 1 mm diameter have also been found in apparently healthy lungs.

Peacock has tested asbestos injected into the air sacs of fowls. Two birds developed tumours, one of which involved the syrinx (bifurcation of trachea), the proventriculus, lungs and ovary; the second tumour had infiltrated the site of injection, humerus and lung. At the New Zealand centre in Otago, Bielschowsky has found that urethane, which is only mildly leukæmogenic for most strains of mice, produces thymomas in a third of treated mice of the *NZB* strain.

At the South African Institute, Oettlé has observed that his colony of mastomys (kangaroo rat), which is the only rodent liable to spontaneous gastric cancer, is now undergoing a decline in this form of neoplasia but remains extremely liable to papillomatosis of the skin, perineum and vagina which occasionally progresses into squamous carcinoma.

Weipers *et al.* at the Glasgow Veterinary School have reported on lymphosarcoma in domestic animals; a proportion of the animals in a cattle herd with the disease showed chromosomal aberrations in blood cultures of circulating lymphocytes; this form of cancer has also been observed in dogs (150 cases) and pigs and in the cat, where it could be transmitted experimentally, and electron microscopy showed virus-like particles 100 m $\mu$  in diameter which were very similar in structure to the viruses of murine leukæmias.

At the Chester Beatty Institute, Delorme, Mikulski and Alexander have investigated the treatment of tumours in rats by infusion of lymphocytes from a donor rat which had been immunized with grafts of tumour cells (benzpyrene-induced fibrosarcoma); some regression resulted in 70 per cent of animals and in 25 per cent the growth of tumour was delayed by more than 60 days. In another experiment  $2 \times 10^6$  antilymphoma lymphocytes from the spleen of a *CBA* mouse which had destroyed grafted lymphoma cells specific to *DBA/2* mice prevented the growth of  $10^4$  lymphoma cells inoculated into *DBA/2* mice which when untreated can succumb to tumour growing from a single injected lymphoma cell.

At Guy's Hospital, Tuffrey and James have shown that epitheliomas produced in *C57BL* mice with dimethyl-benzanthracene are antigenic within the strain of origin, and Wang (King's College Hospital) has found evidence of the partial loss of normal tissue antigens in human tumours, and indications of the presence of additional specific tumour antigens. Watkins has made a detailed investigation of the serum  $\gamma$ -globulin system of the mouse during immunization with tumour cells and extracts.

Smith at Leeds immunized *C57BL* mice with *A* strain mammary carcinoma, and suspensions of the lymph nodes were then injected in  $F_1$  hybrid mice (*A*  $\times$  *C57BL*) which next received a subcutaneous graft of the same *A* tumour; some inhibitions and failures to engraft were found, while 100 per cent of control grafts 'took'.

Ingleton has examined by diffusion technique the antigens of mouse liver and finds that, after only 48 hours' treatment with *O*-aminoazotoluene, two slow-moving components unique to mouse liver fractions are lost or masked.

Burch at the Radiotherapy Centre in Leeds has brought out a theory of carcinogenesis based on immunity which is a gem of its kind. He writes: "Many observations and certain theoretical considerations indicate that immune factors are important in carcinogenesis. In a theoretical analysis of the aetiology of human cancers (Burch,

1963a) it has been concluded that in the absence of viral or hormonal factors, both homologous structural genes at an autosomal locus need to be mutant before malignant conversion can occur. Several structural loci may be at risk, each with a similar phenotypic expression. When only one member of the homologous pair of structural genes is mutant, it appears that under certain conditions, hyperplasia but not malignancy can result.

"From a logical argument (based on autoimmune data) it is concluded that the target tissue antigen (or 'tissue coding factor'—TCF) in disturbed tolerance autoimmunity has the same polypeptide structure as the normal (pre-mutant) cell bound 'antibody'. Genotypic homozygosity at one (at least) of the two autosomal loci associated with the synthesis of cell bound 'antibodies' and TCF's appears to be a general prerequisite for the development of a pathological autoimmune reaction. This gives some support for the conclusion (Burch, 1963b) that in carcinogenesis the autoimmune transformation of a 'carcinogenic' into a malignant cell requires that homologous autosomal structural genes in the former cell must be both mutant."

Schild at University College, London, has investigated specific tumour antigens using anaphylactic tests; the complexities of this line of research can be appreciated from his statement: "It is apparent that positive anaphylactic tests with serum are obtainable with some tumours but not with others. Experiments are at present under way to determine whether, in the case of tumours which give a high percentage of positive results, similar positive results are obtained with non-malignant conditions, i.e., whether the response is specific for tumours or not".

Stuart in Edinburgh reports that he has "found it possible to prepare lymphoid cells which exert a direct action on the Landschütz tumour and have no effect whatsoever on normal host tissue (Stuart and El Hassan, 1964). Thus we now have an experimental model in which an immunological stimulus reacts specifically with a rapidly growing neoplasm and fails entirely to damage the host tissues".

### Radiotherapy and the Physics and Physical Chemistry of Radiations

As in previous years experiments involving ionizing radiations constitute a very substantial part of the research supported by the Campaign. Recent advances in radiobiology have opened the way to gaining an understanding of the biological basis of radiotherapy, surely the first step in the discovery of improvements in this method of treatment. The killing by X-rays *in vitro* can now be determined with precision and the field has been opened to the investigation of the role of host factors and physiological state on radiation response *in vivo*. Investigators at St. Bartholomew's Hospital have shown that skin reactions can only be explained if the behaviour of the cells *in vivo* is very different from that found *in vitro*. It is believed that these investigations may make it possible to determine an optimum fractionation régime which achieves the maximum differential between damage to tumour and normal tissue. The possible role of pharmacologically active factors released in the skin on irradiation is being examined in connexion with the same problem at the General Hospital, Northampton. The ability of different organs, containing rapidly dividing cells, to withstand continuous irradiation varies widely and indicates the complexity that is encountered when radiation is investigated at the tissue level. At the Physics Department of the Institute of Cancer Research, it was found that while the small intestine can maintain a steady state of cell population even when challenged continuously with  $\gamma$ -rays at a dose rate of 415 r./day, the growth of tumours was stopped by continuous irradiation at an intensity of 176 r./day. An immunological method of increasing the radiosensitivity of primary

chemically induced tumours has been developed at the Chester Beatty Research Institute. To eradicate a tumour it is not always necessary to kill (or sterilize) the last cell and in some cases radiotherapy may achieve a 'cure' by reducing the number of cancer cells to a small fraction which can then be eliminated by immunological processes. This is supported by the finding that no correlation could be found between the widely varying radiosensitivity of sarcoma cells *in vitro* and the *in vivo* response to X-rays of the tumour from which the cells were derived.

Much effort continues to be devoted to the factors which determine the sensitivity of isolated cells and to methods of modifying it. The problem is very complex, since the radiation-induced lesions can be restituted, often very effectively, by post-irradiation repair processes. If these are assisted or impaired the radiosensitivity can be greatly altered even when the nature of the primary radiation lesion is not affected. The sensitizing action of oxygen is well known and is believed to occur at the level of the initial radiochemical events. Workers at Mount Vernon Hospital investigated the possibility that this effect was due to the trapping by oxygen of radiation-produced hydrated electrons. If this were the case there should be no oxygen effect at low pH values. By using a sulphur bacillus capable of withstanding strong acid they were able to demonstrate pronounced sensitization by oxygen at pH 1, and this suggests that the hydrated electron is unlikely to play a major part in this system at least. The same workers have made an exhaustive investigation of the two most effective types of protective agents for bacteria, that is, aliphatic substances containing either SH or OH compounds. The key difference is that the SH substances are active at one-hundredth the concentration of those containing alcoholic groups. That there is a complex interplay of many factors in the determination of radiosensitivity is illustrated by nitric oxide, which can be made to sensitize or to protect micro-organisms depending entirely on the conditions used (Christie Hospital and Holt Radium Institute). At the Chester Beatty Research Institute it was shown that the greater radiosensitivity of mammalian cells and of bacteria grown in medium containing bromodesoxyuridine was largely due to the fact that post-irradiation repair was inhibited. Cells with little recuperative capacity were sensitized only to a very small extent.

Almost nothing is known about the nature of the repair processes which rescue a cell from a lesion that would otherwise have been fatal. The majority of chemical and biochemical work is concentrated on the nature of the primary radiation lesion. Our understanding of radiation chemistry is being advanced by the use of physical tools; electron spin resonance is being used at half a dozen centres to investigate the radicals formed when organic substances are exposed to radiation. The technique of flash photolysis used at the Christie Hospital in Manchester and at Mount Vernon Hospital is providing much information about the nature of the unstable species that are formed when aqueous solutions are irradiated. Chemists had speculated rather widely about the nature of radical intermediates in radiochemical reactions and many of these ideas can now be checked. Although the basic concepts of radiation chemistry have not been affected these new techniques have made it necessary to re-evaluate much of the finer detail. The implications of all this to radiobiology lie in the future; before the details of radiation chemistry can be considered the nature of the organelles within the cell that are critical for radiation damage have to be determined. Elegant experiments at the Strangeways Laboratories with microbeams of  $\alpha$ -particles have confirmed that mammalian cells *in vitro* are extremely resistant to irradiation when it is confined to the cytoplasm, and that part of the nucleus has to be included to achieve effective killing. Biochemical investigations also point to the nucleus, and in murine lymphoma

cells irradiation by a few hundred r. was shown to interfere within a few minutes with nuclear RNA (Chester Beatty Research Institute). However, the cytoplasm undoubtedly plays an important part in radiation injury, possibly because it is the site of a repair system which is itself radiosensitive. The lethal lesion may be in the nucleus, but whether it is restituted or not may depend on the integrity of structures in the cytoplasm. Workers at the Chester Beatty Research Institute concluded that in bacteria the site of action of some powerful radiosensitizers and radioprotectors appeared to be at or near the cell membrane. Damage immediately after irradiation of the DNA in cells, as measured by physico-chemical methods, was not altered by treatments which greatly varied the radiosensitivity in terms of lethality.

Radiation is a universal and most powerful carcinogenic and leukæmogenic agent. Without doubt, it promises to be a most valuable tool in the search for the causes of cancer. At St. Bartholomew's Hospital, lung cancers following whole-body irradiation have been examined in great detail. Those of aveolar origin are produced by relatively small amounts of irradiation and become less frequent as the dose is raised above 150 r. Thus in animals that have received 470 r. the incidence is considerably lower than in unirradiated controls. Tumours of bronchial origin are more rare, but their number increases at high doses. These results have been interpreted in terms of the radiosensitivity of cells once they have undergone the malignant transformation. Host factors cannot, however, be neglected, as is shown by the observation from the Strangeways Laboratory that radiation acts synergistically with carcinogenic hydrocarbons in inducing tumours of the genital tract of castrated rats, while for intact rats radiation reduces the incidence of chemically induced tumours.

### Clinico-pathology of Cancer

An example of the way in which expertise in a specialized field may aid an investigation in an apparently unrelated one is provided by Dent's group at University College Hospital. For nearly 20 years Dent has been a leading investigator of calcium and phosphorus metabolism, specializing in problems of renal dysfunction, parathyroid tumour, and vitamin D metabolism. He now reports that about 50 per cent of a group of patients suffering from superficial, uncomplicated squamous cell and basal cell carcinoma have a urinary calcium-level above the usually accepted limit of normality and about one-third have a low plasma phosphorus. The plasma calcium and plasma alkaline phosphatase are normal in all cases. These findings are unaltered after the tumour has been removed or treated by radiotherapy and it appears most likely that there is a metabolic predisposition to the development of skin cancer in these patients. The changes are similar to those of idiopathic hypercalciuria, but skin cancer patients, unlike idiopathic hypercalciurics, do not form renal stones. Nor is the formation of renal stones a feature of other hypercalciuric cancer patients, for example, those cases of cancer of the breast with widespread skeletal metastasis. A hypothesis that cancer patients possess a mechanism which protects them against the formation of renal stones is to be investigated: the results will be awaited with interest, since there is a great difference between the behaviour of a basal cell carcinoma, which may appear micro-anatomically as a slowly growing, locally destructive proliferating malformation of skin appendages, and the behaviour of the common mammary carcinoma, which is an anaplastic tumour. It would be interesting to know whether there were similar findings in patients with tumours which are comparable but not malignant, in patients with a tumour, at present benign, which has malignant potentialities, and so on. All manner of avenues for exploration are suggested, and there will be no shortage of material; the

bottleneck will be the time and staff required to extend these investigations.

Metabolic associations of consequences of malignancy are matters for which all workers in the cancer field should be on the *qui vive*. Many of the correlations so far recorded, for example, hypokalaemic alkalosis resulting from adrenal cortical hyperplasia in patients with carcinoma of the bronchus, diabetes mellitus and carcinoma of the body of the uterus, are difficult to explain using our present knowledge, but the patients invariably suffer metabolic disorders and investigations of these are almost certain to be fruitful.

A group of workers from the gas industry has been investigated for taste appreciation, colour blindness, right- and left-handedness, as well as the social background, in an attempt to discover whether there is a particular type of constitution which renders a man susceptible to tar warts or skin cancer.

As a result of careful trials, a group at the Royal Marsden Hospital has reported that chemotherapy (chlorambucil) has a definite part to play in the treatment of advanced ovarian adenocarcinoma. A regression was found in 60 per cent of patients treated with alkylating agents. This is a good example of empiricism in medicine, for at present it appears to be pure chance whether or not an 'irremovable' adenocarcinoma responds to irradiation, radiomimetic drugs or alkylating agents. Clinical behaviour and pathology are not useful guides and those conducting the trials, provided as they are with quite hopeless advanced cases of disease, cannot be selective until they have gained sufficient experience in the management of a group of cases. This will come rapidly with, for example, carcinoma of the bronchus, where 10-20 patients may be gathered in a few weeks, but in the case of the sarcomata, which are all relatively rare, experience in the use of drugs is hard to come by.

No significant advances in the techniques or results of regional perfusion with chemotherapeutic agents are recorded.

There is once again a first-class lesson in tumour pathology from St. Mark's Hospital, in which benign and malignant epithelial tumours of the large intestine are discussed. Each year the report from St. Mark's Hospital exemplifies the very best of scientific tradition—the painstaking, meticulous collection of data, their rearrangement, and the thought processes leading to conclusions. The methods are essentially those of surgical pathology—methods which have not significantly changed in forty years, but which continue to provide fundamental contributions to knowledge.

Nephroblastoma (Wilm's tumour) arises before the age of eight, and usually before the fourth year. Twenty-five years ago the prognosis was that 10 per cent would survive two years or more with a variety of treatments. The Children's Hospital (Great Ormond Street) between 1952 and 1960 has achieved a 47.5 per cent survival rate using a standard procedure. This consists of immediate nephrectomy by a route which permits early ligation of the renal blood vessels (to forestall dislodgement of the tumour into the blood stream as a result of manipulating the organ) followed by X-irradiation.

Gluckmann at the Strangeways Laboratories continues his clinico-pathological investigations of human carcinoma of the cervix in an attempt to avoid the blunderbuss irradiation methods at present employed, and substitute a more critical approach based on the known response of the individual tumour to an initial dose of irradiation as seen in serial biopsies. (In this context the term 'human' is really redundant since carcinoma of the cervix is virtually unknown in other species.) Those showing a favourable response were treated by radiotherapy only, and those showing an unfavourable response by radiotherapy followed by surgery.

Inevitably much of the clinical survey work is concerned with the frequent, lethal tumours. The Marie

Curie Hospital surveys its results in the management of carcinoma of the uterine cervix treated by a variety of combinations of surgery and irradiation; unfortunately the presentation is far from clear.

Bloch has shown that under mild hypothermia (30°–33° C) in men there is an increase in sensitivity of cerebral tissue to irradiation, that tumour tissue shows a relatively greater sensitivity than normal brain tissue but that life is not prolonged by adding hypothermia to irradiation in the management of brain tumours (to which can be added the fact that the results of treatment of the gliomata—primary malignant tumours arising in brain from neuroglia—except in the relatively uncommon type which can be removed surgically at the expense of a cerebellar hemisphere—are very poor).

St. George's Hospital provides a very detailed review of tumours arising from the acoustic (8th cranial) nerve. These are histologically benign tumours of the nerve sheath cells and in general are called 'neurilemmomas' or 'schwannoma'. Such tumours can affect any nerve in the body, but the 8th nerve is a site of predilection. Although this tumour is histologically benign, it has been held responsible for the death of 107 of a series of 280 cases in which it was known to be present. The tumour occurs in a notoriously inaccessible site within the skull, is hazardous to remove and virtually radioresistant.

P. ALEXANDER  
I. HIEGER  
A. L. LEVENE

## OBITUARIES

### Prof. V. A. Bailey

It may be said that Prof. Victor Albert Bailey, who died on December 7, 1964, was one of a decreasing number of physicists who lived and worked through what is now thought of as the 'heroic era' in physics.

He had the good fortune to be associated at an early age of his life with Sir John Townsend and his school in Oxford. Townsend soon discovered in his young associate a remarkable combination of mathematical facility and experimental skill. Much of the classical work on the electrical breakdown of gases was published conjointly by Townsend and Bailey.

When he accepted a chair of physics in the University of Sydney in 1924 (in 1936 this became the chair of experimental physics), Bailey immediately established a research school engaged in the elucidation of various phenomena occurring in ionized gases and other ionized media such as the upper atmosphere. Although a large number of publications arose from this work, Prof. Bailey will probably be best remembered for the elucidation of an effect observed by early wireless operators and sometimes referred to as the 'Luxemburg effect'. Briefly, it was observed that the station *Radio Luxemburg* could also be received on neighbouring frequencies but only under certain rather puzzling conditions. Bailey showed by theoretical calculations and experiments that radio waves can interfere with each other when passing through a medium such as the ionosphere, that is, they can impress their modulation eventually on to each other. In addition he showed that the effect would be considerably increased if the 'disturbing' transmitter should happen to be tuned to the so-called 'gyro-frequency' which depends on the strength of the Earth's magnetic field at the point of interference of the two waves. This gyro-magnetic resonance has long since been confirmed experimentally and developed into a standard method of investigating the properties of the ionosphere.

During the Second World War the Australian Government entrusted Prof. Bailey with the task of forming, within the University of Sydney, a school of basic scientific instruction in radar techniques for officers of the Australian armed forces. From this school several hundred graduates emerged, some of them taking regular science courses after the War. This shows that at that stage Prof. Bailey's scientific reputation was already looked on as unquestionable.

After the War he returned again to the subject of wave propagation in ionized media, taking into account more and more of the numerous variables involved in these modes of propagation, such as the relativistic motion of the charge carriers. Some of this work is considered classical in the field that is now called 'plasma physics'.

After his retirement in 1960, Prof. Bailey turned his attention to cosmology and in particular investigated the

possibility that some of the celestial bodies such as the Sun may carry permanent electrical charges of considerable magnitude. He read three papers on the subject just recently at the Galileo celebration convention in Rome. He intended to continue this work, and in fact had accepted the offer of a visiting professorship in the Department of Space Science and Applied Physics of the Catholic University of America, in Washington, D.C., for February 1965.

His death will be mourned by the great number of his colleagues, students and friends all over the world.

KURT LANDECKER

### Prof. Umberto D'Ancona

It is with very great regret that biologists learnt of the sudden death of Prof. Umberto D'Ancona on August 24. Only in the previous month he had been taking active and far-sighted part at the meeting of the International Union of Biological Sciences at Prague, and at the subsequent meeting on the International Biological Programme at Paris, where his wisdom and wide biological outlook seemed destined to exert an important influence on the future development of that Programme.

He was an outstanding example of that handful of biologists who carried and adapted the traditions of nineteenth-century evolutionary biology to the wider and very different outlook of to-day.

Born in Fiume on May 9, 1896, he first studied science at the University of Budapest. Afterwards he became an Italian citizen and transferred to the University of Rome, where in due course he became an assistant in the Institute of Comparative Anatomy under Prof. Grassi and Prof. Cotronei. From 1929 until 1936 he held the chair of zoology at Siena and then for a brief spell at Pisa. Since 1937 he has been professor of zoology at Padova and director of the Institute of Zoology.

D'Ancona took a full share in the administrative responsibilities of his University. But his enduring interest was in limnology and marine biology. The Hydrobiological Station at Chioggia was founded and directed by him, and also the Istituto Nazionale di Studi Talassografici at Venice, and he was a governor of the Marine Biological Institute of the Adriatic. In the end he was a man naturally called on by international authorities to further limnological and marine biological science. He was president of the Limnological Section of the International Union of Biological Sciences, and recently played a major part in initiating the international programme of productivity in relation to the sardine fisheries of the Mediterranean-Biscay-Channel area.

His researches were recorded in some 250 valuable papers and books. These illustrate clearly the development and expansion of his ideas. He gained a rigorous training in cytology and histology, the embryology of

fish, and by his work on sex-determination and differentiation in fish. He would turn to systematics in his work on the amphipod *Niphargus* as well as to the biology and systematics of fish, particularly of sturgeons and of eels; and these widening interests led him into his researches in hydrobiology and oceanography, and to his book *The Struggle for Existence*, well known in both the Italian original and its English translation.

He received many well-deserved honours both in his own country and in others. He was awarded the National

Prize of the Accademia dei Lincei in 1955 and the Médaille Memorial of the Fondation Albert 1<sup>er</sup> Prince de Monaco. Only last year he received the Manley Bondell Prize.

Learned and kindly, he saw what should be done and how to get things done, and how to promote international goodwill and co-operation to extend our knowledge of Nature. We offer our sympathy to his wife, herself a distinguished zoologist and his collaborator, and to his daughter, a pathologist in the University of Padova.

C. F. A. PANTIN

## NEWS and VIEWS

### Chief Scientist of the Army Department:

Mr. E. C. Cornford

MR. E. C. CORNFORD has been appointed chief scientist of the Army Department in succession to Dr. W. Cawood, who has been appointed chief scientist of the Ministry of Aviation (*Nature*, 204, 923; 1964). Mr. Cornford graduated in mathematics at the University of Cambridge in 1938 and in the same year entered the Ministry of Supply. In 1945 he joined the Guided Weapons Department of the Royal Aircraft Establishment and stayed there until 1951. During this period he was concerned not only with guided weapons development but also with weapons research, air warfare and radar aids to navigation. From 1951 until 1954 Mr. Cornford served on the Staff of the Scientific Adviser to the Air Ministry. He then rejoined the Royal Aircraft Establishment, eventually to become the head of the Guided Weapons Department. In 1961 he joined the Ministry of Defence as chairman of the Defence Research Policy Staff. Since then he has been assistant chief scientific adviser (projects) at the Ministry of Defence. Mr. Cornford took up his new appointment on January 1.

### Department of Genetics in the University of Leeds

A DEPARTMENT OF GENETICS has been established in the Faculty of Science of the University of Leeds. The new Department will be closely associated with the other biological departments in a School of Biological Sciences, a new building for which is at present being planned. It will be responsible for the elementary and advanced teaching of genetics both to specialists and to students from other departments, and the members will undertake, supervise and promote research in the subject, and co-operate with other departments in which research in genetical topics is already being undertaken.

Prof. J. R. S. Fincham

DR. J. R. S. FINCHAM, head of the Department of Genetics at the John Innes Institute, has been appointed to the chair of genetics and the headship of the new Department of Genetics in the University of Leeds. Dr. Fincham was educated at Hertford Grammar School and Peterhouse, Cambridge, where he read the Natural Sciences Tripos and gained first-class honours in Parts I and II. He was awarded an Agricultural Research Council research studentship in 1946 and spent two years in the School of Botany, Cambridge, followed by one year at the California Institute of Technology. During 1949-50 he was a Bye Fellow of Peterhouse and in 1950 he was awarded the degree of Doctor of Philosophy for his dissertation on "Genetic and Biochemical Studies on *Neurospora*". Dr. Fincham joined the staff of the Department of Botany at University College (later the University of) Leicester as lecturer in 1950 and was appointed to a readership in genetics in 1954. He has held his present post as head of the Department of Genetics at the John

Innes Institute, Bayfordbury, Hertford, since 1960. He spent the academic year 1960-61 as visiting associate professor of genetics at the Massachusetts Institute of Technology. In 1964, Dr. Fincham was awarded an Sc.D. degree of the University of Cambridge. His main research interests are in the field of microbial and molecular genetics and will complement work already in progress in the biological sciences in the University under the direction of Prof. R. D. Preston in the Astbury Department of Biophysics, and Prof. F. C. Happold in the Department of Biochemistry.

### U.S. National Academy of Engineers

A NATIONAL ACADEMY OF ENGINEERING has been formed under the charter of the U.S. National Academy of Sciences. Acting under the authority granted by its Congressional Act of Incorporation of 1863, the National Academy of Sciences has approved Articles of Organization which bring the National Academy of Engineering into being as part of its own structure, operating on an autonomous and parallel, but co-ordinated, basis. The new Academy will share in the responsibility of the National Academy of Sciences of advising the Federal Government, on request, in all areas of science and engineering.

The adoption by the National Academy of Sciences of the Articles of Organization of the National Academy of Engineering brings to a climax the discussions which began in 1960 when the Engineers Joint Council, in co-operation with the Engineering Foundation and the Engineers Council for Professional Development, and with representatives from the National Academy of Sciences and the National Research Council, appointed a committee to make an intensive examination of the need and feasibility of an engineering academy. The President of the National Academy of Sciences will sit *ex officio* on the Council of the National Academy of Engineering, and the National Academy of Engineering will, in turn, have *ex officio* representation on the Governing Board of the Academy-Research Council. Concerning membership of the National Academy of Engineering, the Articles of Organization state that, in addition to the primary qualifications of important contributions to engineering theory and practice or unusual accomplishment in technology: "A candidate for membership shall be recognized by his associates and others for his professional integrity, as well as for his engineering accomplishments. Effectiveness and efficiency in leadership of organizations that have conducted pioneering or complex programs, have made noteworthy contributions to the field of engineering education, should be weighed as supplementing the primary qualifications . . ."

### Aims and Objectives

THE aims and objectives of the National Academy of Engineering, as formulated by the committee appointed by Dr. F. Seitz and set forth in the Articles of Organization



for the National Academy of Engineering, are: (1) To provide means of assessing the constantly changing needs of the United States and the technical resources that can and should be applied to them; to sponsor programmes aimed at meeting these needs; and to encourage such engineering research as may be advisable in the national interest. (2) To explore means for promoting co-operation in engineering in the United States and elsewhere, with the view of securing concentration on problems significant to society and encouraging research and development aimed at meeting them. (3) To advise the Congress and the executive branch of the Government, whenever called on by any department or agency thereof, on matters of national import pertinent to engineering. (4) To co-operate with the National Academy of Sciences on matters involving both science and engineering. (5) To serve the nation in other respects in connexion with significant problems in engineering and technology. (6) To recognize outstanding contributions to the nation by leading engineers.

#### Officers

At a meeting held on December 10, by-laws were adopted and the following officers were elected: *President*, Dr. A. B. Kinzel, vice-president (research), Union Carbide Corporation; *Vice-President*, Dr. E. A. Walker, president, Pennsylvania State University; *Secretary*, Dr. H. Work, director of the Research Division and Associate Dean, School of Engineering and Science, New York University; *Treasurer*, Dr. T. C. Kavanagh, Praeger-Kavanagh-Waterbury, Engineers-Architects. The founding membership of the National Academy of Engineering has been formed from a committee of twenty-five appointed by Dr. F. Seitz, president of the U.S. National Academy of Sciences (see *Nature*, 202, 750; 1964), on nomination by the Engineers Joint Council to deal with the primary organizational tasks of the new Academy.

#### Charter Members

THE twenty-five charter members of the National Academy of Engineering are: H. W. Bode, vice-president, Bell Telephone Laboratories, Inc.; Walker L. Cisler, chairman of the Board, Detroit Edison Co.; Hugh L. Dryden, deputy administrator, National Aeronautics and Space Administration; Elmer W. Engstrom, president, Radio Corporation of America; William L. Everitt, dean, College of Engineering, University of Illinois; Antoine M. Gaudin, Richards professor of mineral engineering, Massachusetts Institute of Technology; Michael L. Haider, president, Standard Oil Co. of New Jersey; George E. Holbrook, vice-president, E. I. du Pont de Nemours and Co., Inc.; J. Herbert Holloman, jun., Assistant Secretary of Commerce for Science and Technology; Thomas C. Kavanagh, Praeger-Kavanagh-Waterbury, Engineers-Architects; Augustus B. Kinzel, vice-president (research), Union Carbide Corporation; James N. Landis, vice-president, Bechtel Corporation; Clarence H. Linder, former vice-president and group executive, General Electric Co.; Clark B. Millikan, professor of aeronautics, California Institute of Technology; Nathan M. Newmark, head, Civil Engineering Department, University of Illinois; W. H. Pickering, director, Jet Propulsion Laboratory, California Institute of Technology; Simon Ramo, executive vice-president, Thompson Ramo Wooldridge, Inc.; Arthur E. Raymond, consultant, RAND Corporation; Thomas K. Sherwood, professor of chemical engineering, Massachusetts Institute of Technology; J. A. Stratton, president, Massachusetts Institute of Technology; C. G. Suits, vice-president and director of research, General Electric Co.; F. E. Terman, provost and vice-president, Stanford University; Charles Allen Thomas, chairman of the Board, Monsanto Chemical Co.; Eric A. Walker, president, Pennsylvania State University; and Ernst Weber, president, Polytechnic Institute of Brooklyn.

#### Part-time Course in Chemical Engineering in Britain

NOTWITHSTANDING the present-day shift in emphasis towards full-time education for all capable of being educated, the part-time course, which is a tradition in the technological ways of life in Britain, continues to flourish. In 1962-63, for example, there were 108,000 advanced students taking part-time courses, 53,000 in the day and 55,000 in the evening, as compared with 43,000 full-time advanced students in institutions of further education. More than ten years have elapsed since the Institution of Chemical Engineers first published its *Scheme for a Part-time Course in Chemical Engineering*. The purpose of that publication was to encourage the establishment of Higher National Certificate courses in chemical engineering, and a number of such part-time courses have since been in successful operation. The Institution (in co-operation with the Joint Committee for Higher National Certificate in Chemical Engineering in England and Wales) has now published a completely revised scheme designed for the boy or girl leaving school who wishes to become a chemical engineer by means of part-time study (Pp. 20. London: The Institution of Chemical Engineers, 1964). The student who completes the course for the Higher National Certificate will be considered in future to have qualified academically as a chemical engineering technician. The more ambitious student can proceed, through the endorsement course and Part 3 (Design Problem) of the Institution examination, to become a fully qualified professional chemical engineer, provided he or she can satisfy the experience requirements for corporate membership of the Institution. Copies of the scheme can be obtained free of charge from the Institution of Chemical Engineers, 16 Belgrave Square, London, S.W.1.

#### The Future of the National Parks

THE *Report and News Letter of the Friends of the Lake District* for August 1964, to which are appended the full text of the pamphlet *Traffic in the Lake District* (see *Nature*, 204, 508; 1964) and the statement on the report on future water resources made to the Manchester Corporation by their engineering consultants and issued in April 1964, makes some comments on the future of the National Parks in general (Pp. 21. Ulverston: Friends of the Lake District, 1964). It is urged that any attempt to cater for the more gregarious forms of recreation in National Parks must run counter to their essential character and function, and provisions for such forms of recreation should be made elsewhere. Attention is also directed to the way in which the new large-scale agricultural development also clashes with its surroundings and calls for much more effective control for agricultural buildings than exists at present. On water supply it is urged that the only real protection for the Lake District against further extraction lies in the development of alternative sources of supply and points out that in future the costs of desalinated sea-water are likely to match those of fresh water very closely. (See also p. 113 of this issue of *Nature*.)

#### Scottish Wildlife Trust

THE Scottish Wildlife Trust has recently been established to help to secure conservation in Scotland. The chairman is Sir Charles Connell. The new Trust will act as a co-ordinating body and will endeavour to do on a smaller scale what the Nature Conservancy is doing on a national scale. One of its functions will be to establish wildlife reserves on a small scale throughout Scotland. There are many places in Scotland where particular species occupy a restricted habitat, always in danger of being lost through ignorance or carelessness. There are also areas of special interest, particularly cliffs and forests, which the Trust hopes to protect from exploitation inimical to the maintenance of an attractive countryside and the conservation of its flora and fauna. Further information

and terms of membership can be obtained from the Secretary at 21 Regent Terrace, Edinburgh 7.

### Mutation Research; a New Journal of Genetics

THE increase in the bulk, and in the specialization, of research in the field of genetics is reflected in the appearance of another new journal, *Mutation Research*, an international journal on mutagenesis, chromosome breakage and related subjects (Amsterdam: Elsevier Publishing Co.). The editor is Prof. F. H. Sobels, of the University of Leiden, assisted by a large international editorial board which includes most of the active workers in this field. The first issue, dated May 1964, contains 10 major papers ranging from viruses to mice and generally of a high standard; the first paper, most appropriately, is by H. J. Muller whose fundamental discovery, in 1927, of the mutagenic action of X-rays inaugurated the whole field of mutation research. The volume will comprise approximately 600 pages; the annual subscription is £6 10s.

### Reproduction of Mammals

THE Eighth Oliver Bird Lecture given in London on November 23 by Dr. Alan F. Guttmacher, on intra-uterine devices used to control human fertility, was followed by a three-day international symposium on the "Comparative Biology of Reproduction in Mammals", held at the Zoological Society of London. The symposium was organized jointly by the Zoological Society and the Society for the Study of Fertility, and supported financially by the World Health Organization. A large proportion of the twenty-eight invited speakers came from overseas and included many distinguished biologists from the United States, the Commonwealth countries and Europe. Some of the topics were of a general nature, such as the evolutionary trends in the physiology of reproduction and the factors controlling the gestation period. The majority of the papers, however, dealt with particular aspects of the reproductive process in the male or female of a particular species or group of mammals. A complete session, at which five papers were read, was devoted to the consideration of reproduction in marsupials. To make the proceedings of the symposium available as soon as possible to all interested workers, abstracts of the papers will be published in the *Journal of Reproduction and Fertility* in the early part of this year and the full papers will appear later in the *Symposia of the Zoological Society of London*. Midway through the programme the overseas speakers visited the newly established Wellcome Institute of Comparative Physiology in Regent's Park. At its conclusion, Dr. Carl G. Hartman was presented with the first Marshall Medal of the Society for the Study of Fertility, by its chairman, Prof. A. S. Parkes. Looking back on these meetings, one was struck not only by the great volume of work that is being done on this subject at the present time, but by the enormous amount that is still to be learned before anything like a fully comprehensive picture can be presented. Dr. Guttmacher's lecture underlined the problems of population control, and the symposium did much to reveal the potential value of comparative studies in the regulation of the reproductive process of mammals.

### Radiation Preservation of Foods

IN Russia and Canada, potatoes are already being irradiated for commercial purposes and, following the recent Ministry statement on the subject, it is quite possible that food preservation by irradiation may begin in Britain very soon. Those interested in radiation preservation of food should read the report *Radiation Preservation of Foodstuffs* (Second Scandinavian Meeting on Food Preservation by Ionizing Radiation, Stockholm, September 9-11, 1963. Arranged by the Royal Swedish Academy of Engineering Sciences. Edited by Per-Olof Kinell and Vera Runnström-Roio. IVA Meddelande Nr.

138. Pp. 87. Stockholm: Ingeniörsvetenskapsakademien, 1964, 25 kr.). Five of the papers in the report are concerned with irradiation facilities, source strength, dose measurement, and dose distribution. The electron linear accelerator at Risø, Denmark, has been operated since 1961 for the industrial radiation sterilization of disposal surgical supplies at the rate of about six tons a month. In conventional heat treatment of canned foods the centre of the can must, of necessity, receive less severe heat treatment than the periphery. This problem can be overcome to some extent with radiation treatment by irradiating the cans from both ends.

One of the chief problems after sprout inhibiting or radurization doses is the recontamination of the food with micro-organisms. This increased susceptibility to storage rot is due either to: (1) tissue damage facilitating the access of micro-organisms; (2) residual radiation-resistant organisms now deprived of competitors; or (3) a combination of (1) and (2). Radiation can affect the wound healing mechanisms of plants to such an extent that flora which are normally harmless can become an important cause of storage rot. After irradiation, therefore, foods must be stored under refrigeration as close as possible to 0° C. *Aureobasidium pullulans*, also known as *Pullularia pullulans* or black yeast, is a very widely distributed fungus which is known to cause damage to wounded soft fruits in the United States. This organism has been shown to be very radiation resistant. The report also discusses the irradiation of fruit juices. The best method of preservation appeared to be irradiation with 0.5 Mrad, storage for a day at room temperature, and then heat treatment at 50° C. The yeasts were severely attacked both by the radiation and the heat treatment. When semi-preserved fish (tidbits) were irradiated, it was found that 300 krad was the highest acceptable dose from an organoleptic point of view.

There is a description of the use of ultra-violet radiation in the food industry, especially for meat storage when it is used to prevent the growth of psychrophilic bacteria. By irradiating either crystalline or dissolved D-glucose with 10 Mrad a substance toxic to *Pseudomonas* has been produced.

### Research in Mental Health

THE Schizophrenia Research Fund has been established to support research into problems connected with mental illness in general and schizophrenia in particular. The fund is administered by a Board of Trustees, and Mr. L. Kelly (Schizophrenia Research Fund, City Gate House, Finsbury Square) is acting as secretary. Initial impetus has been given to the fund by a gift of £50,000 from the Rothschild family, and the establishment of a Schizophrenia Research Fellowship, to which Dr. D. Straughan has been appointed. Dr. Straughan, who is at present at the Institute of Animal Physiology, Babraham, Cambridge, will work in the Department of Psychological Medicine in the University of Edinburgh. The fund will provide greater security of tenure than has hitherto been possible in research in this field, and Dr. Straughan's initial contract is for seven years. His work at Babraham has been concerned with pharmacological aspects of mammalian brain physiology, and he will concentrate on the biochemical basis of schizophrenia. It is hoped that this initial effort will attract interest in, and support for, work in the immense field of research bearing on the problems of mental health.

### The Royal Society and Nuffield Commonwealth Bursaries Scheme

AWARDS under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows: Dr. S. I. Ali, lecturer in botany, University of Karachi, to enable him to study taxonomic problems of Leguminosae of West Pakistan at Kew and the British

Museum (Natural History); Dr. R. G. R. Bacon, reader in organic chemistry, Queen's University, Belfast, to assist him to gain information on the application of new instrumental methods, particularly nuclear magnetic resonance and mass spectrometry, to the study of complex products from organic oxidation-reduction reactions, at Melbourne; Dr. A. J. Cawley, associate professor, division of small animal medicine and surgery, Ontario Veterinary College, to enable him to learn research techniques in the study of bone and bone diseases of both man and animals, at Bristol and Oxford; Prof. I. M. Goodbody, professor of zoology, University of the West Indies, to enable him to visit British Columbia from April to June 1965, to extend his knowledge of living ascidians (Tunicata) and their ecology by a study of Pacific coast species, and with particular reference to the ecology of closely related species; Dr. Janet E. Harker, university lecturer in zoology, Cambridge, to enable her to extend her studies on malignant cell proliferation, caused by upsetting the time-control of endocrine secretion, by working on the relationship between this phenomenon and the time-control of regeneration, at the University of New England, Armidale; Dr. H. H. Huang, lecturer in physical chemistry, University of Singapore, to enable him to study the apparatus and techniques used in the field of the Kerr effect, at Sydney; Dr. R. Kretz, senior lecturer in geology, University of Queensland, to enable him to obtain data on the diffusion of alkali elements in silicate crystals and especially in natural aggregates of silicate crystals, to elucidate the kinetics of mass transfer and solid-state reactions in metamorphic rocks, at the Imperial College of Science and Technology, London; Dr. A. Lazenby, university lecturer in agricultural botany, Cambridge, to assist him to study some problems in techniques in selection criteria in grass-breeding; Dr. A. W. J. Lykke, lecturer in pathology, University of Adelaide, to assist him to study experimental techniques applied to the study of inflammation, at St. Bartholomew's Hospital Medical School, London; Dr. Enid A. C. MacRobbie, university demonstrator in botany, Cambridge, to enable her to visit Australia and to work, at Adelaide, on light effects on ion transport in *Nitella* and to learn the techniques used there in the study of ion transport in plant mitochondria; Dr. D. C. T. Pei, assistant professor of chemical engineering, University of Waterloo, Ontario, to enable him to spend about one year, commencing May 1965, with the applied heat group at the Imperial College of Science and Technology, London; Dr. C. R. Twidale, senior lecturer in geography, University of Adelaide, to assist him to study the weathering and landforms of the granite areas of south-western England, at Exeter.

### Mass Spectroscopy

A MASS SPECTROSCOPY GROUP has been set up for the principal purpose of arranging regular open meetings on the subject. The committee consists of: Dr. J. H. Beynon (Imperial Chemical Industries, Ltd., Manchester) (chairman); Dr. J. Cuthbert (United Kingdom Atomic Energy Authority, Harwell); Prof. A. Maccoll (University College, London); Prof. W. C. Price (King's College, London); Dr. J. D. Waldron (Associated Electrical Industries, Ltd., Manchester); R. M. Elliott (Associated Electrical Industries, Ltd., Urmston) (secretary). A meeting organized by the Group will be held at University College, London, during September 21-22. The meeting will be open to all and will cover all aspects of mass spectroscopy. Those interested are invited to submit titles of short papers on present work before February 26. The Committee will also be grateful to receive suggestions for topics of special interest which might be given prominence or generally discussed at the meeting. Further information can be obtained from the secretary, R. M. Elliott, Associated Electrical Industries, Ltd., Scientific Apparatus Department, Barton Dock Road, Urmston, Lancs.

### Churchill College, Cambridge :

#### Junior Research Fellowships

CHURCHILL COLLEGE offers at least three junior research fellowships in arts, mathematics, science or engineering. Applicants must be under the age of thirty on July 1, 1965, and not have spent more than four years in research work by that date. Candidates should apply to the Senior Tutor, Churchill College, Cambridge, for an application form, which must be returned by February 1, or submit a copy of a thesis by March 1. The junior research fellowships are for an initial period of three years and are of the value of £750 a year. An accommodation allowance of £250 and a children's allowance of £100 for each child are also paid to Fellows not resident in College.

#### Gulbenkian Fellowship

THE College also offers a Gulbenkian Fellowship which is open to graduates of any university other than those in the United Kingdom who wish to carry out advanced work, and is of the same value as the junior research fellowships. The tenure is normally for one year but may be extended. Applicants must be under the age of thirty on July 1, 1965, and not have spent more than four years in research work by that date. Applications should be submitted in the same way as for junior research fellowships.

### Announcements

PROF. JONATHAN W. UHR, director of the Irvington House Institute for Rheumatic Fever and Allied Diseases, and associate professor of medicine, New York University School of Medicine, was awarded on December 28 the Newcomb Cleveland Prize, the senior award of the American Association for the Advancement of Science, for his paper entitled "The Heterogeneity of the Immune Response". The prize, including an award of 1,000 dollars and a medal, is awarded annually for an outstanding scientific contribution to the author of a paper given at the previous year's meeting of the American Association for the Advancement of Science.

THE Pacific Science Center announces the institution of the Arches of Science Award, made possible through the financial support of the Pacific Northwest Bell Telephone Co., and consisting of a gold medal and a cash prize of 25,000 dollars. It will be awarded in 1965 to a citizen or a resident of the United States in recognition of "outstanding contributions to the better understanding of the meaning of science to contemporary man". Further information may be obtained from Pacific Science Center, Inc., 200, 2nd Avenue N., Seattle, Washington 98109.

THE sixth International Soil Mechanics Conference will be held in Montreal during September 8-15. Further information can be obtained from the secretary of the Organizing Committee, M. K. Ward, c/o National Research Council, Ottawa 2.

THE eighth European Congress on Molecular Spectroscopy will be held in Copenhagen during August 14-20. Further information can be obtained from H. C. Ørsted Institute, Chemical Laboratory Five, 5 University Park, Copenhagen Ø.

THE annual symposium on "Molecular Structure and Spectroscopy" will be held in the Ohio State University, Columbus, during June 14-18. Further information can be obtained from Prof. K. Narahari Rao, Molecular Spectroscopy Symposium, Department of Physics, Ohio State University, 174 West 18th Avenue, Columbus.

AN international conference on "Electron Diffraction and the Nature of Defects in Crystals" will be held in Melbourne during August 16-21. Further information can be obtained from Dr. R. I. Garrod, Aeronautical Research Laboratories, Box 4331, G.P.O., Melbourne, Victoria.

## THE ORDNANCE SURVEY

"I AM told," said Robert Louis Stevenson, "that there are people who do not care for maps, and I find it hard to believe." As if in substantiation of his belief, the Ordnance Survey during 1963-64 printed no less than 4 million maps and issued 3 million for sale, the highest total ever in one period of twelve months. Of these, the incomparably popular *One Inch to One Mile Series* accounted for almost half, the *Quarter Inch Series* for another quarter. In all, there are now just less than 100,000 different present-day sheets on all scales, and most of the small-scale series (one inch and smaller) are complete. The bulk of the outstanding programme comprises the 1:1,250 and 1:2,500 plan series, each of which is being produced at an annual rate of several thousand sheets, though so vast is their coverage that 150,000 are still needed. Moreover, the extent and rapidity of new building, motorway construction and other developments over the changing face of Britain necessitate a perpetual programme of revision, according to a careful scheme of priorities. This new material is sometimes required so urgently by public users that the Ordnance Survey makes it available through its Advance Revision Information Service.

Apart from this steady long-term progress, 1963-4 saw the appearance of a number of individual sheets of special interest. These include the beautiful map of Hadrian's Wall, plotted on a modern topographical base-map on a two inches to a mile scale, and a composite sheet of the Isles of Scilly on the 1:25,000 scale. A two-sheet edition of a *Route Planning Map*, in six colours, on a scale of 10 miles to an inch, has proved to be of such value to road-users, with its wide range of supplementary information, that it has already been reprinted three times since its first appearance in January 1964. New editions of the attractive *Tourist Maps* of the Lake District, the North York Moors and the Peak District have appeared, joined by a new recently published Cairngorms sheet which reflects the growing popularity of this mountain group as a ski-ing and climbing area. Preliminary work has been carried out on a proposed tourist map of the Cambridge district.

All this has been accomplished by a total personnel of only 4,500, whose headquarters in the near future will be the fine building under construction in Southampton. The Ordnance Survey first came to that town in 1841, following a fire in the Tower of London which had

destroyed its premises. A century later, though Southampton still remained the centre of the drawing and printing activities of the Ordnance Survey, the administrative headquarters were transferred to Chessington in Surrey. When the new building is completed, the disadvantages of scattered and inadequate accommodation will be overcome, and the Ordnance Survey will have returned home.

The accomplishment of so much with such a relatively small personnel has been practicable only by the constant introduction of technological improvements: the extension of aerial photography (though badly hindered in 1963-64 by long periods of poor flying weather), the acquisition of new stereo-plotting machines to make use of the photographs, the use of electromagnetic distance-measuring equipment (by this means precise traverses were measured from Dover to Cape Wrath and from Land's End to East Anglia), the trials of an automatic stereocomparator using punched cards, the installation of automatic reading planimeters for measuring areas on the 1:2,500 plans, and new economical methods of multi-coloured printing.

One of the most interesting achievements has involved the completion of a new geodetic connexion across the English Channel, in conjunction with the Institut Géographique Nationale. Both angular and electromagnetic distance measurements were made from points near the coast of the Isle of Wight, Portland Bill and the Cotentin peninsula, with the collaboration of the Royal Corps of Signals and a Hydrographic Survey Ship in mid-Channel. Similar enterprises in recent years have linked the geodetic systems of Scotland and Scandinavia, using radar and a high-flying aircraft, and have tied Rockall into the triangulation network.

The Ordnance Survey's annual report for 1963-64\*, with a few pages of text, numerous statistical tables, and some 'progress maps', summarizes in prosaic form a wealth of fascinating cartographical activity. A gross expenditure of almost £5 million was partially offset by receipts of nearly £1½ million; the balance has indeed been spent to good account. In the words of the Director General, this work is "... not only of the highest importance but also of abundant and abiding interest to those who do it".

F. J. MONKHOUSE

\* The Ordnance Survey Annual Report, 1963-64. Pp. 11 + 12 appendix + 9 plates. (London: H.M.S.O., 1964.) 6s. net.

## EARTH SCIENCES IN THE U.S.S.R.

THE following account is based on articles in the journal *Priroda*. A general review of the whole field of present-day research is provided by D. I. Shcherbakov (the editor of *Priroda*) and A. L. Yanshin (1, 44; 1963), who discuss geochronology, biostratigraphy, palaeomagnetism, tectonics, tectonic maps, spores and algae of Precambrian age, ore deposits and methods of survey and prospecting. The last subjects are also discussed by M. F. Grin (7, 2; 1954), who describes exploration in search of petroleum, natural gas, coal, phosphorites and iron ores.

Geological cycles and tectonics are discussed by N. F. Balukhovskiy (2, 54; 1963), E. D. Sulidi-Kondratiev and V. V. Kozlov (1, 102; 1964), G. P. Tamrasyan (1, 107; 1964) and D. A. Frank-Kamentsky (1, 110; 1964). A. I. Rybin (7, 87; 1964) discusses the causes of the deep-seated gravitational tectogenesis and outlines a hypothesis which would link-up deep-seated movements of the Earth's crust with the Earth's contraction and the phenomenon of isostasy.

Geochemistry, a branch of science highly cultivated in the Union, is represented by the articles of A. A. Drobkov (8, 45; 1963), who discusses chemical elements present in the living matter; V. V. Kovalsky (3, 44; 1964) discusses geochemical ecology in living matter, while A. I. Perel'man (5, 8; 1964) proposes a geochemical classification of chemical elements based on their abundance, distribution and migration capacity in the outer geospheres, introducing a new term "coefficient of hydrous migration". Finally, L. S. Tarasov (8, 3; 1963) most ably presents the hypothesis of the formation of geospheres as proposed by A. P. Vinogradov and his collaborators. According to this hypothesis the original Earth in its composition was similar to the composition of chondrite meteorites, and in the course of time, under the action of localized radiogenic heat, a process analogous to that of 'zone melting' in metals separated the volatile-enriched portions from the refractory components. Laboratory experiments of zone melting of a chondritic meteorite showed the

'frontal' zone enriched in silica and the 'rear' zone enriched in olivine. Applying this process to the primitive Earth one would postulate a degasification of the original material and formation of the atmosphere, hydrosphere and lithosphere through geochemical migration of elements.

Methods of study are discussed by V. N. Florovskaya and L. I. Ovchinnikova (11, 69; 1963), who describe the study of coals, bitumens and fossil plants by means of the 'luminescent microscope', using ultra-violet light. Ya. L. Blikh and V. M. Bondarenko (9, 85; 1964) describe

a 'deep-seated' geological survey method, measuring the intensity of cosmic rays in boreholes and mines.

Among accounts of new discoveries is the article by V. P. Solonenko (9, 102; 1964), who describes a newly discovered volcanic region in Eastern Siberia, in which 15 volcanoes of Quaternary age have been mapped and numerous traces of earthquakes recorded. This region is centred on the Udokan ridge situated between the rivers Vitim and Olekma, both tributaries of the River Lena. Siberian mosses of the Permian era, first discovered by M. F. Neiburg in 1941, are described by S. V. Meien (5, 73; 1963).

## TWENTY-FIVE YEARS OF CRUDE OIL PRODUCTION IN GREAT BRITAIN

IN 1939 production of crude petroleum from English oilfields amounted to 3,145 tons; in 1963 the total was 122,764 tons. After a quarter of a century it is interesting to take stock of the enterprise and expertise which have made possible this progress, humble though it may seem when measured by overseas standards. This has recently been done by R. G. W. Brunstrom of the Exploration Department, British Petroleum Co., Ltd., London, in an article entitled "BP's First Quarter Century of Crude Oil Production in Britain" (*BP Magazine*, No. 13, 1964; London). Actually, the first oilfield discovered in Britain was at Hardstoft, Derbyshire, in 1919, drilled for the then Ministry of Munitions, which ceased further explorations for oil in 1922. For various reasons private companies did not pursue the quest until some years later; it was, in fact, the Petroleum (Production) Act, 1934, which created the fillip to industrial enterprise in oil finding. This act vested in the Crown the ownership of all mineral oil not discovered to that date; it supplied just the attractive operating conditions under which oil companies felt justified in spending energy, time and particularly large sums of money in the search for oil in Britain. British Petroleum pioneered this new phase by drilling wells in the southern counties between 1936 and 1938, for example at Portsdown, near Portsmouth, and in Dorset, but with no success. But in the latter year natural gas was struck at Cousland, Midlothian; early in 1939 it was found at Eskdale, Yorkshire. A freak find was that of a shallow field at Formby, near Southport, Lancashire, in May 1939, which has produced more than 9,800 tons of crude oil since then, but with no geological encouragement of commercial extension. Soon after Formby came the well-known discovery of oil at Eakring, Nottinghamshire, a significant turning point in this history. "Eakring, apart from being a significant oilfield in its own right, was

immediately seen to be the first of a group of similar fields awaiting discovery. Formby brought hope of further success, but Eakring brought certainty." Eakring owed much to geological acumen at the time; coal mine and borehole evidence, confirmed by seismic refraction surveys, helped to establish it and, by continuation particularly of the geophysical technique, other discoveries in that region were made—Kelham Hills, 1941, Dukes Wood, 1941, Caunton, 1943. A small field discovered at Nocton, south of Lincoln, in 1943 yielded 40 tons of oil, then went to water. Although numerous exploration wells were drilled during the ensuing 10 years, they were not successful until a field was discovered at Plungar, Leicestershire, in 1953. A technique known as 'secondary recovery', that is, injection of water through wells drilled around the periphery of an oilfield, driving oil towards the production wells by a water flood, had been successfully employed to arrest decline in production in the Eakring group, and by 1953 had become standard practice in most British fields. The discovery of Egmont oilfield in 1955 started another bout of exploration activity which has continued to the present time. "An average of one new oilfield has been found in the East Midlands in each of the last seven years, and three new gas fields have been found but are not yet in production. The new fields are of varying sizes, and the largest, Gainsborough, is probably as big as Egmont." One particularly gratifying success is recorded from Kimmeridge, Dorset, where a 943-ft. well was drilled in 1937 and abandoned as a dry hole; a well drilled to 1,791 ft. near the same site in 1959 found oil and thus the Kimmeridge oilfield came into being. It is noteworthy that: "Up to the present there have been only three non-BP oil wells in Britain—one at Hardstoft and two at the small Esso field named Midlothian, near Dalkeith, Scotland".

## ARID ZONE FORESTRY

THE subject of afforestation and reforestation in arid zones is dealt with comprehensively in a publication entitled *Tree Planting Practices for Arid Zones\**, which has recently been revised by Dr. A. Y. Goor for the Food and Agriculture Organization of the United Nations.

About one-third of the world's land surface lies in the arid and semi-arid zones where the annual rainfall is less than 24 in. The arid zone, with less than 12 in. of rainfall a year, includes some of the great deserts of the world, but it is in parts of the semi-arid zone that afforestation and reforestation schemes have been and are being tried. These schemes and others aimed at improving the natural tree growth can make a useful contribution to the general

welfare of the peoples by bringing to localized areas a lessening of wind erosion, a reduction of evaporation from the soil and of plant transpiration, and in fixing moving sand, arresting gully and sheet erosion, in providing aesthetic benefits and in supplying timber and other products. But where the forester is called on to try to bring about these improvements through trees, he is usually faced with a set of climatic and edaphic factors which does not make his task an easy one. Very often he has to deal with soils that have been degraded by over-grazing, fire or over-cutting. He is often very restricted in the choice of species for planting and has to rely on a limited number of drought-resistant ones. The forester working in these conditions will find this handbook very useful, for it includes methods of seed collection and its handling, nursery and planting techniques. Perhaps some

\* Food and Agriculture Organization of the United Nations. FAO Forestry Development Paper No. 16: *Tree Planting Practices for Arid Zones*. Pp. xii+233+2 maps. (Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1963.) 15s.; 3 dollars.



reference might have been made to the use of *Opuntia* or some other live hedging for nurseries.

The last two chapters on plantations are interesting, and irrigation is dealt with in some detail. Afforestation in such conditions is usually thought of as being uneconomical and written off as necessary protective measures on difficult sites. However, there is a body of opinion which considers that trees have sometimes been planted for protection of a watershed when, in the particular circumstances, a well-managed pasture would have provided just as much protection and possibly, in an arid zone, allowed more water to filter down to the valleys below. Indeed, it has now been demonstrated in some arid regions that forest trees established on better soils than has been

usual to allocate to this type of work in the past are providing favourable returns on the investment. In fact, forestry has proved itself to be the most economic of the various land uses possible. But why should these plantations be given the special name of "forest tree orchards"?

There are two appendixes on tree species used in arid zone afforestation and on seed collection, extraction, storage and pre-treatment practices, also listed by species. The two maps show the distribution of arid homoclimates for the eastern and western hemispheres. The list of selected references is adequate, but the more recent *Exotic Forest Trees of the British Commonwealth* (Streets, Oxford University Press, 1962) should have replaced Troup's publication with a similar title. C. J. TAYLOR

## NEW FOSSIL APES FROM EGYPT AND THE INITIAL DIFFERENTIATION OF HOMINOIDEA

By PROF. E. L. SIMONS

Department of Geology, and Peabody Museum, Yale University, New Haven, Conn.

THE third Yale Paleontological expedition to Egypt (November 1963–March 1964) supported by a U.S. National Science Foundation grant in geology (P-433) concentrated fossil-collecting efforts on a locality (I) in the upper levels of the Qatrani Formation, Oligocene of Egypt, which had yielded a few surface finds of early Anthropoidea in the previous season (January 1963). Extensive excavation at this quarry last winter, primarily during December and January, produced a considerable series of continental vertebrates, particularly rich in primates. Eleven mandibles and more than thirty isolated teeth of primitive Anthropoidea were recovered in addition to two jaws found in the previous season.

These finds add considerably to our understanding of the origins of Old World Higher Primates including, potentially, the ancestors of man at that period. Among these new primate materials are several interesting juvenile specimens of *Apidium phiomense* Osborn<sup>1</sup>; another represents a new large species of *Parapithecus*. The primary purpose of this article, however, is to describe briefly two wholly new and somewhat unexpected genera and species of Primates which were recovered in the collections from Quarry I. These and some 90–100 other Fayum primate specimens from lower horizons are to be illustrated and discussed by me in a forthcoming monograph on early Cainozoic mammalian microfaunas of the Fayum. However, in view of widespread interest in higher primate origins it seems advisable to record taxonomically at the present time the two most significant new species.

### Geological Horizon

The locality which yielded the new primates described here is appreciably higher stratigraphically than the classic fossil-vertebrate localities of the Fayum region, and is approximately that of the "upper level" of Osborn<sup>1,2</sup>. From this upper fossil wood zone came specimens which Osborn described as the types of a rodent *Metaphiomys beadnelli*, a primate *Apidium phiomense*, and a creodont carnivore *Metasinopa fraasi*. Evolutionary advances in the fauna at Quarry I, compared to those of the lower levels, suggest that considerable time elapsed between accumulation of these faunas, but at present it is not possible to say more than that most species appear to be different from those of the lower zones. For example, examination of Fayum rodents by A. E. Wood, soon to be published, have shown that a common rodent of this upper level, *Metaphiomys beadnelli*, is not present at the next lowermost Quarry (G) in the section, but that a

smaller, more primitive species of *Metaphiomys* that could be ancestral to *M. beadnelli* occurs in Quarry G. Similarly at Quarry G a small species of *Apidium*, *A. moustafai*, is abundant. *A. moustafai* is almost certainly ancestral to the much larger *A. phiomense* of the Quarry I level. Apparently the type specimen of the archaic hominoid primate *Propithecus haeckeli* Schlosser<sup>3</sup> came from about the level of Quarry G, or even lower in the section. For a left  $M_1$ , indistinguishable from that of *P. haeckeli*, has been recovered from Quarry G. Other teeth of *Propithecus* sp. from Quarry G are appreciably larger and somewhat different in morphology from those of the type species, *P. haeckeli*. At the Quarry I level no teeth of *Propithecus haeckeli* have been found, but instead only those of a generally larger and more advanced primate species. Taken together these evolutionary differences between the faunas of the two upper quarries, G and I, their stratigraphical separation by more than 250 ft. of varying, partly cyclothemmic sediments, including riverine sands, marine limestones and marls, indicate that the fauna of Quarry I represents the latest known Oligocene fauna of Africa and one which is distinctly different from the classic Fayum faunas examined by Andrews<sup>4</sup> and Schlosser<sup>3</sup>.

### Systematics

Order, PRIMATES

Superfamily, HOMINOIDEA

Family, Pongidae

Genus *Aegyptopithecus*<sup>5</sup>, gen. nov.

Type: *Aegyptopithecus zeuxis*<sup>5</sup>, new species.

**Generic characters:** Lower dental formula 2.1.2.3, size approximately that of a gibbon and 25 per cent larger than the type of *Propithecus* in most comparable measurements. Differs from its contemporary *Propithecus* in showing relatively larger canine, premolar heteromorphy and relatively larger  $M_2$  and  $M_3$ . Resembles *Proconsul* in marked molar size increase posteriorly.  $M_1 < M_2 < M_3$ , but differs from members of the latter genus, *Pliopithecus* and *Dryopithecus*, in possessing a more triangular  $M_3$ , narrowing posteriorly; unlike most *Proconsul*, entoconid and hypoconulid (rather than hypoconid and hypoconulid) joined by distinct crest. Resembles *Dryopithecus* in rounded outline of  $M_{1-2}$  but unlike later dryopithecines retains lower and more rounded molar cusps, as in *Propithecus*. Ascending ramus of mandible approximately 40 per cent broader from front to back compared with depth of horizontal

ramus at  $M_2$  than in *Propithecus*; ascending ramus of mandible more vertical relative to the tooth row than in *Propithecus* or *Proconsul*, probably indicating comparatively short face.

While resembling *Propithecus*, *Aegyptopithecus* is clearly much closer to Mio-Pliocene dryopithecines particular to East African Miocene species of this group which it agrees with in showing unusually marked molar size increase posteriorly. At the symphysis, however, *Aegyptopithecus* is more typically pongid than some members of the East African species of *Proconsul*, and shows comparatively less anteroposterior thickening of the symphysis and a more backward-directed simian shelf. The characters of symphyseal robusticity and degree of development of simian shelf are notably variable in living hominoids<sup>6</sup>. In *Proconsul nyanzae* and *Proconsul major* this shelf is usually more ventrally directed than in *Aegyptopithecus*.

*Aegyptopithecus* is close in size to *Pliopithecus* (*Limnopithecus*) from East Africa but differs from it and from European *Pliopithecus* in showing more distinct labial cingula, relatively smaller premolar and molar trigonids, less distinct cross-crests between protoconid-metaconid and entoconid-hypoconid, relatively much larger  $M_3$  with narrower  $M_3$  talonid, and comparatively larger  $M_3$  hypoconulid. Being much earlier and more generalized than *Dryopithecus* or *Proconsul*, *Aegyptopithecus* does show some likeness to *Pliopithecus* (*Limnopithecus*), which Ferembach<sup>7</sup> has maintained may have greater affinity with the great apes (pongines) than with the gibbons. However, resemblance between all the East African and Egyptian hominoids of the Oligocene and Miocene is great. In general, they exhibit the sort of morphological interrelationship to be expected of various members of a category comparatively close to the time of its initial radiation. These interrelationships which are reasonably apparent in early hominoids naturally affect all attempts to place given species in higher categories. Dental features now seen only in gibbons or only in the African apes (*Pan*, *Proconsul*) were then combined in one species as Ferembach's observations show.

Since it is characteristic of African *Pliopithecus* (*Limnopithecus*), European *Pliopithecus* and modern gibbons to have lower third molars which are shorter or equal in size to  $M_2$  (and other features cited here), this new Fayum primate would appear to relate more definitely to the ancestry of *Proconsul*. The comparatively enlarged  $M_3$  of *Aegyptopithecus* and of *Proconsul* species is one of the few features which seem to differentiate species of the latter from the type species of *Dryopithecus*, *D. fontani*. Moreover, if some Fayum primates are near the ancestry of later Hominoidea, as certainly appears to be probable, another contemporary of *Aegyptopithecus* (described here) would seem to be a much better candidate for possible ancestry to the gibbons of Miocene-Recent times.

*Aegyptopithecus zeuxis*<sup>8</sup>, new species

Type: C.G.M. 26901, left mandibular ramus with  $P_4$ - $M_2$ ; partial alveoli of  $C$ ,  $P_3$  and  $M_3$  and symphysis.

*Hypodigm*: Type and Yale Peabody Museum (Y.P.M.) 21032, left horizontal and vertical ramus with broken  $P_4$ ,  $M_1$ - $M_3$ , American Museum of Natural History (A.M.N.H.) 13389, left mandibular ramus with broken  $C$ ,  $P_3$  roots and alveoli of  $P_4$ - $M_3$ .

*Horizon and Locality*: Yale Expedition Quarry I, upper fossil wood zone, Qatrani (Fluviomarine) Formation, Oligocene, Fayum Province, Egypt, U.A.R.

*Specific characters*: Not distinguished from generic.

The three known mandibular rami complement each other in parts preserved although the reference of A.M.N.H. 13389 to this species remains slightly in doubt in view of its fragmentary condition and uncertain locality and horizon; it was collected in 1906.

MEASUREMENTS (MM) OF *Aegyptopithecus zeuxis* AND *Propithecus haeckeli*

Dentition	<i>A. zeuxis</i>		<i>P. haeckeli</i>	
	C.G.M. 26901 type	Y.P.M. 21032	A.M.N.H. 13389	S.M.N. 12638 type (left side)
Length of lower tooth series $\bar{C}$ - $M_3$ inclusive	—	—	40.5+	27.0
Length of series $P_4$ - $M_3$	—	25.1	27.5+	18.5
Length of series $P_4$ - $M_2$	17.7	16.8	18.0+	13.1
$P_4$ maximum transverse diameter (width)	4.2	—	—	4.0
$P_3$ maximum anteroposterior diameter (length)	4.4	—	—	3.5
$M_1$ maximum transverse diameter	5.2	5.4	—	4.5
$M_1$ maximum anteroposterior diameter	6.1	5.9	—	4.8
$M_2$ maximum transverse diameter	6.3	6.3	—	4.5
$M_2$ maximum anteroposterior diameter	7.1	6.8	—	4.8
$M_3$ maximum transverse diameter	—	6.8	—	4.1
$M_4$ maximum anteroposterior diameter	—	8.6	—	5.6
Mandible				
Depth beneath $M_2$	14.9	14.1	13.0+	12.4
Depth behind $M_3$	16.0e	16.2	15.0+	14.0

Abbreviations: A.M.N.H., American Museum of Natural History, New York. C.G.M., Cairo Geology Museum, Cairo. S.M.N., Stuttgart Museum of Natural History, Stuttgart, Germany. Y.P.M., Yale Peabody Museum, New Haven, Connecticut.

Family, (?) Hylobatidae

Genus *Aeolopithecus*<sup>8</sup> gen. nov.

Type: *Aeolopithecus chirobates*, new species.

*Generic characters*: Lower dental formula 2.1.2.3. Slightly smaller than *Propithecus* and much smaller than all other Oligocene-Recent hominoids. Differs from the contemporary genus *Propithecus* in showing marked premolar heteromorphy (sectorial  $P_3$ ), comparatively much larger canines and more procumbent incisors and from both *Aegyptopithecus* and *Propithecus* in having tooth rows more divergent posteriorly and in showing relatively higher and deeper genial fossa, with horizontal ramus of mandible shallowing posteriorly. Differs from *Pliopithecus* in possessing relatively larger canines, and greater degree of premolar heteromorphy. Resembles some *Hylobates* and differs from *Aegyptopithecus*, European *Pliopithecus* and *P. (Limnopithecus)* in having much reduced  $M_3$ .

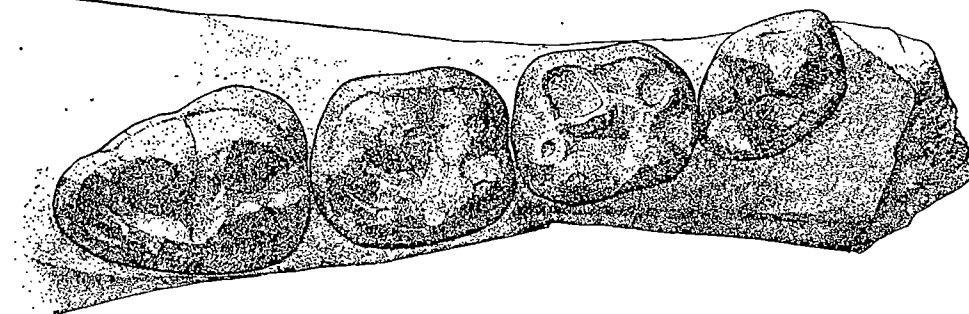


Fig. 1. Crown view of the left mandibular teeth of the type of *Aegyptopithecus zeuxis*, C.G.M. 26901, with  $M_3$  restored from Y.P.M. 21032. ( $\times 4.00$ )



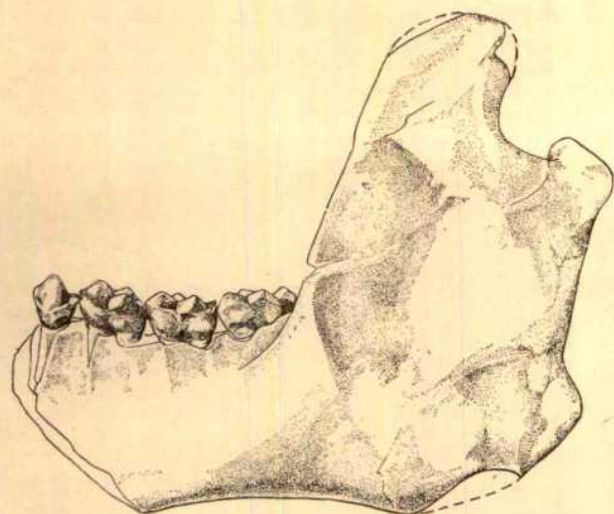


Fig. 2. Lateral view of the left lower jaw of *Aegyptopithecus zeuxis* Y.P.M. 21032 with  $P_4$  restored from C.G.M. 26901. ( $\times c. 1.50$ )

Although molar crown patterns are obscured by destruction of the enamel the observable characters of the mandible and teeth all suggest that this primate is related to the gibbons, living and fossil. Particularly pertinent to this conclusion are the large and long canines, reduced  $M_3$  and posterior shallowing of the mandibular ramus. As in some *Hylobates* the genial fossa is remarkably high and deep.

In size *Aeolopithecus chirobates* is rather larger than *Oligopithecus savagei* and *Parapithecus fraasi*. It also comes from a higher horizon than either of the latter species. *A. chirobates* differs from *Oligopithecus savagei* not only in size and proportion of the mandibles (the mandible shallows less posteriorly in the latter), but also in that the mental foramen is located much farther forward, the canines are relatively larger and the premolars more heteromorphic. Molar crowns of *Aeolopithecus chirobates* also appear to lack the cross-crests of *Oligopithecus savagei*. Close relationship of *Aeolopithecus chirobates* to *Apidium phiomense*, *Apidium moustafai* or *Parapithecus fraasi* is ruled out by their differing dental formulae and wholly dissimilar tooth morphologies.

This is one of the most distinctive of Fayum primates known so far and it is regrettable that only one specimen exists. However, it is the second most complete specimen of an Oligocene primate mandible known, after the type of *Parapithecus fraasi* (S.M.N. 12639a), and it should provide data for useful comparative analysis when early gibbons become better known.

The most direct relationships of this species are with *Pliopithecus*, particularly sub-genus *Limnopithecus* which possesses a similarly large simian shelf and with which *Aeolopithecus* agrees in most other observable features except for its much smaller size. Conceivably *Aeolopithecus* is closer to the line that gave rise to modern *Hylobates* and *Symphalangus*, that is, *Pliopithecus*, in view of its third molar reduction, as is also observed in some species of *Hylobates*. Nevertheless, adoption of such a conclusion on the strength of this one feature alone seems inadvisable. It remains entirely possible that none of the known fossil gibbons is actually ancestral to present-day south-east Asian species.

In the early part of this century Fourtau<sup>10</sup> described a species, *Prohylobates tandyi*, from the supposed Burdigalian (early Miocene) fauna of Wadi Moghara, west of Alexandria, Egypt. As the name implies, he concluded that this fossil was related to the gibbons. Consequently, it is necessary to consider the relation of this primate to *Aeolopithecus*. Examination shows clearly that *Prohylobates tandyi* is a cercopithecoid monkey, a fact missed

by most previous commentators on its status (including Remane<sup>11</sup>, MacInnes<sup>12</sup>), although Le Gros Clark and Leakey<sup>13</sup> did remark "the molars [of *P. tandyi*] appear to be more cercopithecoid in their cusp pattern". This is a typical example of the danger of publishing discussion on fossils which have not actually been seen and compared with other pertinent materials. *Prohylobates tandyi* is clearly congeneric and probably conspecific with a still undescribed cercopithecoid monkey which occurs in the Miocene Rusinga Island fauna of East Africa, and consequently bears no taxonomically significant relationship to *Aeolopithecus*.

#### *Aeolopithecus chirobates*<sup>18</sup> new species

**Type:** C.G.M. 26923, complete horizontal rami of mandible fused at symphysis with incisor alveoli and left and right  $\bar{C}-M_3$ .

**Hypodigm:** Type.

**Horizon and locality:** Yale Expedition, Quarry I, upper fossil wood zone, Qatrani (Fluviomarine) Formation, Oligocene, Fayum Province, Egypt, U.A.R.

**Specific characters:** Not distinguished from generic.

MEASUREMENTS (MM) OF *Aeolopithecus chirobates* IN COMPARISON WITH OTHER FAYUM PRIMATES OF SIMILAR SIZE<sup>14</sup>

	<i>Aeolopithecus chirobates</i> type, C.G.M. 26923		<i>Oligopithecus savagei</i> type, C.G.M. 29627	<i>Parapithecus fraasi</i> type, S.M.N. 12639a
Dentition	Left	Right	Left	Left
Length of $\bar{C}-M_3$ inclusive	28.3	28.0	—	23.5
Length of $P_3-M_3$ inclusive	22.0	22.0	—	21.0
Length of $P_4-M_3$	18.0	17.9	—	17.7
$\bar{C}$ transverse diameter	3.5	3.5	3.3	2.5
$\bar{C}$ anteroposterior diameter	5.9	5.6	3.7	3.2
$P_3$ transverse diameter	2.4	2.6	3.1	2.5
$P_3$ anteroposterior diameter	4.3	4.5	4.2	3.3
$P_4$ transverse	2.4	2.7	3.0	2.5
$P_4$ anteroposterior	3.5	3.6	3.3	3.3
$M_1$ transverse	3.7	3.6	3.4	3.2
$M_1$ anteroposterior	5.0	4.9	4.2	4.2
$M_2$ transverse	4.0	3.9	3.5	3.4
$M_2$ anteroposterior	5.2	5.0	4.2	4.3
$M_3$ transverse	3.1	3.0	—	3.3
$M_3$ anteroposterior	4.0	4.0	—	4.2
Mandible				
Maximum symphyseal thickness	6.8		—	—
Maximum symphyseal depth (along axis)	16.1+		—	9.0
Depth of mandible below $P_3$	11.7		10.4	7.5
Depth of mandible below $M_1$	9.8		9.4	7.6

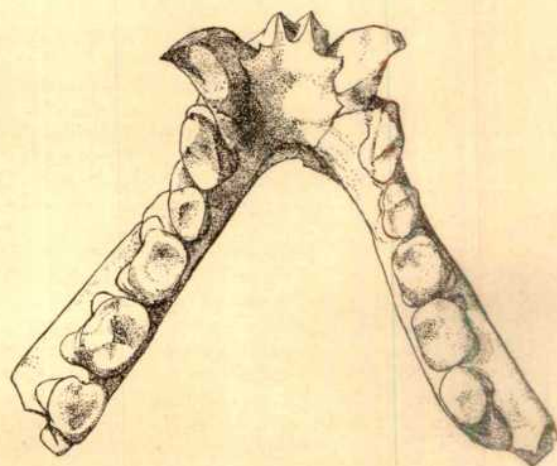


Fig. 3. Type mandibles of *Aeolopithecus chirobates* C.G.M. 26923 ( $\times c. 2.00$ )



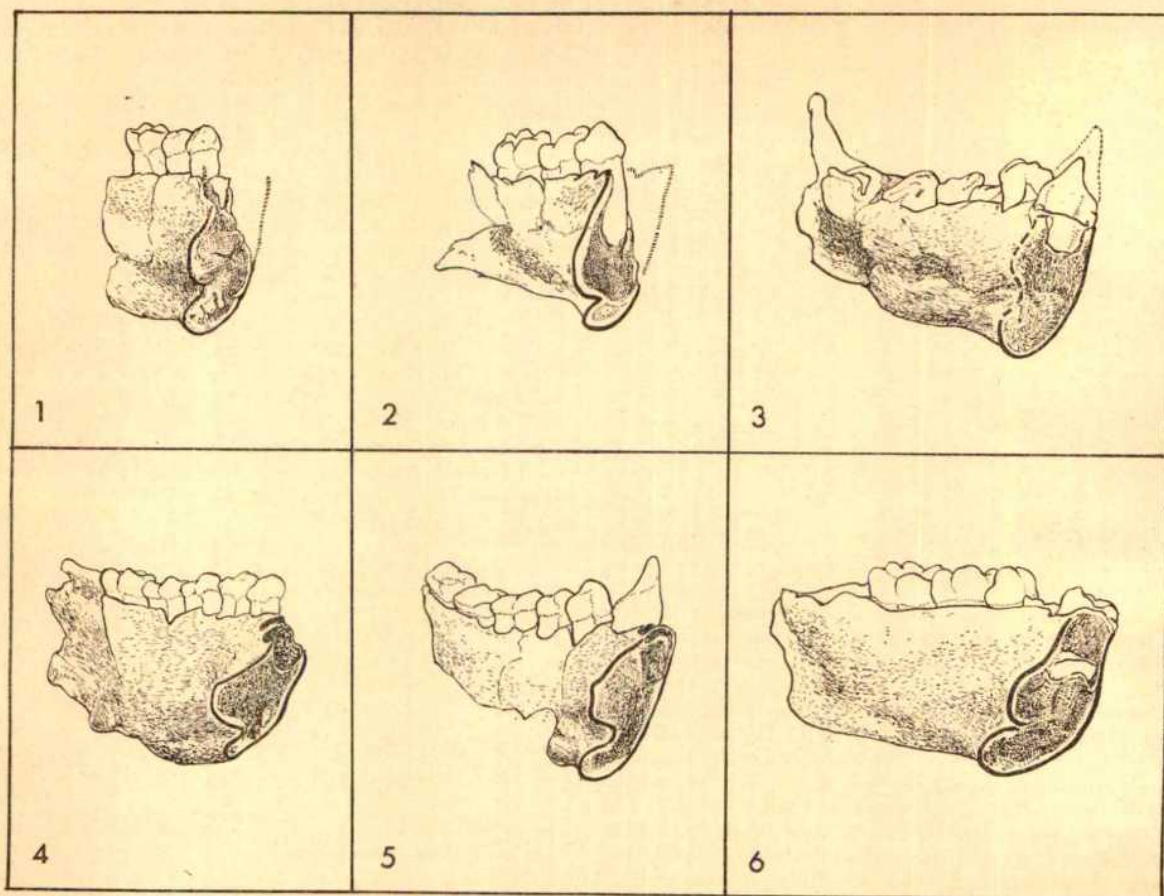


Fig. 4. Symphyseal cross-sections in several early primates, showing variations in symphyseal thickness and in expression of simian shelf (not to the same scale). (1) *Amphipithecus mogaungensis* A.M.N.H. 32520, type Eocene of Burma; (2) *Aegyptopithecus zeuxis* C.G.M. 26901 type; (3) *A. zeuxis* A.M.N.H. 13389; (4) *Parapithecus* sp. nov. Yale Coll.; (5) *Aeolopithecus chirobates*, C.G.M. 26923 type; (6) *Sugrivapithecus salmantanus*, Y.P.M. 13811 type

### General Discussion

In determining the position of given fossils in evolutionary trees it is often necessary to make use of inferences which cannot be tested in full but which often serve as a basis for further investigations. One of these is that many Egyptian Oligocene primates are likely to be in or near the ancestry of later monkeys, apes and men. Students of fossil mammals know that there is little evidence of the occurrence of higher primates (Anthropoidea) in the known Eocene, Oligocene and early Miocene faunas of Eurasia; thus pointing to the continent of Africa (where Oligocene and Miocene Primates are abundant) as the most probable place of differentiation of monkeys, apes and men. Of course, were early and middle Cenozoic faunas better known in Asia, distribution of early Anthropoidea might be extended. Recent review of the early Cainozoic primates of Europe<sup>16,17</sup> has shown that none of the known Eocene primate species of that continent is a good candidate for possible relationship to the ancestry of monkeys, apes or men. One species, *Alsaticopithecus lemani*, described by Hürzeler<sup>18</sup> from middle Eocene deposits in Alsace, has been tentatively suggested as having molar resemblance to Anthropoidea by several authors, as indeed it does. However, McKenna<sup>19</sup> has correctly identified this species as a member of the family Microsyopidae. It thus belongs to a doubtfully primate family known otherwise only from the Eocene of North America. Microsyopid molars parallelistically resemble those of certain hominoids, but it is unlikely that Old World higher primates are derived from Microsyopidae or anything like them.

The only remaining, possibly hominoid, primates of early age outside Africa are from the late Eocene of

Burma: *Pondaungia cotteri*<sup>20</sup> and *Amphipithecus mogaungensis*<sup>21</sup>. The original materials of both these species have been examined during the process of preparing this article, but the fragmentary condition of the types of these two forms precludes a definite statement that the origins and early geographic distribution of Old World higher primates were extended or were centred outside Africa.

The type of *Pondaungia cotteri*, which was quite inadequately figured and described by Pilgrim, consists of partial mandibular rami of both sides of the lower jaw carrying chemically corroded second and third molars. As nearly as can be judged, in size, outline and cusp placement, these molars resemble those of *Propliopithecus*. Associated with the two mandibular fragments of *Pondaungia* at the site of discovery was a left maxillary fragment with  $M1-2$ , apparently belonging to the same individual.

Pilgrim's figures<sup>20</sup> and discussion of *Pondaungia* left much to be desired and indeed, on the basis of the data he then provided, it was scarcely possible to make an ordinal placement for the form. Consequently, most publications on early Old World primates since 1927 either omit *Pondaungia cotteri* or question placement of this species as a primate.

Pilgrim noted that web-like chemical erosion of the enamel of lower right and left  $M2-3$  obscured their crown patterns. He also supposed this to be the case for the upper molar surfaces. However, microscopic examination of the type at Calcutta indicates that, while the lower molars do appear to have suffered erosive damage, the grooves and ridges on the upper teeth represent natural surfaces except in one or two broken areas. Thus it is

possible to observe upper molar structure in considerable detail in *Pondaungia*. These molars have the typical placement of the four primary cusps, pronounced lingual cingula and crenulate occlusal surface characteristic of most early hominoids, but multiple cusps exist in the region of paraconule and metaconule and there are no ridges connecting any of the main cusps.

In the combination of these molar features, *Pondaungia* somewhat resembles species of the European Eocene tarsioiid genus *Microchoerus* and members of the North American omomyid primate genera *Hemiacodon* and *Ourayia*. It is tempting to interpret molar crown structure in *Pondaungia* as morphologically transitional between that characteristic of Omomyidae and that of Pongidae, but there are non-Primates which *Pondaungia* also resembles, particularly some species belonging to the hypsodontid Condylarthra. This archaic mammalian family is not definitely known to have occurred in Asia, but its presence there in the late Eocene would not be particularly surprising. The evidence on the taxonomic relationship of *Pondaungia* is therefore equivocal but, rather than regarding it as *incertae sedis*, the balance would seem to weigh in favour of a questioned placement among *Hominoidea*—archaic members of which it resembles in known comparable parts.

From the Pondaung Formation of Burma is the type of *Amphipithecus mogauensis* Colbert<sup>21</sup> (Fig. 4). Colbert's reasons for placing this animal among the higher primates remain acceptable and have been strengthened by its dental resemblance to the recently discovered Fayum primates *Oligopithecus savagei* and *Aegyptopithecus zeuxis*. In its mandibular depth, foreshortening of the anterior dentition, and symphyseal cross-section this species is typically hominoid. Indeed, Colbert made a queried reference of the species to the taxonomic family of the apes—Simiidae, now Pongidae. Unfortunately, only two premolars and first molar of the left side are preserved in the type and only specimen. Roots of a canine and a  $P_3$  are also evident and if his identification of these teeth is correct this means that three premolars were present. Although all later Old World Anthropoidea, with the exception of the Parapithecidae, possess only two premolars above and below, the occurrence of three here is presumably a primitive feature. Most other Eocene Primates had at least three premolars. Taken altogether, the structure of the teeth and jaw fragment of *A. mogauensis* permits its close taxonomic association with the early apes of the Fayum, but it is regrettable that so little is known of this species. Since this is the case, understanding of its full significance must wait until more material is secured. It is clear, as Colbert quite adequately demonstrated, that this fragment is more like comparable parts of early catarrhines than like members of any other known order of Mammalia. The conclusion which must be drawn is that known materials of *Pondaungia* and *Amphipithecus* indicate, but do not prove, that early hominoids or hominoid-like primates existed in the Asian tropics before the end of the Eocene Epoch. The question whether these species bear any relation to the latter radiation of apes, man and monkeys of the Old World cannot really be settled on the basis of evidence available at present.

Consequently, the Fayum primates of Egypt remain the only adequate basis for speculation as to the nature of the early stages of differentiation of the higher primates of the Eastern Hemisphere. The following tentative conclusions as to their phylogenetic meaning are consequently presented.

*Propithecus haeckeli* Schlosser<sup>3</sup> has most often been treated as representing an ancestral gibbon or at least a good morphological antecedent for Mio-Pliocene *Pliopithecus* species. Although Schlosser<sup>3</sup> in his original description stressed this point of view, his comparisons between *Propithecus* and *Pliopithecus* are not very specific and he equally considered a number of other

phyletic relationships for *Propithecus*, including the possibility that it was ancestral to *Pithecanthropus* and modern man. Of course, at the time of his writing there were not many other fossil Higher Primates for comparison. Afterwards, with more comparative material available, Gregory<sup>22</sup>, Abel<sup>23</sup> and Le Gros Clark<sup>24</sup> also stressed resemblances between *Propithecus* and European or East African *Pliopithecus*. More recently, in the most comprehensive investigation of *Pliopithecus* published so far, Hürzeler<sup>25</sup> questioned that *Propithecus haeckeli* could be ancestral to the Mio-Pliocene gibbons. He noted that in this Egyptian species there is no  $P_3$  sectoriality, that the anterior fovea of  $P_4$  does not open on the lingual side as in *Pliopithecus* species and the metaconid crest of  $P_4$  of *Pliopithecus* is lacking in the Fayum primate, which instead possesses a labial cingulum not seen in *Pliopithecus*. Moreover, in *Propithecus* a weak crest exists between  $M_3$  hypoconid and hypoconulid while in *Pliopithecus* instead a crest joins entoconid and hypoconulid. In *Propithecus* the lower molar cusps are lower and more rounded and lack much of the crown-crenulation and development of accessory tubercles common in *Pliopithecus*. All these distinctions, when taken together with the now documented presence of a rather more *Pliopithecus*-like species, *Aeolopithecus chirobates*, contemporary with at least one species of *Propithecus* in the Fayum, suggest that *Propithecus haeckeli* should not be thought of as taxonomically close to the gibbons.

In its overall morphology, the type mandible and dentition of *Propithecus haeckeli* (S.M.N. 12638) is more reminiscent of what one would expect to see in an Oligocene forerunner of the family Hominidae rather than in an early dryopithecine or gibbon. The probability that this primate indicates that, by early Oligocene times, species ancestral to living man had already differentiated from those which led to *Dryopithecus* and subsequent great apes is thus increased. Confirmation of this interesting possibility will not come unless Oligocene-Miocene forms transitional between *P. haeckeli* and *Ramapithecus punjabicus* of the late Miocene of Africa and Eurasia are recovered by future field research in relevant areas of the Old World.

<sup>1</sup> Osborn, H. F., *Bull. Amer. Mus. Nat. Hist.*, **24**, 255 (1908).

<sup>2</sup> Osborn, H. F., *Bull. Amer. Mus. Nat. Hist.*, **26**, 415 (1909).

<sup>3</sup> Schlosser, M., *Beitr. Palaont. und Geol. Österreich-Ungarns*, **24**, 51 (1911).

<sup>4</sup> Andrews, C. W., *Brit. Mus. (Nat. Hist.) Mono.*, **1** (1906).

<sup>5</sup> Named in reference to the Egyptian provenance of the type.

<sup>6</sup> Schultz, A. H., *Viking Fund Pub. Anthro.*, **37**, 85 (1963).

<sup>7</sup> Ferembach, D., *Ann. Paleont.*, Paris, **44**, 1 (1958).

<sup>8</sup> From Greek, yoke, join. In reference to the mandibular and dental morphology which shows intermediary between *Propithecus* and *Dryopithecus*.

<sup>9</sup> Named with reference to the fact that the type specimen was exposed in Yale Quarry I by a wind-storm, for Aeolus god of the winds, and in analogy with *Oreopithecus*, *Limnopithecus*, etc.

<sup>10</sup> Fourtau, R., *Cairo, Min. Finance, Surv. Dept. Pub.*, **1** (1918).

<sup>11</sup> Remane, A., *Centralbl. Min. Geol. Palaont. Jahrg.*, **220** (1924).

<sup>12</sup> MacInnes, D. G., *J. East Africa and Uganda Nat. Hist. Soc.*, **17**, 141 (1943).

<sup>13</sup> Le Gros Clark, W. E., and Leakey, L. S. B., *Brit. Mus. (Nat. Hist.) Foss. Mamm. African*, **1**, 1 (Brit. Mus. (Nat. Hist.), 1951).

<sup>14</sup> From Greek *cheir*, hand, and *bates*, trader or climber, with reference to the use of hands in arboreal progression presumed for early hominoids and in analogy with the modern gibbon, *Hylobates* (forest climber).

<sup>15</sup> All measurements are decreased by surface erosion or corrosion of enamel in the type and are therefore 1–2 mm less than they would be in an undamaged individual. As nearly as can be judged the removal of enamel has affected all tooth dimensions about equally, so that the same proportionate relations are retained as between any two given teeth.

<sup>16</sup> Simons, E. L., *Bull. Brit. Mus. (Nat. Hist.)*, **5**, 43 (1961).

<sup>17</sup> Simons, E. L., *Bull. Brit. Mus. (Nat. Hist.)*, **7**, 1 (1962).

<sup>18</sup> Hürzeler, J., *Ecl. Geol. Helv.*, **40**, 343 (1947).

<sup>19</sup> McKenna, M. C., *Bull. Dep. Geol. Univ. Calif.*, **37**, 1 (1960).

<sup>20</sup> Pilgrim, G. E., *Mem. Geol. Surv. India*, **14**, 1 (1927).

<sup>21</sup> Colbert, E. H., *Amer. Mus. Novitates*, **951** (1937).

<sup>22</sup> Gregory, W. K., *Bull. Amer. Mus. Nat. Hist.*, **35**, 239 (1916).

<sup>23</sup> Abel, O., *Die Stellung des Menschen im Rahmen der Wirbeltiere*, **1** (Jena, 1931).

<sup>24</sup> Le Gros Clark, W. E., *Proc. Zool. Soc. Lond.*, **122** (2), 273 (1952).

<sup>25</sup> Hürzeler, J., *Ann. Paleontol.*, **40** (Paris, 1954).



## ACTIVATION OF LYSOSOMES BY OXYGEN

## Oxygen-induced Resorption of Cartilage in Organ Culture

FOLLOWING the *in vivo* observations of Johnson on the distribution of tissue types in bone tumours and the correlation between this distribution and the blood supply<sup>1</sup>, it has been demonstrated that exposure of skeletal tissues in culture to elevated partial pressures of oxygen causes resorption and may affect the differentiation of the tissue<sup>2,3</sup>. The mechanism of this effect has not been explained.

In the experiments recorded here, the effect of high partial pressures of oxygen on the cartilaginous limb-bone rudiments from embryonic chicks has been investigated in organ culture. The histological changes produced in the explants closely resemble those caused by exposure to excess vitamin A (ref. 4), and the possibility that both agents act through the same mechanism, increased release of proteolytic lysosomal enzymes<sup>5</sup>, has therefore been explored.

Tibiae and femora from eight-day chick embryos were cultivated on stainless-steel grids<sup>6</sup> in a chemically defined medium based on that described by Biggers *et al.*<sup>7</sup> and containing 0.5 per cent bovine serum albumin. In certain experiments cortisol hemisuccinate was added at either 1.0  $\mu\text{g}/\text{ml}$ . or 0.1  $\mu\text{g}/\text{ml}$ . One of each pair of rudiments received experimental treatment and the other was grown under control conditions. The cultures were incubated at 37.5° centigrade in air-tight glass chambers<sup>8</sup> in which the gas phase was either 85 per cent oxygen with 5 per cent  $\text{CO}_2$  or 20 per cent oxygen with 5 per cent  $\text{CO}_2$ . The medium was collected and the cultures re-fed and gassed at two-day intervals. At the termination of the experiment, usually after six days, representative rudiments and their controls were fixed for histological examination and the remainder were assayed for enzyme content.

The medium was cooled in ice and adjusted to pH 5.0 with hydrochloric acid. Acid phosphatase was estimated with sodium nitrophenol phosphate as substrate<sup>9</sup> and acid protease with haemoglobin as substrate<sup>10</sup>. The cartilaginous rudiments were homogenized in 0.25 M sucrose and a particulate preparation sedimented at 200,000g/min. The enzyme activity of this pellet (the bound activity) was determined after re-suspension in a neutral detergent, and the activity of the supernatant (the free activity) was also estimated. Since both acid phosphatase and acid protease are of lysosomal origin, the ratio of their free to total activities was taken as a measure of the stability of the lysosomes to the stress of homogenization.

The effect of high oxygen was quite striking. The longitudinal growth of the rudiments was greatly reduced and they became abnormally translucent and soft. Histological sections showed a severe loss of metachromatic material throughout the rudiment and especially marked in the hypertrophic zone (Fig. 1). The periosteal layer of osteoblasts was much thicker in the oxygen-treated explants. Accompanying these histological changes were a greatly increased production and release of acid phosphatase and protease, and the percentage of free enzyme was elevated in the oxygen-treated rudiments (Table 1).

Table 1. OXYGEN-INDUCED RELEASE OF ACID PROTEASE  
Figures are percentages of the control values in that experiment

	Released into medium	Total synthesized	Free/total
Air	100	100	100
85% $\text{O}_2$	130	136	186
85% $\text{O}_2$	100	100	100
85% $\text{O}_2$ with cortisol (1.0 $\mu\text{g}/\text{ml}$ .)	55	73	78

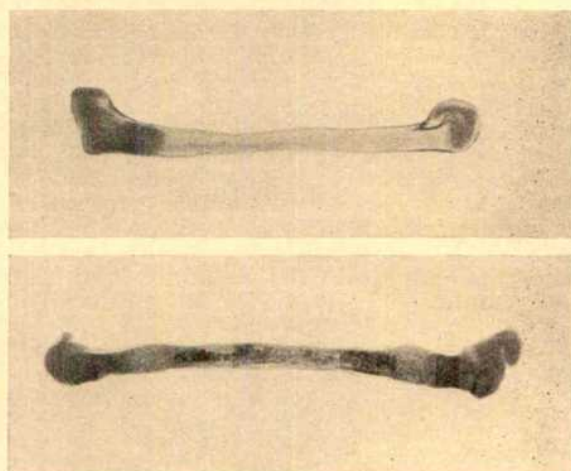


Fig. 1. Top, 8-day tibia cultured 6 days with gas phase of 85 per cent oxygen; bottom, paired rudiment in air. (Toluidine blue,  $\times c. 7.3$ )

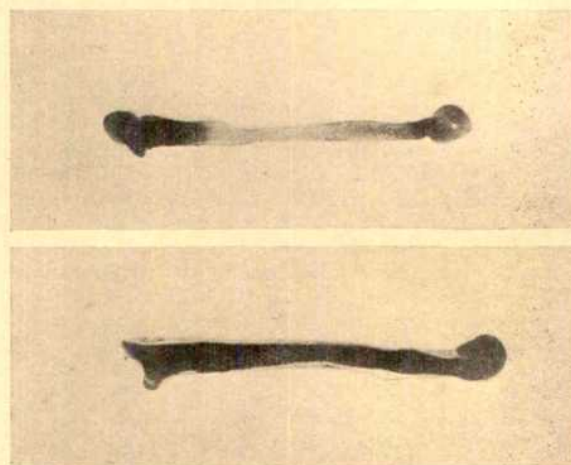


Fig. 2. Top, gas phase 85 per cent oxygen; bottom, paired tibia with gas phase 85 per cent oxygen; 0.1  $\mu\text{g}/\text{ml}$ . cortisol. (Toluidine blue,  $\times c. 7.3$ )

Since cortisol largely inhibits lysosomal instability produced by a number of agents<sup>11,12</sup>, it was tested in this system. At both 1.0  $\mu\text{g}/\text{ml}$ . and 0.1  $\mu\text{g}/\text{ml}$ . this steroid prevented the excessive release of enzyme (Table 1) and the loss of metachromatic ground-substance (Fig. 2). The increased stability of the lysosomes was reflected in the reduced percentage of free enzyme (Table 1).

To correlate the loss of ground-substance specifically with the release of acid protease, cultures were grown in the presence of the protease inhibitor, epsilon-amino-n-caproic acid<sup>13</sup>. This agent, at 0.1 M concentration, has been found to be non-toxic and to inhibit the vitamin A-induced loss of ground-substance in the limb-bone rudiments of embryonic chicks<sup>14</sup>. The oxygen-induced loss of ground-substance was also largely prevented by this protease inhibitor (Fig. 3).

These results suggest that, in this system, high partial pressures of oxygen act like vitamin A, that is, by alteration of the lipoprotein lysosomal membrane, allowing escape of hydrolytic enzymes and consequent degradation of the ground-substance<sup>15</sup>. This view is supported by the inhibitory effect of cortisol and of epsilon-amino caproic acid.



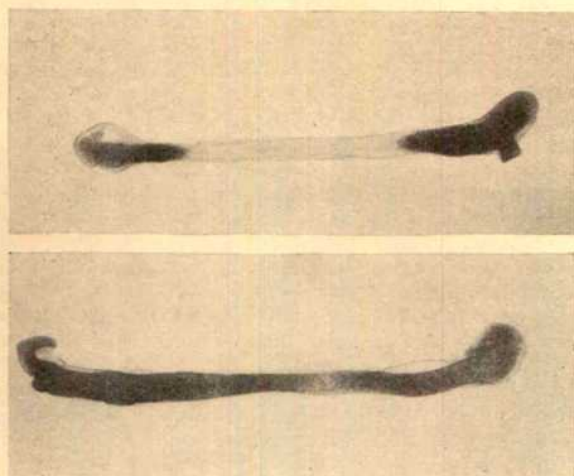


Fig. 3. Top, gas phase 85 per cent oxygen; bottom, paired tibia with gas phase 85 per cent oxygen; 0.1 M  $\epsilon$ -amino caproic acid. (Toluidine blue,  $\times c. 7.3$ )

To ascertain whether oxygen acts directly or indirectly, lysosome-rich suspensions were prepared from rat liver and kidney and incubated with 100 per cent oxygen, 100 per cent nitrogen, and air. There was no significant release of lysosomal enzymes under any of these three conditions. This confirms the results of de Duve<sup>16</sup> carried out with slices and crude lysosomal preparations from rat liver. The possibility remains that the lysosomes of skeletal tissues may respond differently to oxygen. Preliminary experiments indicate that lysosomal fractions prepared from embryonic chick cartilage are more susceptible to oxygen than are fractions prepared from liver and treated in the same manner. The question of whether oxygen *in vivo* acts directly on the lysosome or indirectly, perhaps through alteration of cell membrane permeability, remains unanswered. We had planned to pursue this point with histochemical investigations on monolayer cultures, when we became aware of the work of Allison, reported in the following article.

This phenomenon has important physiological implications. Cartilage is normally an avascular tissue. Invasion by blood vessels leads to its resorption and replacement by a tissue capable of existing in an oxygenated environment; this is the normal sequence of events in the epiphyseal plate. We suggest that a local rise in oxygen tension may cause an increased release of lysosomal enzymes from the cartilage cells. These enzymes include an acid protease which degrades the protein-polysaccharide complex of the ground-substance and facilitates invasion by vascular tissue.

We thank Dame Honor B. Fell for her advice.

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## Role of Lysosomes in Oxygen Toxicity

THERE is substantial evidence that prolonged exposure to 95 per cent oxygen at atmospheric pressure or to hyperbaric oxygen produces toxic effects in animals<sup>1,2</sup> and cells in culture<sup>3,4</sup>. We have confirmed the toxicity of 95 per cent oxygen at atmospheric pressure using three systems of cultured cells and have made observations on the sequence of changes following exposure to hyperoxia.

HeLa cells and chick embryo cells were propagated and counted as previously described<sup>5,6</sup>, and counts of viable cells were made on the basis of resistance to staining with 0.01 per cent eosin in saline<sup>7</sup>. In the presence of air + 5 per cent CO<sub>2</sub>, logarithmic growth of HeLa cells continued for at least 6 days (Fig. 1). In the presence of 95 per cent oxygen + 5 per cent CO<sub>2</sub> limited growth was seen for 3–4 days, after which the number of viable cells decreased, paralleling observations of Rutter<sup>3</sup> on AH cells. The toxicity was evidently not due to deterioration of the medium, since medium taken from degenerating cells after growth under 95 per cent oxygen for 5 days supported normal growth of other cells under 20 per cent oxygen, and a change of medium had no beneficial effect on cells kept under 95 per cent oxygen.

Similarly, chick embryo cells in control cultures equilibrated with air + 5 per cent CO<sub>2</sub> remained in good condition for at least one week, whereas cultures exposed to 95 per cent oxygen + 5 per cent CO<sub>2</sub> showed toxic

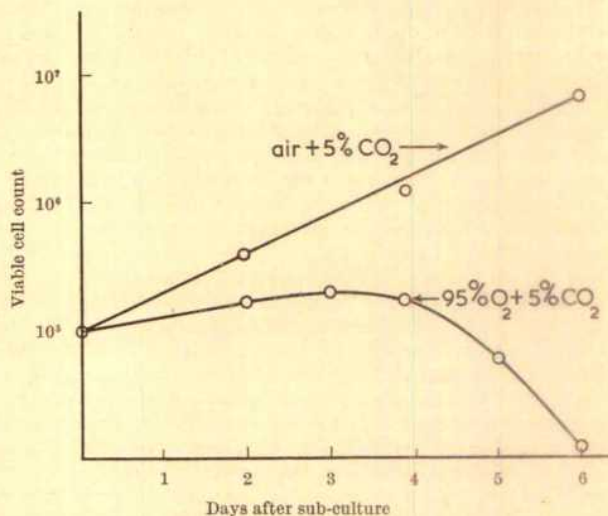


Fig. 1. Counts of HeLa cells per culture when grown in the presence of air + 5 per cent CO<sub>2</sub> and 95 per cent oxygen + 5 per cent CO<sub>2</sub>.

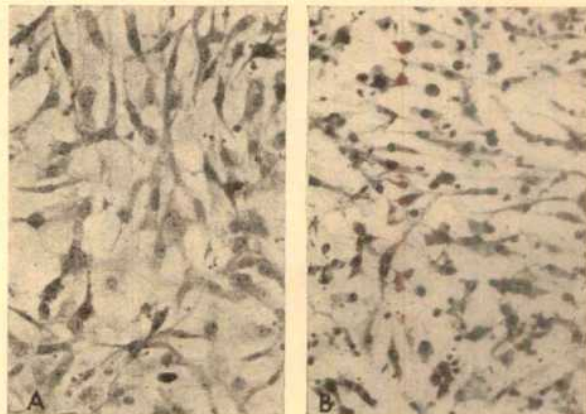


Fig. 2. Chick embryo cells grown for 5 days in the presence of air + 5 per cent CO<sub>2</sub> (A) and 95 per cent oxygen + 5 per cent CO<sub>2</sub> (B) ( $\times c. 180$ ).



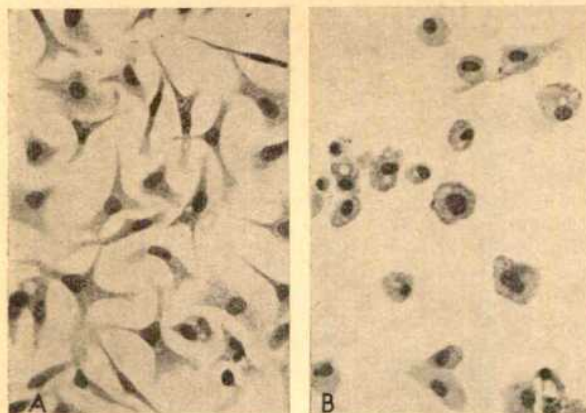


Fig. 3. Mouse macrophages cultivated for 3 days in the presence of air + 5 per cent  $\text{CO}_2$  (A) and 95 per cent oxygen + 5 per cent  $\text{CO}_2$  (B) ( $\times c. 160$ )

effects after the third or fourth day, including rounding, cytoplasmic granularity or vacuolation and nuclear changes (Fig. 2). Mouse macrophages were obtained by intraperitoneal inoculation of 3 ml. of culture medium (Parker's solution 199 (Glaxo) with 20 per cent calf serum heated at  $56^\circ$  for 20 min) containing 10 i.u. heparin per ml. into male mice of strain VSBS/NIMR weighing 18–20 g. After brief massage the cells were collected and dispensed in 1 ml. volumes in tubes containing small coverslips. Cultures equilibrated with air + 5 per cent  $\text{CO}_2$  remained in excellent condition for at least 2 weeks. Cultures equilibrated with 95 per cent oxygen + 5 per cent  $\text{CO}_2$  showed some rounding of cells by 36 h and progressively more severe changes thereafter (Fig. 3). A conspicuous change was 'ballooning' of the cells—which were round and much increased in size with some cytoplasmic vacuolation. Later the cells degenerated completely and many left the coverslips on which they had been grown.

To test whether lysosomal enzymes might be involved in these changes, cultures of unfixed macrophages were stained for acid phosphatase by the method of Gomori as described elsewhere<sup>8</sup>. Normal cells showed very little staining, whereas cells incubated in high oxygen showed phosphatase staining of lysosomes within 24 h (Fig. 4). As Bitensky<sup>9</sup> has pointed out in another context, this implies that the permeability of lysosomal membranes has been increased, because the  $\beta$ -glycerophosphate substrate does not penetrate into intact, healthy lysosomes. This stage—which we term first stage activation<sup>8</sup>—is reversible, and most macrophages exposed to 95 per cent oxygen for 24 h recover when cultured under 20 per cent oxygen. By 48 h of hyperoxia, second stage activation

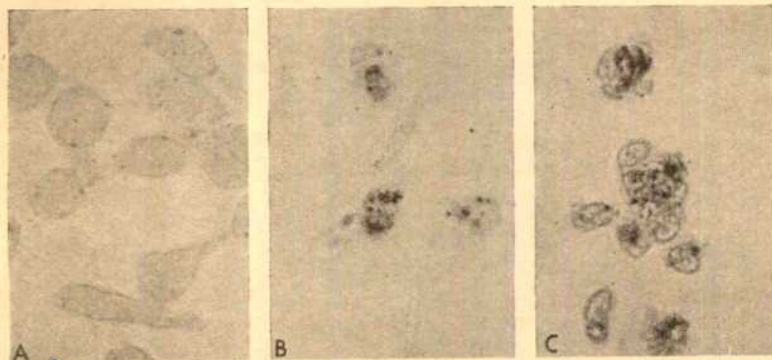


Fig. 4. Mouse macrophages unfixed and stained for acid phosphatase by the Gomori technique. A, normal cells; B, cells exposed to 95 per cent oxygen + 5 per cent  $\text{CO}_2$  for 24 h. Note discrete staining of lysosomes, with some cells still fully extended. C, cells exposed to 95 per cent oxygen + 5 per cent  $\text{CO}_2$  for 48 h. All cells are rounded, and there is some diffuse cytoplasmic staining as well as intense lysosomal staining ( $\times c. 650$ )

is evident: enzyme is released from lysosomes into cytoplasm. This can be shown chemically (Fig. 5) as well as histochemically (Fig. 4). The lower total enzyme activity in cells exposed to hyperoxia may be explicable by release into the medium and, possibly, inactivation.

The question arises whether the increased permeability of lysosomal membranes in hyperoxia is a primary event or is secondary to some other change such as damage to the cell membrane. That cell membranes are sensitive to oxygen is shown by observations such as the lytic effect of hyperbaric oxygen on erythrocytes, especially those of tocopherol-deficient animals<sup>10</sup>. However, in the cells we have examined under high oxygen increased permeability of lysosomes is seen at least 12 h before the cell membranes become permeable to eosin; fluorescence microscopy reveals the penetration of even very small amounts of eosin into the cells. Moreover, the toxic effects of oxygen were markedly reduced by having in the culture medium 10–50  $\mu\text{g}/\text{ml}$ . chloroquine or hydrocortisone, both of which stabilize lysosomal membranes<sup>11,12</sup>.

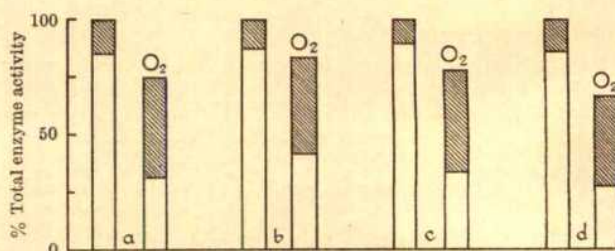


Fig. 5. Enzyme activities in particulate and supernatant (hatched) fractions of macrophages cultured in air + 5 per cent  $\text{CO}_2$  or 95 per cent oxygen + 5 per cent  $\text{CO}_2$  ( $\text{O}_2$ ), homogenized and assayed under conditions similar to those described by Cohn and Wiener<sup>14</sup>. The particulate fraction was disrupted before assay, and results in oxygen-treated macrophages are expressed per mg protein, referred to 100 per cent as total activity per mg protein in controls. a, Acid phosphatase; b, acid DNase; c, acid RNase; d, acid protease

We suppose that hyperoxia increases the permeability of lipoprotein membranes of cells and cell organelles, probably as a consequence of lipid peroxidation. Tappel *et al.* have already demonstrated release of lysosomal enzymes from rabbit liver homogenates by lipid peroxidation damage<sup>13</sup>. Short exposures of liver slices or homogenates *in vitro* to oxygen were not found to release lysosomal enzymes<sup>14</sup>, but the results with cell cultures suggest that longer exposures may be necessary to produce demonstrable changes. In any event, it seems that the lysosomes of some cells are more susceptible to oxygen effects than those of other cells; the cartilage cells described by Sledge and Dingle in the preceding communication would be a case in point. Heppleston and Simnett<sup>4</sup> found that some cells were more susceptible to oxygen damage in organ cultures than other cells; for example, alveolar cells showed more severe changes than lung adenomata. The atrophy of germinal cells of the testis seen in animals exposed to chronic hyperoxia<sup>2</sup>, and in tocopherol deficiency, suggests that these cells are highly susceptible. The role of tocopherol as an antioxidant protecting lysosomes, which has already been raised by Tappel *et al.*<sup>13</sup>, deserves further investigation. It might be expected that tocopherol-deficient animals, and the lysosomes of cells isolated from them, would be very sensitive to hyperoxia. Moreover, the additive carcinogenic effect of hyperoxia and hydrocarbon carcinogens, which are selectively concentrated in lysosomes, directs attention to the role of these organelles in carcinogenesis<sup>15</sup>.

Although these results suggest that lysosomal enzymes play an important part in



oxygen toxicity, they do not exclude the possibility that other effects may exist, for example, oxidation of thiol groups of enzymes.

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## DELINEATION OF THE THYMIC AND BURSAL LYMPHOID SYSTEMS IN THE CHICKEN

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INVESTIGATIONS of clinical immunological deficiency syndromes have long suggested the existence in man of two distinct populations of lymphoid cells, a division seen most clearly in the Bruton type of sex-linked recessive agammaglobulinemia in which there is virtually complete failure of plasma cell formation<sup>1,2</sup> and  $\gamma$ -globulin production<sup>3</sup>. Many of these patients have never formed detectable antibody. Their immunological failure is only partial, however; they are able to express delayed hypersensitivity<sup>4,5</sup> and are usually able to reject homografts, although the rejection process is slower and less efficient than normal<sup>6</sup>. Circulating lymphocyte-levels are relatively normal in most instances. Thus, their immunological defect represents a relatively isolated absence of antibody-producing capability and of its cellular and humoral correlates, plasma cells and the immunoglobulins.

It has been possible, since the discovery of the immunological role of the mammalian thymus and the avian bursa of Fabricius, to produce immunological deficiency syndromes in experimental animals, provided the organs are removed in the early neonatal or post-hatching<sup>7-17</sup> period. However, none of the mammalian models of immunological defect, in the mouse, rabbit, rat, or hamster, has involved absence of immunoglobulins or lack of plasma cells<sup>13,15,18-20</sup>, although all involve some loss of antibody-producing capability. The only model approaching classical agammaglobulinemia has been that of the chicken bursectomized in the immediate post-hatching period or hormonally bursectomized by treatment with 19-nortestosterone or similar agents during incubation. Such birds produce low levels of antibody to most antigens<sup>7,8,21,22</sup> and are usually deficient in plasma cells and in  $\gamma$ -globulin, particularly the 7S component<sup>21,23-25</sup>. Data relating the thymus and bursa to reactions of delayed hypersensitivity are inconclusive<sup>21,26</sup>. Skin homograft immunity has been variably affected by neonatal thymectomy in different series of experiments<sup>21,27,28</sup>, but has been quite consistently uninfluenced by bursectomy.

Szenberg and Warner<sup>29</sup>, in 1962, on the basis of investigations of hormonally bursectomized chickens, some of which also had an atrophied thymic cortex, originally postulated a dissociation of immunological function based on thymic versus bursal influence. Since that time, additional evidence for this immunological dissociation has been forthcoming from several laboratories<sup>26,27,29-31</sup>. Further, the investigations of Peterson *et al.*<sup>32</sup> established

the role of the bursa in visceral lymphomatosis. This virus-induced malignancy seldom if ever occurs in bursectomized birds, while thymectomized chickens seem to be as susceptible as controls.

The chicken model, then, seemed to offer the greatest potential for separating the two cell systems experimentally, systems so clearly separated in that extraordinary 'experiment of Nature', sex-linked recessive agammaglobulinemia. The work to be described involved a combination of sub-lethal irradiation with bursectomy, thymectomy, or both in the immediate post-hatching period in chickens. It has enabled us to produce an experimental model of agammaglobulinemia, and to define the thymic and bursal systems in the chicken. The findings suggest that the thymus-dependent tissue is basic to immunological recognition, and that it interacts with the bursa-dependent lymphoid tissue which might be termed the production system for antibody. They suggest, too, that all the experiments in mammals demonstrating immunological deficiency following removal of the thymus or appendix, or both, have affected thymus-dependent lymphoid tissue, that is, recognition.

On the day of hatching, White Leghorn chickens were divided into five groups. One group was surgically thymectomized, another surgically bursectomized, and a third subjected to both procedures. The following day these birds and an unoperated group were irradiated with 600 r. (conditions of irradiation: 220 kV, 15 m.amp, with 0.25 mm copper filtration at a dose rate, in air, of 45 r./min). In a previous experiment 700 r. was the LD<sub>50</sub> dosage for 2-day-old chickens. All the irradiated animals plus a control unirradiated group were housed together under standard poultry housing conditions.

At the age of 40 days each animal was injected intra-abdominally with 20 mg of crystallized bovine serum albumin (Armour) in saline and 10<sup>6</sup> killed *Brucella abortus* organisms (U.S. Department of Agriculture). Nine days later the birds were bled and killed. Spleen sections from each animal were prepared for staining with methyl green-pyronin<sup>33</sup> and haematoxylin and eosin. Thymus and bursa tissue sections were also stained with haematoxylin and eosin. Assays for antibody to bovine serum albumin were performed by a tube haemagglutination technique using bis-diazotized benzidine linkage of bovine serum albumin to rabbit erythrocytes<sup>34</sup>. Antibody to *Brucella* was assayed by a standard tube bacterial agglutination method. Microimmunoelectrophoresis of sera from each group of animals was performed by the method of Scheidegger<sup>35</sup> with a 0.05 M borate phosphate

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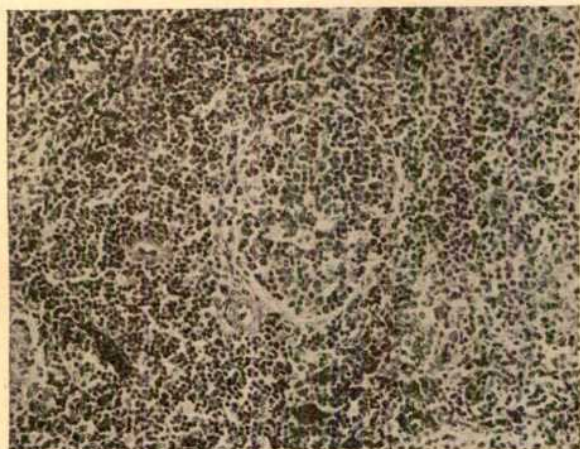


Fig. 1. Section of a normal chicken spleen showing the bursa-dependent follicle and surrounding thymus-dependent lymphoid component (haematoxylin and eosin;  $\times$  c. 166)

buffer at pH 8.2, using serum from rabbits hyperimmune to whole chicken serum.

In the chicken spleen there are two distinctly different types of lymphoid tissue. One is seen along the small arteries and arterioles, as sheaths of small lymphocytes or as scattered clusters composed of large and small lymphocytes, lymphoblasts, primitive reticular cells, and frequent mitotic figures. This represents the white pulp of the spleen, the bulk of its lymphoid tissue, and it becomes apparent in normal chickens in the immediate post-hatching period. We found that it was markedly reduced in thymectomized-irradiated and thymectomized-bursectomized-irradiated chickens. Such depletion has been noted previously in chickens subjected to neonatal surgical thymectomy<sup>30</sup> and in birds with testosterone-induced thymic cortical damage<sup>31</sup>. This component was not significantly altered in our other experimental groups. This lymphoid tissue we consider the *thymus-dependent* lymphoid tissue of the chicken.

There is a second type of lymphoid tissue in the chicken spleen, clearly differentiable from the thymus-dependent type both in morphologic appearance and in the time of its development. It is seen as sharply circumscribed round or oval lymphoid follicles (Fig. 1) which seem to be encased by a thin fibrous membrane and which always lie in juxtaposition to a small artery. These follicles bear a striking morphological resemblance to the follicles of the bursa of Fabricius itself (Fig. 2). They are even more clearly separable from the other spleen components when

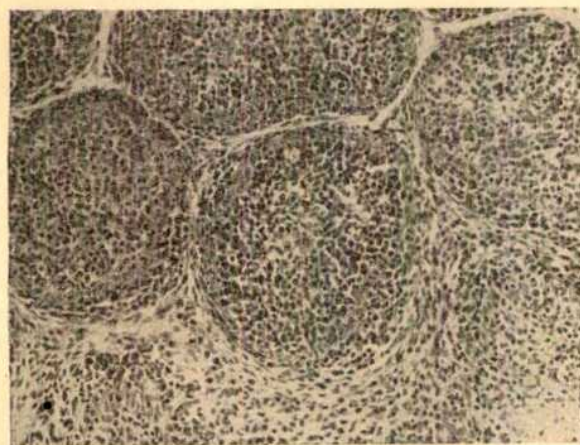


Fig. 2. Bursa section showing follicular structure (haematoxylin and eosin;  $\times$  c. 166)

stained with methyl green-pyronin because of the cytoplasmic pyroninophilia of the constituent cells (Fig. 3). These structures, in contrast to the thymus-dependent white pulp, develop relatively late in the normal chicken, about the fourth to fifth week of life. Thus, the thymus-dependent tissue is quite well developed by the time the bursa-like follicles are first recognizable morphologically.

These bursa-like follicles were not found in spleen sections of any bird irradiated following neonatal bursectomy or bursectomy-thymectomy, but were consistently present in members of all the other groups regardless of prior manipulation (Table 1). Plasma cells were also consistently absent in the bursectomized-irradiated or bursectomized-thymectomized-irradiated chickens, but were readily found in all the other groups. Thus, we consider the *bursa-dependent* lymphoid system to include these bursa-like lymphoid follicles and the plasma cell system.

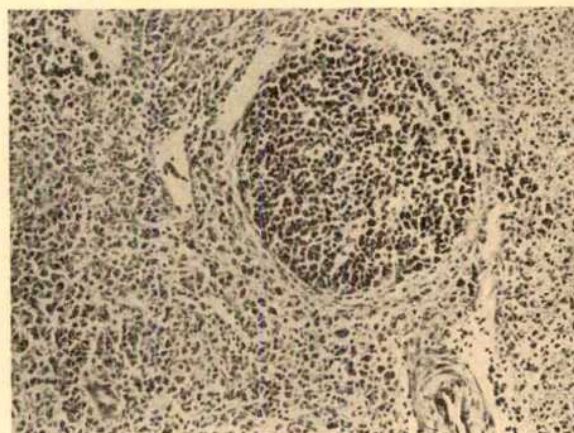


Fig. 3. Bursa-dependent follicle in the spleen showing the pyroninophilia of the enclosed cells (methyl green-pyronin;  $\times$  c. 166)

Table 2 gives the antibody titres to bovine serum albumin and *Brucella* in the five experimental groups. None of the animals subjected to both bursectomy and irradiation, and lacking bursa-like follicles and plasma cells in the spleen, developed detectable antibodies to these antigens. In addition, the sera of these birds showed no detectable  $\gamma^M$  (19S) or  $\gamma^G$  (7S) globulins on immunoelectrophoresis, although these bands were regularly seen in the sera of chickens of the other groups (Fig. 4). This is not a quantitative technique, but the

Table 1. BURSA-DEPENDENT FOLLICLES AND PLASMA CELLS IN SPLEENS OF IRRADIATED CHICKENS SUBJECTED TO PRIOR THYMECTOMY AND/OR BURSECTOMY\*

Group	Bursa-dependent follicles	Plasma cells
Bursectomized-irradiated	0/9	0/9
Bursectomized-thymectomized-irradiated	0/5	0/5
Thymectomized-irradiated	11/13	13/13
Control-irradiated	14/15	15/15
Control-non-irradiated	15/15	15/15

\* The denominator in each case is the number of animals examined; the numerator is the number of animals in which the structure or cell type was found in any of the several sections from each animal.

Table 2. PRIMARY ANTIBODY RESPONSE OF IRRADIATED CHICKENS SUBJECTED TO PRIOR THYMECTOMY AND/OR BURSECTOMY

Group	<i>Brucella abortus</i>			Bovine serum albumin		
	No. positive*	Mean titre†	S.E.	No. positive*	Mean titre†	S.E.
Bursectomized-irradiated	0/8	—	—	0/8	—	—
Bursectomized-thymectomized-irradiated	0/8	—	—	0/8	—	—
Thymectomized-irradiated	7/12	4.83	1.12	7/12	2.83	0.67
Control-irradiated	16/17	7.30	0.86	14/18	4.18	0.52
Control-non-irradiated	22/22	7.95	0.71	16/20	4.30	0.61

\* The denominator is the number of chickens for which sera were available; the numerator is the number of chickens with detectable antibody.

† The reciprocal of each antibody titre was converted to  $\log_2 + 1$ ; the means and standard errors were calculated using 1 as the value for the negative sera.



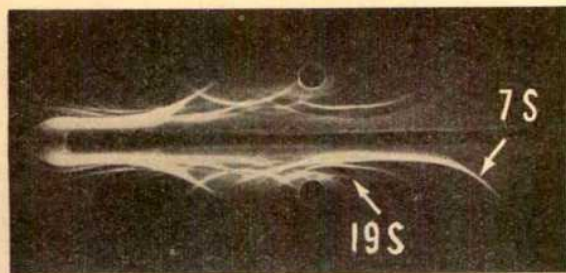


Fig. 4. Microimmunoelectrophoresis of sera from bursectomized-irradiated chicken (top) and normal chicken (bottom). The antiserum used was from rabbits hyperimmune to whole chicken serum

results suggest that  $\gamma$ -globulin is absent in the bursectomized-irradiated animals.

Thus, we have 7-week-old chickens, bursectomized and irradiated in the early post-hatching period, which are uniformly agammaglobulinemic, entirely lacking in plasma cells, and incapable of producing detectable antibody to two antigens of different types. Their spleens have no follicular development of the bursa type; their thymuses are morphologically normal (Fig. 5) as is the thymus-dependent lymphoid development in their spleens.



Fig. 5. Normal-appearing thymus of a bursectomized-irradiated chicken (haematoxylin and eosin;  $\times c. 27$ )

It is of interest that significant suppression of antibody-forming ability was also observed in the thymectomized-irradiated birds. Significant depression of antibody responses has occasionally been observed in groups of chickens thymectomized at hatching<sup>21</sup>, but in most instances such birds have had relatively normal antibody levels. The results of the present series suggest that the thymus-dependent tissue in the chicken spleen is sufficiently developed at hatching to respond adequately to antigenic challenge, and that it can continue to do so in the maturing chicken despite the absence of the thymus itself.

These results offer new perspectives not only of the many bursectomy experiments, beginning with the historical work of Glick *et al.*<sup>7</sup>, but also on the recent thymus work.

Generally, the bursectomy investigations, whether the ablation was surgical or chemical, have involved a quantitative suppression of plasma cells, immunoglobulins, particularly the 7S component, and antibody production. Results within series and between series have been quite variable, even allowing for differences in method and strain of chicken. Quite consistent with this picture of residual capacity for antibody production has been the failure to find characteristic morphological changes in the spleens of hormonally or surgically bursectomized chickens, except for the dearth of plasma cells<sup>21,26</sup>.

The effect of bursectomy on the peripheral component of the bursal lymphoid system is seen clearly only in the irradiation model in which the unmistakable bursa-like follicles are eliminated. Since surgical bursectomy alone immediately after hatching does not prevent the development of these follicles, 'peripheralization' of this lymphoid development is apparently well under way at the time of hatching although the follicles themselves are not recognizable morphologically until 4-5 weeks of age.

Treatment of chickens with testosterone propionate during the early embryonic period produces a striking reduction in the bursa-dependent follicles in some of the treated birds. The thymic damage in these birds led Warner *et al.*<sup>21</sup> to associate this reduction or absence of splenic follicles with the influence of the hormone on the thymus. We believe, however, that the splenic follicles they were quantitating were the bursa-dependent follicles, although they did not differentiate these two forms of lymphoid tissue.

The bursa system is brought out in bold relief in another context. As noted earlier, Peterson *et al.*<sup>22</sup> recently showed that the presence of the bursa of Fabricius is necessary for the development of the avian leukosis, visceral lymphomatosis. We might expect, if the concept of the bursa system is valid, to find exaggeration of the bursal follicles in visceral lymphomatosis. This was described ten years ago by Lucas *et al.*<sup>27</sup>, who found that infection of chickens with the virus of avian leukosis resulted in a three-fold increase in the quantity of follicular lymphoid tissue (bursa-dependent tissue in our view). They stated clearly that visceral lymphomatosis did not involve the white pulp (thymus-dependent tissue). They regarded the abnormally proliferating structures of the spleen as ectopic lymphoid structures, although they noted similar structures in normal chickens which were less numerous and less dramatic. The similarity of the structures involved in this form of malignancy and the normal bursal follicle is apparent when Fig. 6 is compared with Fig. 1.

Since the peripheral component of the thymic lymphoid system develops very early compared to the bursal follicles, the role of the thymus has evidently been partially masked in the earlier investigations involving thymectomy immediately after hatching. The variable effects on skin grafts and delayed allergy may well reflect slight strain differences, or even individual differences, in the degree of this peripheral development at the time of hatching. That the chicken thymus, like the thymus of mice, rats, rabbits, and hamsters, has a role in antibody production is shown in the work recorded here in which the peripheral development was reduced by radiation. The response of the thymectomized-irradiated animals



Fig. 6. Spleen section of chicken with early visceral lymphomatosis, showing exaggeration of involved bursa-dependent follicles (haematoxylin and eosin,  $\times c. 166$ )



was significantly lower than that of the irradiated controls. In the postulated two-cell system of antibody production, the thymus-dependent cells apparently provide the recognition mechanism. The bursa system will continue to form plasma cells and produce immunoglobulins when the thymus system is awry, but its efficiency in terms of specificity is much reduced. We believe that this is what we and other investigators have observed in thymectomized mice: appreciable numbers of plasma cells, relatively normal levels of immunoglobulins, but complete loss of capacity for specific antibody response to some antigens, a variable loss of responsiveness to other antigens, and a normal level of reactivity to still others<sup>11,12,15</sup>.

It seems probable that a thymus-type of function is exercised by the appendix of the rabbit, and perhaps by other organs in that and other species. The investigations which demonstrated this function of the rabbit appendix experimentally had as their point of departure morphological similarities of the appendix to the chicken bursa<sup>38-40</sup>. In addition, both of these tissues, as well as the thymus, have a similar embryological origin, either in close association with or directly from the gut epithelium<sup>41,42</sup>. Early removal of the rabbit thymus or appendix or both curtailed specific antibody production<sup>39,40</sup>, but it left intact the plasma cells and a portion of the follicular lymphoid structure in such tissues as the spleen and lymph nodes. Indeed, we<sup>43</sup> observed plasmacytosis in the lymphoid tissues, 'autoimmune' processes, amyloidosis, and at least normal immunoglobulins. It appears that even the thymectomized-appendectomized rabbit retains the mammalian equivalent of the bursal system, and that its locus is another of the gut-epithelium-associated lymphoid organs.

In sex-linked recessive agammaglobulinæmia, the Bruton type, the patients seem to have a loss analogous to that of the bursectomized-irradiated chickens of the present study, as noted earlier. These children have normal thymic morphology and usually have normal numbers of small lymphocytes in the peripheral blood. They recognize 'foreignness', develop delayed allergy, and usually reject skin homografts, though by a slow abnormal process. By contrast, they are almost completely lacking in plasma cells, in true follicles in the lymphoid tissues, and in capacity to form any of the immunoglobulins or circulating antibody. Especially striking in the present context is the failure of development of palatine or pharyngeal tonsils in these patients<sup>44,45</sup>. The human tonsils show close morphological resemblances to the chicken bursa of Fabricius, develop in close association with the epithelium of the gut, and have a similar ontogenetic history, reaching maximum development prior to puberty and then undergoing striking involution.

Thus, it seems more important than ever to test the hypothesis that a mammalian equivalent of the bursa may exist in the palatine and pharyngeal tonsils, or in other gut-epithelium-associated lymphoid tissue such as the intestinal tonsil or sacculus rotundus of the rabbit. This work is in progress.

Many problems, both clinical and basic, take on new perspectives if we consider that there is a two-way division in the lymphoid system. The first, which might be termed 'horizontal', is the distinction between 'central' gut-epithelium-associated lymphoid tissues (the thymus, bursa of Fabricius, appendix, and other tissues to be defined) and 'peripheral' lymphoid tissues, primarily the spleen and lymph nodes. The central tissues are source tissues, using the terminology of Beard<sup>46</sup>, who described the thymus in these terms before 1900. The peripheral tissues are derivative in ontogenetic development, dependent on the central tissues in late embryonic and early postnatal life, but apparently relatively autonomous in the mature organism.

The second division, termed 'vertical', is based on the Bruton type of agammaglobulinæmia and the chicken

model of agammaglobulinæmia just described. It places in one category the bursa of Fabricius (an equivalent unidentified tissue in mammals), the bursa-like follicles of the spleen, nodes, and other tissues (clearly present in mammals), and the plasma cells; and in the other the thymus, other thymus-like central lymphoid tissue, the white-pulp type of lymphoid development in the peripheral tissues, and the small lymphocytes. Functionally, we see this vertical division as immunological recognition and information on one hand, and specific antibody production on the other.

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# MOLECULAR MECHANISM OF FORMATION OF AN ANTIGEN-ANTIBODY COMPLEX

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IT is usual to describe the formation of antigen-antibody complexes in terms of the lattice theory of Marrack<sup>1</sup>, in which bivalent antibody molecules cross-link determinant sites on antigenic particles or molecules and thus build up a complex network. The (7S  $\gamma$ -globulin) antibody molecule has been described by Oncley<sup>2</sup> as a cigar-shaped particle, of axial ratio 5:1 and length more than 200 Å. In terms of this latter model, it has seemed reasonable to assign the two binding sites to opposite ends of the elongated antibody molecule, and to devise models for the lattice on this basis. Recently, Almeida, Cinader and Howatson<sup>3</sup>, and Lafferty and Oertlis<sup>4</sup>, have attempted to interpret negative-contrast electron microscope pictures of antibody bound to virus particles in these terms.

We have examined pure 7S  $\gamma$ -globulin preparations in the electron microscope (by shadowing and by negative contrast), and complexes of horse-spleen ferritin with rabbit anti-ferritin (by negative contrast). Both rabbit and human pure 7S  $\gamma$ -globulin have been examined, but no differences were observed. Our results suggest a different

mechanism for the building up of antigen-antibody complexes from that described here.

Shadowed pictures of 7S  $\gamma$ -globulin (Fig. 1a) show fields of particles the width (for 90 per cent of the particles in random fields) of which varies between 80 Å and 120 Å, and the thickness (estimated from shadow length) of which is constant at about 34 Å. By negative contrast (Fig. 1b), using a low-temperature method of preparation (to be discussed elsewhere), the particles seen have a low degree of asymmetry, and a maximum linear dimension of about 105 Å. The results from the two techniques are

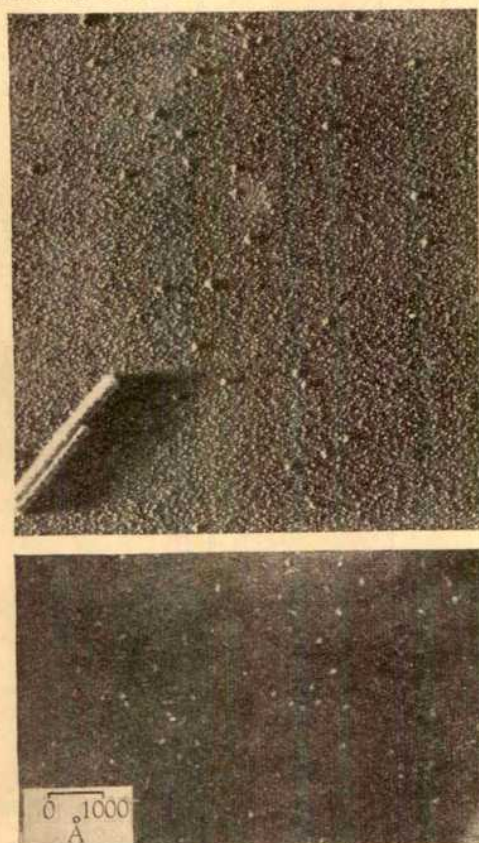


Fig. 1. Electron micrographs of purified 7S  $\gamma$ -globulin, using the Siemens Elmiskop, examined by (a) shadowing, (b) negative contrast. (a) Rabbit  $\gamma$ -globulin, platinum shadowed at an angle of 15 to one with tobacco mosaic virus as marker; photographed at initial magnification  $\times 3,500$  and an accelerating voltage of 40 kV. (b) Human  $\gamma$ -globulin in 8 per cent sodium tungstoborate, pH 6.2, dried down over holes in carbon film (ref. 9); photographed at initial magnification  $\times 40,000$  and an accelerating voltage of 80 kV.

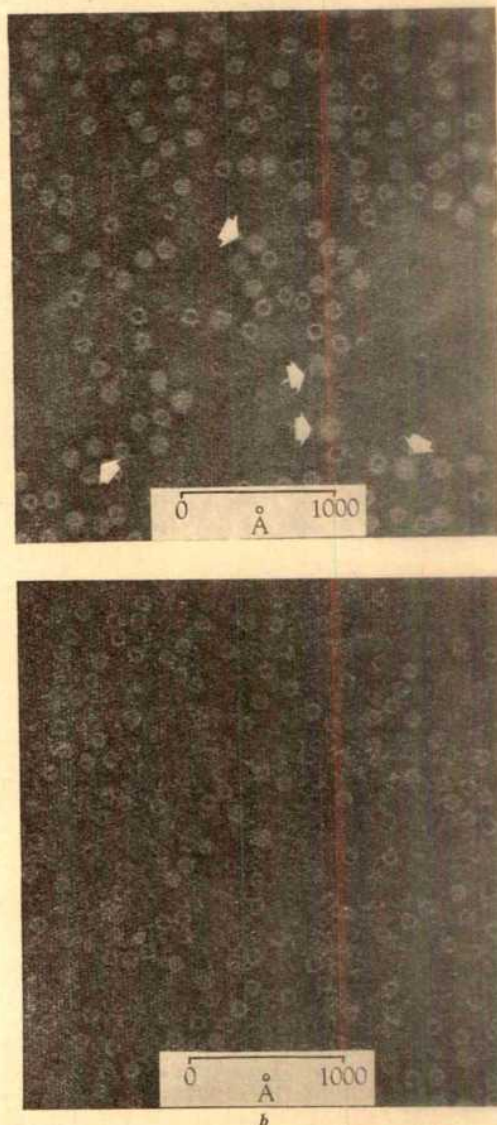
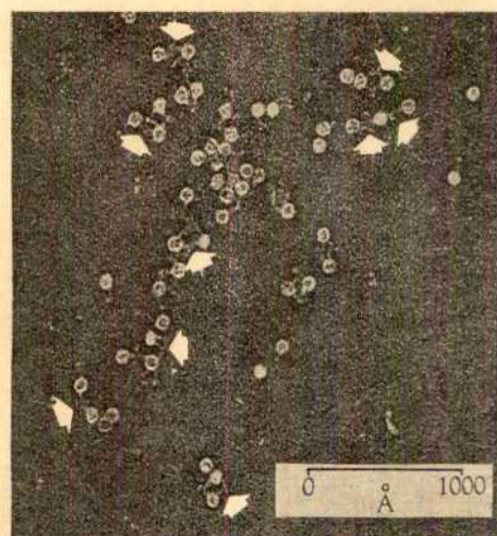
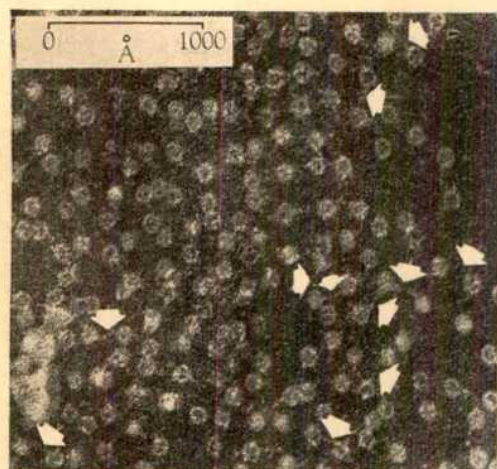


Fig. 2. (a) Electron micrograph of a mixture of ferritin and antiferritin, with a large excess of the ferritin antigen. Ferritin molecules with antibody bound to them are indicated by arrows. (b) Control ferritin. Microscopy details as for Fig. 1b.

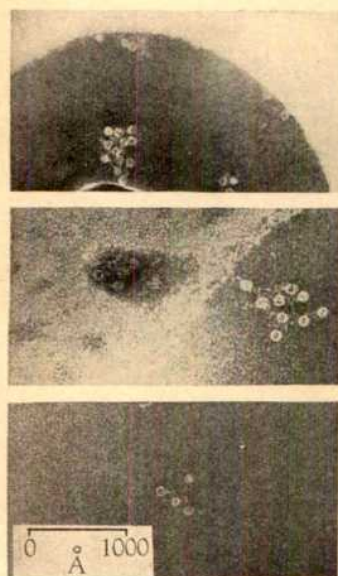




a



b



c

Fig. 3. Electron micrographs of ferritin-antiferritin complexes at proportion near to equivalence, showing cross-linking strands between ferritin molecules. (a) Intact antibody, (b) pepsin-treated antibody, (c) selected areas, with intact antibody. Microscopy details as for Fig. 1b

in good accord, and contradict the picture of 7S  $\gamma$ -globulin as a cigar-shaped particle.

Pictures of ferritin-antiferritin systems at large antigen excess, where no insoluble complex is formed, can readily be explained in terms of one or more such molecules binding without change of shape to the ferritin molecules (approximate diameter 105 Å, by negative contrast) as shown in Fig. 2. The entire absence of cross-linking strands between the ferritin molecules is to be noted. However, at antigen-antibody ratios nearer to equivalence, when a lattice is formed, a large number of rod-like strands, of diameter approximately 15 Å, can be seen cross-linking the ferritin molecules (Fig. 3). If pepsin-treated antibody is used<sup>5</sup>, the strands seen are similar, but show rather less complexity of form (Fig. 3b), presumably owing to the known removal by pepsin of part of the molecule not essential in precipitation. We interpret these strands as corresponding to a 'backbone' feature in the tertiary structure of the antibody molecule, this backbone being non-penetrated by the negative contrast medium. Where these linkages are straight, their maximal length is a little more than 200 Å, which is twice the length of a single, intact antibody molecule; however, it is more usual to see a kink in the linkage, located midway along the length, and the angle of this kink may vary over a wide range (Fig. 4).

We interpret these observations as meaning that in the original antibody molecule the two binding sites are situated fairly close to each other in a compact structure. This compact structure is retained if only a single antigen is bound by the antibody. When, however, cross-linking of antigenic particles occurs, then the antibody molecule 'clicks open' to varying degrees about a 'hinge point' at one end of the molecule, enabling a linkage twice as long as the original antibody molecule to be formed. This theory is illustrated diagrammatically on the right-hand side of Fig. 4.

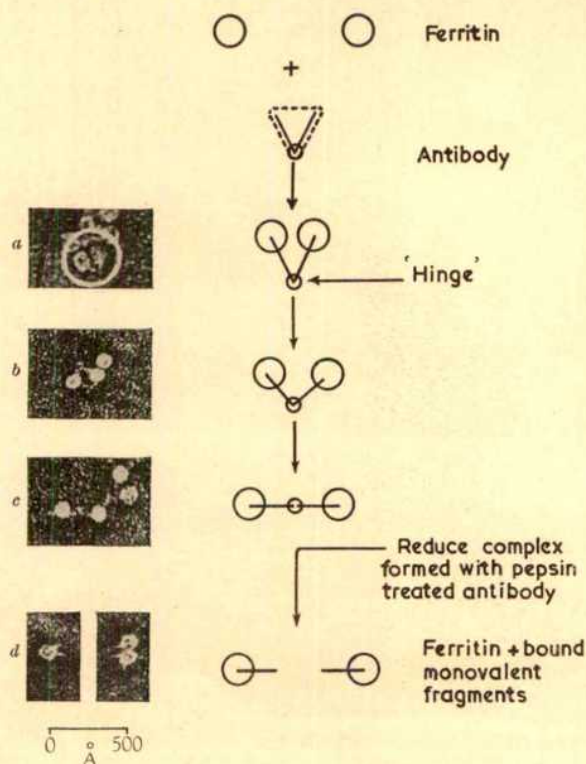


Fig. 4. Selected areas from electron micrographs to show the various types of linkage observed in ferritin-antiferritin complexes (a-c), and the effect of reduction following pepsin treatment (d). Corresponding to the linkages illustrated, the right-hand side of the figure gives a diagrammatic representation of the 'click-open' theory of cross-linking described in the text. Microscopy details as for Fig. 1b



By using complexes formed with pepsin-treated antibody and, in these precipitated and washed complexes reducing a single disulphide linkage per antibody molecule with mercaptoethanol<sup>6</sup>, the precipitate is completely redissolved. From the solution, pictures of monovalent fragments of antibody bound to ferritin have been obtained (Fig. 4d). These fragments are rod-like, not longer than 100 Å, and show no sign of a kink. This experiment demonstrates that the molecular 'hinge' about which the 'click-open' occurs is almost certainly associated with a disulphide linkage.

In connexion with this theory it should be noted that ferritin, like the viruses, is composed of many identical sub-units and thus identical antigenic sites are repeated within the molecule. There is thus the possibility of the two binding sites of the antibody molecule being satisfied within a single antigen molecule. In other antigen-antibody systems, where this saturation of the two sites by one antigen molecule is not possible, a different form of soluble complex from that observed here (Fig. 2) might occur. It is, however, a general feature of soluble complexes formed in antigen excess that they fix little complement<sup>7,8</sup>. Our theory suggests a simple explanation for

this: the 'click-open' mechanism, which occurs only in insoluble complexes, exposes a part of the 7S  $\gamma$ -globulin molecule which was hitherto masked, and enables it to bind with complement.

Our pictures reveal a distinct tendency for the linkage in insoluble complexes to be through two, rather than one, antibody molecules (Fig. 3). It may be that this double linkage is necessary for the formation of a stable cross-link.

A full account of this work and a more detailed interpretation will be published shortly.

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## PORTAL BLOOD SUPPLY TO GLOMUS TISSUE AND ITS SIGNIFICANCE

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THIS article describes a portal system for the flow of venous blood through glomus (chemoreceptor) tissue. The glomera are situated: (1) between the ascending aorta and pulmonary artery (aorticopulmonary glomus tissue); (2) on the arch of the aorta; (3) near the roots of the subclavian arteries (aortic bodies); (4) scattered throughout the cervical and abdominal regions in association with autonomic nerves and ganglia<sup>1-7</sup>. The carotid bodies are not discussed as their blood supply, unlike that of the other glomera, arises directly from the arterial system.

Howe<sup>8</sup> is the only one to have considered the venous drainage of the 'aortic bodies'. He concluded that they were richly vascularized and possessed an arterial supply and a venous drainage. The arterial supply has been investigated many times. The arteries have been described as end-arteries arising from the nearest major vessel.

The usual methods of investigation—microdissection or serial sectioning after injection, or block staining—are time consuming and difficult to interpret. To circumvent these difficulties the present investigator used the well-recognized technique for the investigation of vasa vasorum of injection of Indian ink, with or without gelatin, followed by dehydration and clearing. The blood was washed out with a slightly hypertonic saline solution (1.0 per cent) containing glyceryl trinitrate (1.2 mg/250 ml.). Injection was performed with pulsatile pressure, retrogradely into the descending aorta (160/80 mm mercury) or into the thoracic inferior vena cava (50/20 mm mercury). The cat was chosen as the experimental animal because it is a convenient size and its compact tissue can be recognized in whole-mount preparations<sup>9</sup> by the characteristic appearance of its injected sinusoids (Fig. 1). This was repeatedly confirmed by histology (Fig. 2).

The aorticopulmonary glomus tissue is densest at the base of the heart between the ascending aorta and the pulmonary artery but continues as far as the ligamentum arteriosum. It is also much more intimately associated with the anterior and posterior aspects of the pulmonary artery than has previously been described. Fig. 3 is a

diagram of the typical vascular connexions of a mass of aorticopulmonary glomus tissue. This arrangement was demonstrated in the wall of the pulmonary artery in more than 40 cats. The coronary arteries supply the vasa vasorum of the pulmonary artery and ascending aorta and convey blood to an unusually extensive capillary network specific to the outer half of the media of the main pulmonary artery. Most of the venae vasorum which drain this network enter glomus tissue at the adventitia-medial junction and divide into thin-walled sinusoids in intimate contact with glomus cells (Fig. 4). The sinusoids re-form

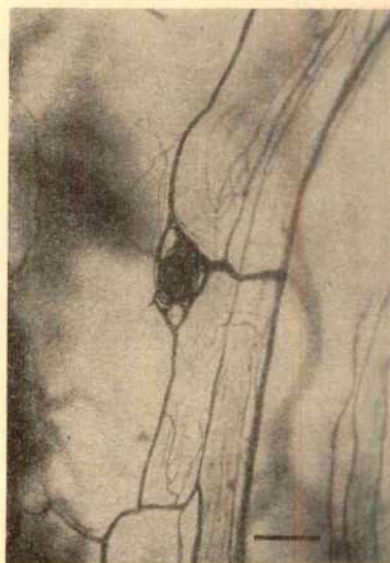


Fig. 1. The glomus, outlined by the indian ink in its sinusoids, is on a vein draining the left depressor nerve and to the left of it as it crosses the anterior surface of the left subclavian artery, just above the arch of the aorta of the cat. The vein leaving the top of the glomus was eventually traced to the superior vena cava. The nerve is also indicated by the ink in its vessels. The line is equivalent to a length of 0.25 mm.





Fig. 2. An 8- $\mu$  paraffin section, stained with haematoxylin and eosin, of the glomus in Fig. 1. Parts of the nerve can be seen on the right, top, and bottom, and parts of the injected vein and sinusoids are present. A small nerve bundle enters the glomus from the right and an arteriole occupies the inverted 'V' made by the vein over the top of the glomus. (Line, 0.1 mm)

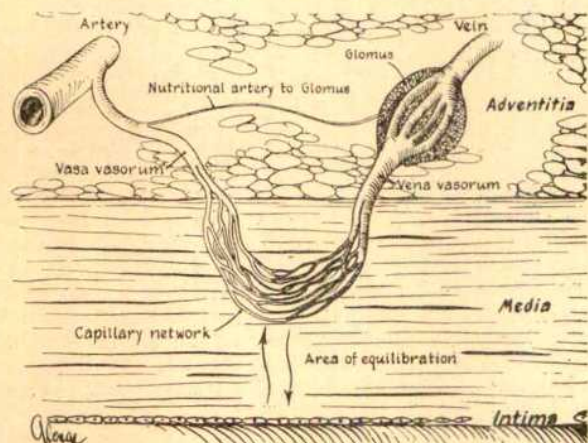


Fig. 3. Diagram of the vascular connexions of the aorticopulmonary glomus tissue of the cat. The blood in the capillary network probably comes into chemical equilibrium with that in the pulmonary artery lumen by diffusion

veins that eventually drain into the superior vena cava or the coronary sinus.

A portal system similar to that in the aorticopulmonary glomus tissue is present on the anterior and posterior aspects of the arch of the aorta. The capillaries, which penetrate deeper into the media than in adjacent aortic areas, often have small dilatations on their course and appear to radiate to and from a central axis of an artery and vein instead of forming part of a continuous network as in the media of the pulmonary artery. The exact location of these sharply circumscribed capillary areas is variable but appears to correspond to known pressoreceptor areas. The glomera, into which this aortic portal system drains, are more widely distributed on the anterior and posterior surface of the aortic arch than has been indicated in the literature.

Glomera were found on the course of veins draining from autonomic nerves in each of twelve preparations of the mediastinal and cervical tissues. The number of glomera varied from three to eight but this variability may be a reflexion of the completeness of the injection. The right aortic body was found in three cats and the left aortic body in seven; this variability confirms previous findings<sup>8</sup>. The aortic bodies appear to be fortuitous examples

of the random glomera scattered in the mediastinum and are intimately concerned with the venous drainage of the depressor nerves and of the areas of vessel wall innervated by the depressor nerves which are recognized pressoreceptor areas.

The retroperitoneal tissues of eight cats were injected through the abdominal aorta or inferior vena cava. In each, masses of chemoreceptor tissue were seen to be closely associated with autonomic nervous structures and to possess a similar portal venous drainage.

That glomera are supplied by one or more fine arteries is not denied (Fig. 5). The large arteries that penetrate glomus tissue—especially the aorticopulmonary—continue beyond it; they are not the end-arteries implied or depicted by previous investigators. This is obvious in cleared specimens, but would not be so by microdissection or from serial sections. These fine arteries (analogous to the hepatic arteries) probably supply oxygen to chemoreceptor cells, which are thought to have a high metabolic rate. No true arteriovenous anastomoses were seen.

The findings recorded here differ from those of Howe and others by the demonstration of this portal system which allows one to postulate the physiological roles and explain the distribution of these enigmatic structures<sup>6,10</sup>.

The aorticopulmonary glomus tissue is intimately associated with the pulmonary artery and its unique



Fig. 4. The posterior aspect of the pulmonary artery of the cat has been injected, dehydrated, and cleared. The specimen is viewed through the intimal surface. The capillary network drains into a vein which passes to the adventitia-medial junction where it divides into thin-walled sinusoids surrounded by glomus cells. (Line, 0.1 mm)



Fig. 5. An 8- $\mu$  paraffin section (haematoxylin and eosin) of an injected aorticopulmonary glomus of a cat. A large muscular artery passes through the glomus and a fine branch leaves its inferior surface. These branches are comparatively infrequent in serial sections. Several injected sinusoids can be seen surrounded by glomus cells. (Line, 0.05 mm)



capillary network<sup>3</sup>. It is possible that its portal circulation carries to the glomera blood that has reached chemical equilibrium with the mixed venous blood flowing in the pulmonary artery by diffusion across the inner half of the media (area of equilibration; Fig. 3). The chemical environment of the glomus cells would therefore be determined partly by the gradient of diffusion of gases from the blood in the pulmonary artery and partly by the rate of flow of arterial blood into the capillary network. The latter is a measure of the blood pressure and of the vaso-motor tone in the vessels of the system. It is thus possible to reconcile the experimental data of Duke *et al.*<sup>11</sup> with the absence of a vessel from the pulmonary artery lumen to chemoreceptor tissue in the adult animal<sup>12-14</sup>.

The physiological role of a chemoreceptor for mixed venous blood is not evident at the present time<sup>15</sup>. The functioning of this tissue in the foetal animal, suggested by a special branch of supply from the pulmonary artery which closes at birth, indicates that the tissue is concerned with the control of the cardiovascular rather than the respiratory system. This could be its function in the adult.

The glomera associated with the autonomic nerves, ganglia, and pressoreceptor areas are probably protective. These autonomic structures are vital to the animal and an adequate local blood supply must be maintained despite the circulatory readjustments demanded by severe exercise, haemorrhage, or asphyxia. The most logical situation for such a protective chemosensitive mechanism

is on the venous side of the capillaries involved. The anatomical evidence presented demonstrates that the sinusoids of the glomera are part of a portal system carrying effete blood from these capillaries. In the same way the sinusoids of the suprarenal medulla carry venous blood from the suprarenal cortex. Thus the medullary glomus tissue is in a position to 'measure' and increase the blood supply to the cortex. This could explain the puzzling association of the two apparently unrelated tissues. The neurones within the substance of glomus tissue may be part of a local reflex mechanism to bring about local circulatory readjustments.

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## AUTORADIOGRAPHIC INVESTIGATIONS OF RNA AND DNA METABOLISM OF HUMAN LEUCOCYTES CULTURED WITH PHYTOHÆMAGGLUTININ; URIDINE-5-<sup>3</sup>H AS A SPECIFIC PRECURSOR OF RNA

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THE pattern of cellular change in cultures of leucocytes from normal peripheral blood, incubated with phyto-hæmagglutinin (PHA), is well established<sup>1-3</sup>, and the metabolic events associated with the process of transformation of numbers of lymphocytes to nucleolated prolymphocytes and then to primitive blast cells during the initial 72-96 h of culture have been further clarified by cytochemical and autoradiographic methods. The periodic acid-Schiff (PAS) reaction, various dehydrogenase reactions, and the use of tritiated thymidine as a specific precursor of DNA have been especially helpful in this regard<sup>4,5</sup>. The increase in cytoplasmic basophilia during transformation suggests that active synthesis of RNA is occurring and that the transforming cell population would be suitable also for investigating the uptake of an appropriate labelled precursor of RNA.

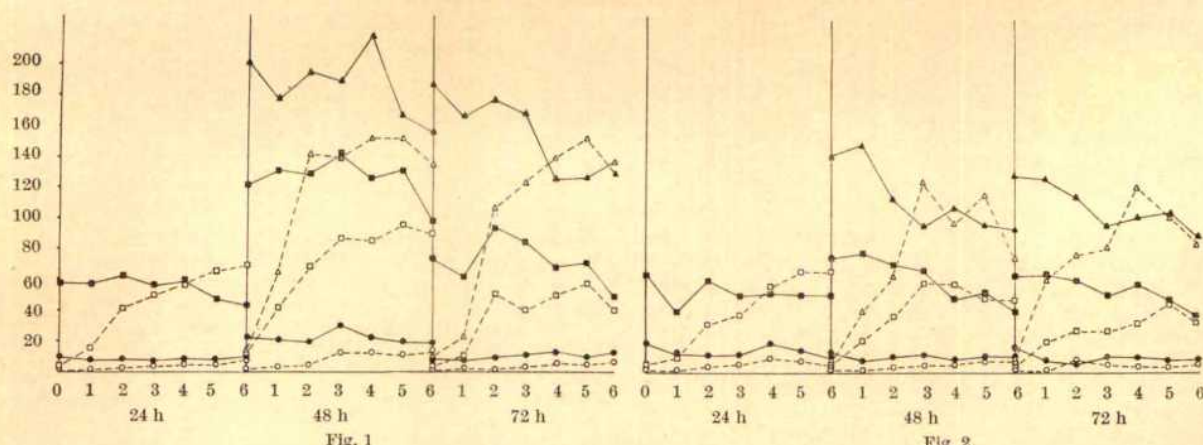
Autoradiographic investigations of RNA synthesis within cells have hitherto been hampered by the lack of labelled precursors which could be specifically incorporated into RNA but not into DNA. Tritiated uridine might be expected to act as a suitable precursor if methylation at the 5-position with subsequent incorporation into DNA of the thymidine so formed could be prevented, or if the process of methylation itself led to a loss of the radioactive label. Uridine tritiated in the 5-position has recently become available from the Radiochemical Centre, Amersham. This compound should lose its tritium label on conversion to thymidine and should therefore serve as a specific precursor of labelled RNA.

We have used this newly available compound, uridine-5-<sup>3</sup>H, to examine autoradiographically the extent of RNA synthesis in lymphocytes, prolymphocytes and blast cells present in cultures of leucocytes from normal peripheral blood, incubated with PHA.

There is much evidence from biochemical and autoradiographic investigations that cells incubated with a radioactive precursor of RNA may rapidly acquire nuclear radioactivity and that when these cells are transferred to non-radioactive medium there is a progressive shift of radioactivity from nucleus to cytoplasm. Whether this shift represents a movement of stable RNA or is a consequence of breakdown of nuclear RNA and diffusion of acid-soluble end-products is still uncertain. Contributions to this problem and references to the relevant literature may be found in several recent articles<sup>6-10</sup>. Although various different strains of cells have been used in the reported examinations they do not include normal human leucocytes stimulated by PHA, and we have therefore made preliminary observations on the movement of label from nucleus to cytoplasm in these cells during a 6-h period of chase following the initial exposure to uridine-5-<sup>3</sup>H and subsequent transfer of cells to non-radioactive medium.

Parallel investigations of DNA synthesis were also performed using tritiated thymidine as precursor, to allow comparison to be made between the respective localizations and time-sequence of uptake of the two labelled compounds. Earlier investigations having shown that prolymphocytes were usually rich in glycogen while blast cells were almost invariably devoid of this substance, the PAS reaction was used in addition to Romanowsky stains to assist in the identification of cells in the autoradiographic preparations and to provide additional data on metabolic relationships during transformation.

At 48 and 72 h when cells with active DNA synthesis were present, the distribution of labelled thymidine was also followed during a 6-h period of chase in non-radioactive medium, in order: (a) to detect the first appearance



Figs. 1 and 2. Nuclear and cytoplasmic grain counts in lymphocytes, polymorphocytes and blast cells from 24-, 48- and 72-h cultures of leucocytes from each of two normal subjects during 6-h periods of chase following incubation for 1 h in uridine-5-<sup>3</sup>H. ○, Lymphocytes cytoplasm; ●, lymphocytes nucleus; □, polymorphocytes cytoplasm; ■, polymorphocytes nucleus; △, blasts cytoplasm; ▲, blasts nucleus

of labelled mitotic figures and thus to assess the duration of the  $G_2$  period of post-synthetic premitotic rest; (b) to determine whether any shift of radioactivity occurred within the cell.

Cultures of leucocytes from the peripheral blood of two normal subjects were set up with PHA in the manner previously described<sup>4</sup>. From each, 3.5 ml. aliquots were removed at 24, 48 and 72 h and incubated at 37° C with uridine-5-<sup>3</sup>H at a final concentration of 0.5  $\mu$ Ci/ml. for a period of 1 h. Further aliquots were similarly incubated with tritiated thymidine at the same concentration and for the same time. From all the uridine-labelled aliquots and from the 48- and 72-h thymidine-labelled aliquots, samples were then aspirated, the cells deposited by centrifugation and smears made on gelatin-coated slides. The remaining suspensions were centrifuged, the radioactive supernatants removed, the cells washed once with fresh medium and finally re-suspended in a further change of fresh medium. Afterwards 0.5 ml. samples were removed at hourly intervals for 6 h and smears made of the centrifuged deposit. All smears were kept in contact with Kodak 'AR10' stripping film for 6 days and after development were appropriately counterstained.

In the uridine-labelled specimens separate nuclear and cytoplasmic grain counts were performed in lymphocytes, polymorphocytes and blast cells and the average scores for each cell type, based on 25-100 cells scored, were calculated. Due allowance was made for the presence of background activity, by appropriate subtraction.

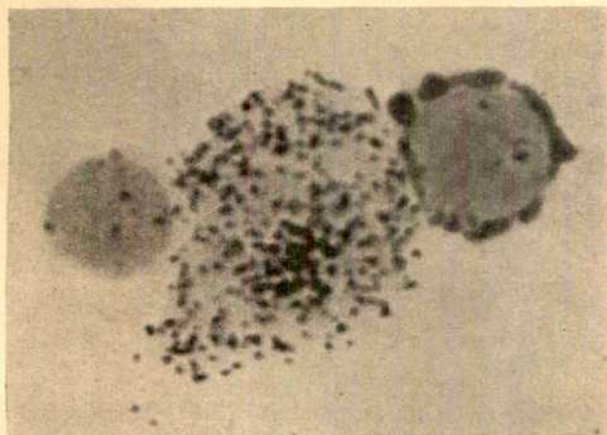


Fig. 3. A polymorphocyte from a 48-h culture after incubation for 1 h in uridine-5-<sup>3</sup>H and subsequent incubation in non-radioactive medium for 5 h. The grain distribution is fairly even throughout the cytoplasm, while there is a strong concentration of radioactivity in the nucleolar region of the nucleus. The two accompanying lymphocytes, one of which has coarse PAS positivity in the cytoplasm, are virtually negative

The results of the uridine-5-<sup>3</sup>H investigations are presented graphically in Figs. 1 and 2. In both cases the patterns of uptake and distribution were essentially similar. The localization of radioactivity was almost exclusively nuclear in all cell-types immediately after incubation in the radioactive medium, whether at 24, 48 or 72 h of culture. Uptake was small in lymphocytes, greater in polymorphocytes and very marked in the blast cells which had appeared in the 48- and 72-h specimens. A conspicuous concentration in the region of the nucleolus was frequently apparent. During the 6-h period of chase there was a tendency for an irregular fall in nuclear grain counts to take place, accompanied by a considerably steeper and more uniform rise in cytoplasmic grain counts especially during the first 2-5 h. The increasing grain density in the cytoplasm was usually fairly evenly distributed, but the nuclear positivity often continued to show local accentuations in the nucleolar areas (Fig. 3). In the 48- and 72-h preparations there was usually a terminal fall in both nuclear and cytoplasmic grain counts. It is clear from the figures that the sum of nuclear and cytoplasmic counts rose during the period of chase. This may have been due to fresh incorporation of label which had not been completely removed during the exchange of medium or which was derived from damaged or degenerating cells. The first of these possibilities appears unlikely in the light of experiments involving

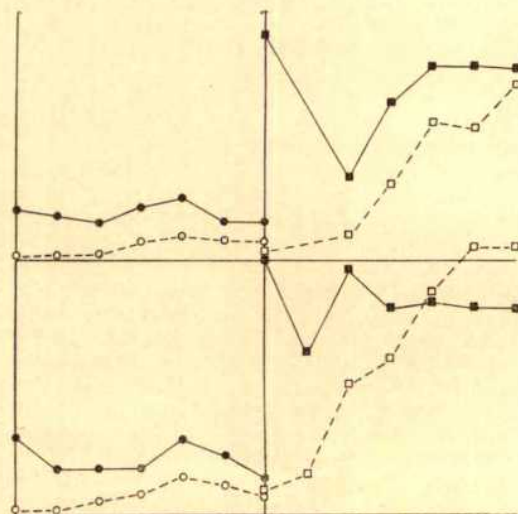


Fig. 4. Grain distribution in lymphocytes and polymorphocytes from a 24-h culture. The lower graphs show findings in cells washed only once, and the upper graphs those in cells washed three times. Symbols same as for Figs. 1 and 2



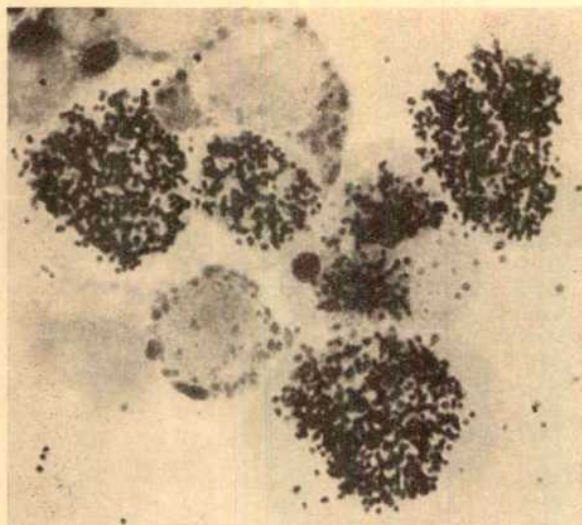


Fig. 5. Restriction of thymidine label to nucleus in blast cells from a 72-h culture after completion of a 6-h period of chase in non-radioactive medium. A cell in anaphase shows clear localization of activity to the chromosomes

Fig. 6

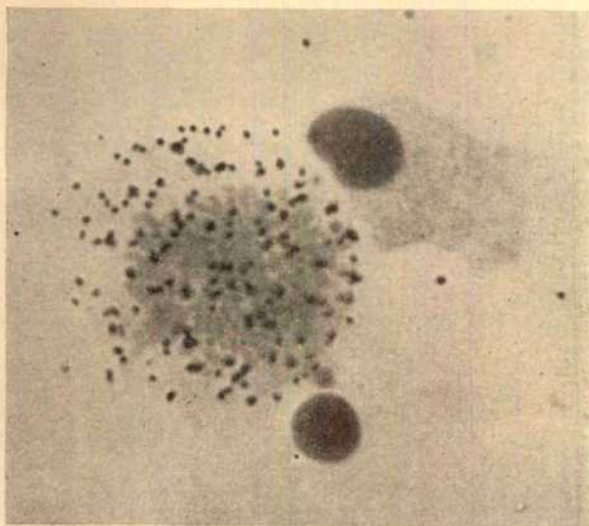
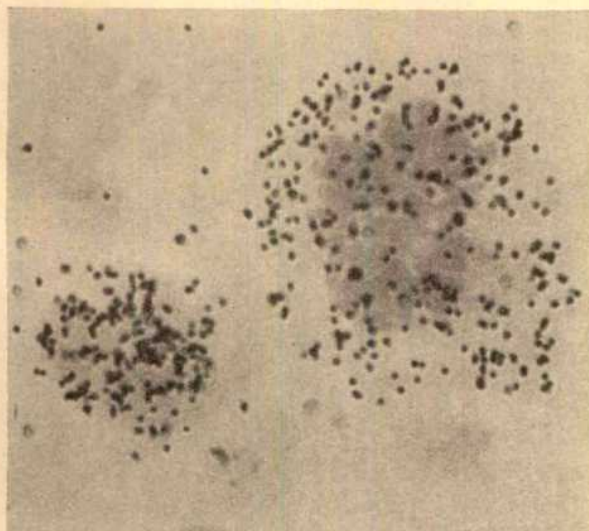


Fig. 7

Figs. 6 and 7. Distribution of uridine label in blast cells at metaphase

repeated washing of incubated cells in saline prior to their re-suspension in non-radioactive medium for the period of chase. Fig. 4 shows that the intensity and distribution of grain counts in triply washed cells from a 24-h culture did not differ greatly from those in cells washed only once, although the rate of appearance of label in the cytoplasm was slightly retarded. The second possibility is more likely, since release of RNA metabolites from cells in tissue culture and their later re-utilization by other cells has been reported<sup>11,6</sup> and a similar transfer has been observed *in vivo*<sup>12</sup>.

The general distribution and movement of the uridine label show several points of contrast with those of the thymidine label. When the cultures were first set up (0 h) approximately 60 per cent of the lymphocytes took up uridine-5-<sup>3</sup>H, and at 24, 48 and 72 h more than 90 per cent did so. These cells did not show thymidine uptake at any stage. Polymorphocytes were present in the cultures at 24, 48 and 72 h; they invariably showed uptake of uridine at all times, but the great majority failed to incorporate thymidine at 24 h and approximately 70 per cent still showed no uptake at 48 and 72 h. Blast cells were present in the cultures at 48 and 72 h; like the polymorphocytes they were always labelled after incubation with uridine, but between 20 and 30 per cent of them failed to take up labelled thymidine. Furthermore, the thymidine label remained in the nucleus with no appearance of cytoplasmic labelling and no increase in grain density during the whole of the 6-h period of chase at all stages of culture (Fig. 5).

When mitotic figures appeared in the preparations the distribution of uridine label within the dividing cells at metaphase was conspicuously away from the chromosomes (Figs. 6 and 7) whereas the thymidine label, when present, was concentrated on the chromosomes (Figs. 8 and 9). During the 6-h chase of cells incubated with tritiated thymidine, mitotic figures seen at the first and second

Fig. 8

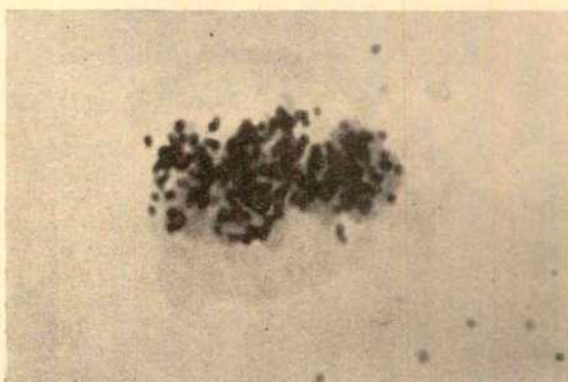
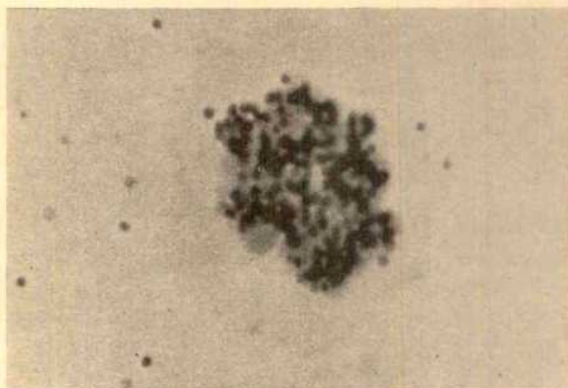


Fig. 9

Figs. 8 and 9. Distribution of thymidine label in blast cells at metaphase



hour were unlabelled, but at the third hour and later most figures showed heavy labelling. This suggests that the  $G_2$  period of rest intervening between the end of DNA synthesis and the onset of mitosis in the cultured cells under these experimental conditions is of the order of 3 h.

The observation that all prolymphocytes and blast cells took up labelled uridine during a 1-h period of incubation would imply that RNA synthesis in these cells is an almost continuous process, the rest periods, if any, being less than 1 h. In this respect the transformed cells from normal human blood appear to resemble HeLa-S<sub>3</sub> cells where RNA synthesis has been found to continue until late prophase and to resume at telophase<sup>7</sup>.

The presence in the 48- and 72-h cultures of 20-30 per cent of blast cells unlabelled after an hour's incubation in tritiated thymidine would be compatible with a duration for the  $S$  period of DNA synthesis in these transformed cells of approximately 2-3 times that of the  $G_1$  and  $G_2$  periods of rest combined.

These preliminary experiments suggest that uridine-5-<sup>3</sup>H may prove to be a satisfactory specific precursor of RNA. The nuclear labelling of many cells which show no tritiated thymidine uptake and are presumably therefore not synthesizing DNA, and the movement of label to the cytoplasm during the period of chase in non-radioactive medium, establish that RNA is being labelled. The absence of chromosomal labelling in cells with strong uptake of uridine during the later stages of culture, when DNA synthesis is also taking place, suggests that DNA is not being labelled by the precursor. Again, if DNA were incorporating a radioactive derivative of uridine-5-<sup>3</sup>H

one would expect some 30 per cent of the prolymphocytes in 48- and 72-h cultures to be much more heavily labelled than the remainder, since only this proportion is synthesizing DNA as judged by parallel experiments with tritiated thymidine. No such discrepancy was observed; the extent of labelling in prolymphocytes was scattered fairly uniformly around the mean.

We have previously shown that accumulation of glycogen precedes DNA synthesis in cells transforming under the influence of PHA (ref. 5). Whether a relation also exists between cellular glycogen and RNA synthesis is less apparent; strong labelling of RNA occurred in cells with much or little glycogen indiscriminately.

We hope that the observations reported here will provide a reference for comparison in future autoradiographic investigations of the behaviour of leukaemic cells cultured *in vitro*.

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## A NEW NEUROHYPOPHYSEAL PRINCIPLE IN AN ELASMOBRANCH, *Raia ocellata*

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**I**NVESTIGATIONS of the neurointermediate lobe (NIL) of the pituitary of various elasmobranch species (*Squalus acanthias*, *Scyliorhinus caniculus*, *Raia clavata*, *Raia batia*, *Eulania milberti*, *Carcharinus leucas*, *Dasyatis* sp., and *Sphyrna* sp.) by Perks, Dodd and Dodd<sup>1-4</sup> and Sawyer, Munsick and van Dyke<sup>5</sup> have shown that, after allowance for the arginine vasopressin content of U.S.P. standard posterior pituitary powder, the milk ejection, oxytocic and antidiuretic activities approximate to a ratio of 3:1:0.05. This indicates the presence of an oxytocin-like principle (or principles) similar to, but not identical with, oxytocin. In *Squalus acanthias* NIL, Sawyer *et al.*<sup>5</sup> also noted high frog bladder activity.

In the present work, an attempt has been made to purify the oxytocic fraction of the NIL of another elasmobranch species, *Raia ocellata*. 377 freshly caught specimens were dissected at sea. The NILs were dehydrated in acetone, desiccated and extracted in 0.25 per cent acetic acid. The oxytocic agent of the crude extract, which assayed at 18.7 (16.6-21.0) mU/mg acetone dried powder (isolated rat uterus<sup>6</sup>), was shown to be destroyed by 0.01 M sodium thioglycollate, by 0.1 N sodium hydroxide, and by ultra-violet light. The low antidiuretic potency of 1.11 (0.29-4.21) mU/mg (anaesthetized rat assay<sup>7</sup>) was in the expected range, but the rabbit milk-ejection activity<sup>8</sup> of 194 (154-245) mU/mg gave a milk-

ejection/rat uterus activity ratio of 10.3 ( $\pm 1.1$ ), approximately three to four times that of all other elasmobranch species so far investigated (for example, *Squalus acanthias*, Table 1).

Purification of the oxytocic agent was achieved by gel-filtration and ion-exchange chromatography in ammonium acetate buffers. The degree of purification was judged by the increase in oxytocic activity (rat uterus) per mg of total 'peptide' (method of Lowry, Rosebrough, Farr and Randall<sup>9</sup>). 13,635 mU of oxytocic activity contained in 24.3 ml. of crude extract, at 86.9 mU/mg Lowry peptide, were passed in three aliquots through a 3.4 cm  $\times$  91 cm column of 'Sephadex G-25', medium grade. A 5.48 fold purification was achieved. The eluate assayed at 476.9 mU/mg Lowry peptide, and 83.1 per cent of the initial oxytocic activity was recovered. The active eluate was diluted to a specific conductivity of 0.111 mmho/cm and applied to a carboxymethyl cellulose column at pH = 5.0. The oxytocic activity was not adsorbed. The eluate was then brought to pH = 7.1, specific conductivity 0.143 mmho/cm, and passed through a DEAE-'Sephadex' column (A-25, coarse grade). Again, the oxytocic activity was not adsorbed. However, after adjustment of the pH of the eluate to 5.5, specific conductivity = 0.150 mmho/cm, the oxytocic activity was completely adsorbed on a 1 cm  $\times$  23 cm 'CM-Sephadex' column (C-25,

Table 1

Species	Oxytocic activity (rat uterus, without Mg) $\mu$ U/mg acetone dried powder, or $\mu$ U/ml. (95% confidence limits in parentheses)	Ratio of assay values indicated/rat uterus value (without Mg) (S.E. in parentheses)				
		Rat uterus with 0.5 mM Mg	Rabbit milk ejection	Rat antidiuresis	Rat vasopressor	Frog bladder
<i>Squalus acanthias</i> * crude extract $\mu$ U/mg powder	30 (26-34)	1.6 ( $\pm 0.12$ )	2.3†	0.03	0.04	50
<i>Raia ocellata</i> crude extract $\mu$ U/mg powder	18.7 (16.4-21.0)	—	10.3 ( $\pm 1.1$ )	0.059 ( $\pm 0.025$ )	—	—
<i>Raia ocellata</i> purified extract $\mu$ U/ml.	32.8 (30-36)	2.6 ( $\pm 0.2$ )	8.5† ( $\pm 1.0$ )	0.04 ( $\pm 0.004$ )	< 0.02	< 0.46

\* Revised from Sawyer *et al.* (1961), Maine Powder.

† Milk ejection assays carried out against U.S.P. Standard Powder; adjusted for arginine vasopressin content of this standard according to  
 $\frac{\text{Synthetic oxytocin ('Syntocinon')}}{\text{U.S.P. Standard}} = 0.8$

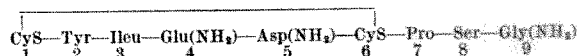
medium grade). Washing with 0.0013 M ammonium acetate, pH = 5.5, specific conductivity 0.135 mmho/cm, did not remove the oxytocic activity, but when the buffer was replaced by 0.02 M ammonium acetate, pH = 6.0, specific conductivity 1.93 mmho/cm, the oxytocic activity eluted in a volume of 336 ml. Final wash with 0.2 M ammonium acetate, pH = 7.0, specific conductivity 16.3 mmho/cm, failed to displace any further oxytocic material from the column. The active eluate contained 80 per cent of the oxytocic activity of the crude extract, but 98.7 per cent of the original Lowry peptide had been removed; the final potency of the peptide was 5,568  $\mu$ U/mg Lowry peptide, which represented a 64-fold purification of the crude extract. Since pure synthetic oxytocin contains about 133  $\mu$ U/mg Lowry peptide, the elasmobranch peptide appears to have only 4.2 per cent of the specific oxytocic activity of oxytocin. The Lowry peptide figure of 133  $\mu$ U/mg for synthetic oxytocin corresponds to a true specific activity of 420  $\mu$ U/mg weight. This suggests that the specific activity of the *Raia ocellata* peptide is 17.6  $\mu$ U/mg weight.

The purified extract assayed at 32.8 (30-36)  $\mu$ U/ml. on the isolated rat uterus without magnesium, and at 86 (77-96)  $\mu$ U/ml. by the same preparation with 0.5 mM magnesium added. The rabbit milk ejection activity was relatively high at 281 (235-337)  $\mu$ U/ml., but the rat anti-diuretic activity assayed at only 1.42 (1.3-1.6)  $\mu$ U/ml. No rat vasopressor activity was found (< 0.8  $\mu$ U/ml.), nor could frog bladder activity be detected (< 15  $\mu$ U/ml.). The ratio of milk ejection to rat uterus activity ( $8.5 \pm 1.0$ ) does not appear to have been significantly altered during purification, since the standard errors for crude and purified extracts overlap one another; the ratio remains more than that for *Squalus acanthias* (Table 1), and more than that for other species so far examined. The absence of any detectable frog bladder activity is also in contrast to the high activity found in crude *Squalus* extracts. Therefore the present evidence suggests that the oxytocic agent of *Raia ocellata* is different from that of *Squalus acanthias* and from the principles found in other elasmobranch species investigated.

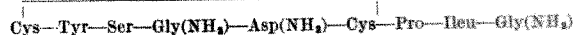
The purified extract was lyophilized to a white powder and then twice re-dissolved and re-lyophilized in order to remove ammonium acetate. In four experiments samples of the dry powder were taken up in triple distilled hydrochloric acid (about 6 N), evacuated and hydrolysed at 107°-110° C for 17-24 h. The hydrolysate was analysed on an EEL amino-acid analyser (Evans Electroscelenium, Ltd.) or by high-voltage electrophoresis (Locarte Co., London). Ammonia and ethanalamine were identified, together with the residues of the following eight amino-acids: cystine, tyrosine, serine, glutamic acid, aspartic acid, proline, isoleucine and glycine. The amino-acids were present in approximately equimolar quantities, except for cystine and tyrosine for which there was evidence of some degradation during hydrolysis. The recovery of the amino-acids indicates that the original

peptide had an activity of approximately 17.8  $\mu$ U/mg (rat uterus), a figure in close agreement with that calculated from the Lowry peptide determination. In one analysis only, lysine was also indicated, but it was not in equimolar ratio to the other amino-acids. As the oxytocic activity of the purified peptide was resistant to the action of crystalline trypsin (Sigma Chemical Co.) when the enzyme was at ten times that concentration which completely destroyed a control preparation of arginine/lysine vasopressin ('Pitressin', Parke Davis Co.), it is unlikely that lysine is a constituent of the active principle. In the same analysis the possible presence of ornithine was indicated, but the evidence was inconclusive, and subsequent analyses by high-voltage electrophoresis failed to confirm the presence of either ornithine or lysine, free or in combination, in the final purified extract.

Since the general properties of the unknown peptide resemble those of oxytocin it is reasonable to suggest that those amino-acids present in both occupy the same positions. The unknown would then be the 8-serine analogue of oxytocin:



This analogue is not available and we cannot compare its activities directly with those of the unknown. There are, however, two reasons to doubt that this is the *Raia ocellata* principle. All known analogues with the intact oxytocin ring have frog bladder to rat uterus activity ratios greater than one. None has milk ejection to rat uterus activities greater than three. These ratios for the unknown are less than 0.46 and more than 8, respectively. Comparable frog bladder and milk ejection activities are only known in analogues containing an amino-acid other than isoleucine in the 3-position. This suggests an alternative hypothesis, that the serine may be in the ring, possibly in the 3-position:



An 8-isoleucine fraction is present in the oxytocin-like principle of teleost fishes. At present it would appear that this second sequence, 3-serine, 8-isoleucine oxytocin, provides the best hypothesis for the structure of the *Raia ocellata* peptide, since it fits most closely the chemical properties, the analyses, and our predictions based on structure activity relationships among known analogues. The final establishment of structure must await degradation investigations on the active molecule, and pharmacological comparison of the unknown to synthetic 8-serine oxytocin and 3-serine 8-isoleucine oxytocin, if they become available.

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## NUCLEAR-CYTOPLASMIC INTERACTIONS

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**I**NDISPENSABILITY of the nucleus for the prolonged continuation of normal cell metabolism has been recognized for many years. Yet, the precise nature of the exchanges which must at some time in the life of the cell occur between nucleus and cytoplasm remains to be fully elucidated.

Pulse tracer investigations such as those carried out by Ficq<sup>1</sup> on frog oocytes, Le Blond and Amano<sup>2</sup> on somatic cells and others on a variety of cells<sup>3,4</sup>, indicate that all or most of the cytoplasmic RNA originates fully labelled from the nucleus. Results obtained from labelled nuclear grafts in amoeba<sup>5</sup> provide further evidence that at least some of the cytoplasmic RNA comes from this source.

Strands of granular materials extending from the region of the nucleolus to the nuclear envelope have been seen in electron micrographs<sup>6,7</sup>. Watson<sup>8,9</sup>, Beams<sup>10</sup> and others provide pictorial evidence for the at least occasional extrusion of such nucleolar material into the cytoplasm. The maximum cytoplasmic basophilia, in many species, corresponds to the moment at which the nucleo-cytoplasmic ratio reaches its peak value.

**Annular 'pores'.** One of the more intriguing features about this apparent wholesale transfer of materials from nucleus to cytoplasm is that in many cells the nuclear 'pores' do not appear to be patent, at least during a major portion of the life of the cell. In the case of the chicken oocyte which has been a primary subject of investigation in our laboratory, two pieces of evidence support this view: (a) in sagittal sections of the 'pores', the true nuclear membrane and the adjacent membrane (which is a part of the endoplasmic reticulum) appear to be pressed closely together but are continuous across the part of the so-called 'pore' which has sometimes been claimed to be patent (Figs. 1 and 3). (b) In tangential cuts across the nuclear membrane (Fig. 2) nearly all the truly bisected 'pores' are observed to contain a single dense and/or osmophilic granule (G) (about 200 Å) positioned in their very centres. (As can be seen in Fig. 1, the granules (G) appear to arrive at the pores from inside the nucleus.)

Wiener *et al.*<sup>11</sup> have provided additional evidence that annular pores, at least in the case of *Drosophila* gland nuclei, do not constitute freely communicating channels between nucleus and cytoplasm. They show that differences in electrical resistance of the nuclear envelope are not related to the numbers of pores in the membrane assayed.

**Passage across the nuclear membrane.** If the nuclear 'pores' are not open across their greater diameters, then how do macromolecules traverse them? One possibility is that the peripheral cores (C), which appear to extend from inside the nucleus to the cytoplasm, may be essentially hollow. The insert in Fig. 2 suggests that this may be possible. However, Feldherr<sup>12</sup> has shown that colloidal gold particles can readily enter the nucleus and do so at the very centres of the annular 'pores' where they are slowed briefly before proceeding inward. It would appear, therefore, that the barriers across the mouths of the 'pores' can be traversed by entities up to 50–60 Å in diameter.

With the hope of gaining more insight into the nature of the materials within the nucleus and the mode of passage of these materials to the cytoplasm, we have

undertaken microscopic investigations on chicken oocytes and cells of the chicken embryo. These cells would appear to be especially favourable subjects because of the rapid growth and differentiation occurring within them. The eggs and embryos were fixed by exposure to 1 per cent osmic acid (pH 7.8) for 2 h, were taken up through 50, 75, 95 and 100 per cent ethyl alcohol steps to propylene oxide and were finally embedded in 'Epon 812'. A 4-week curing period was allowed to elapse prior to cutting (60–90-mμ sections) with a diamond knife and mounting on copper grids coated with collodion and carbon. Staining with lead was carried out according to the method of Karnofsky<sup>13</sup>. The electron microscope used was the RCA model EMU2B.

Although the chromosomes of avian cells appear at interphase to lose almost their basophilia and become invisible or are represented only by a delicately stained reticulum, nucleoli are quite prominent. It would appear, however, that the fine morphologies of these structures are extremely subject to distortion. Following what we consider to be poor fixation, the nucleoli appear to consist of granules. Under possibly better conditions more of the nucleolar sub-structure is revealed to be in the form of vesicles. In Fig. 5 is shown an enlargement from the

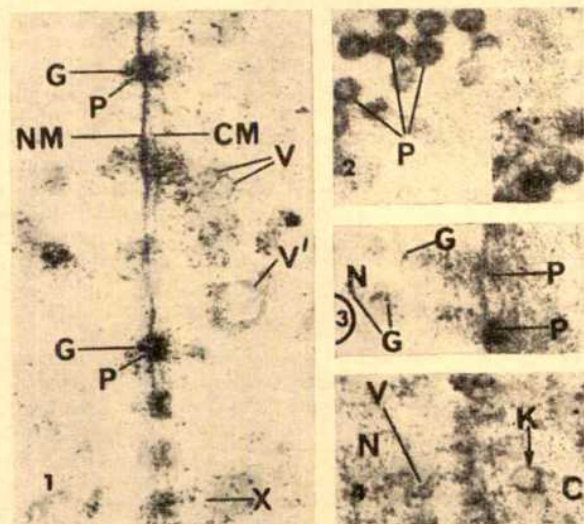


Fig. 1. Nucleocytoplasmic interface in chicken oocyte (sagittal section). The nuclear envelope consists of the true nuclear membrane (NM) and the endothelial reticulum (CM). At the periphery of the top 'pore' (P) short cores appear to pass from nucleus to cytoplasm. Two large dense granules (G) can be seen on the nuclear side of two of the pores, and on the cytoplasmic side vesicles (V, V') of various sizes may be seen. At the site marked X, a vesicle appears to be elaborating from the 'pore'. ( $\times 28,875$ )

Fig. 2. The nuclear—or annular—'pores' (P) are here shown in tangential section. Note (in the insert) that the annular cores appear to be either hollow tubes or rows of vesicles. ( $\times 30,675$ )

Fig. 3. Figure illustrating presence in oocyte nucleus (N) of larger granules (G) within vesicles. These are aligned with respect to the nuclear 'pores' (P). ( $\times 30,675$ )

Fig. 4. Oocyte nucleus (N) showing string of vesicles (V) (similar to those comprising 'stacks' in nucleolus, Fig. 5) in association with nuclear membrane. Larger vesicle (K) appears to be forming from smaller ones on cytoplasmic (C) side. ( $\times 48,675$ )



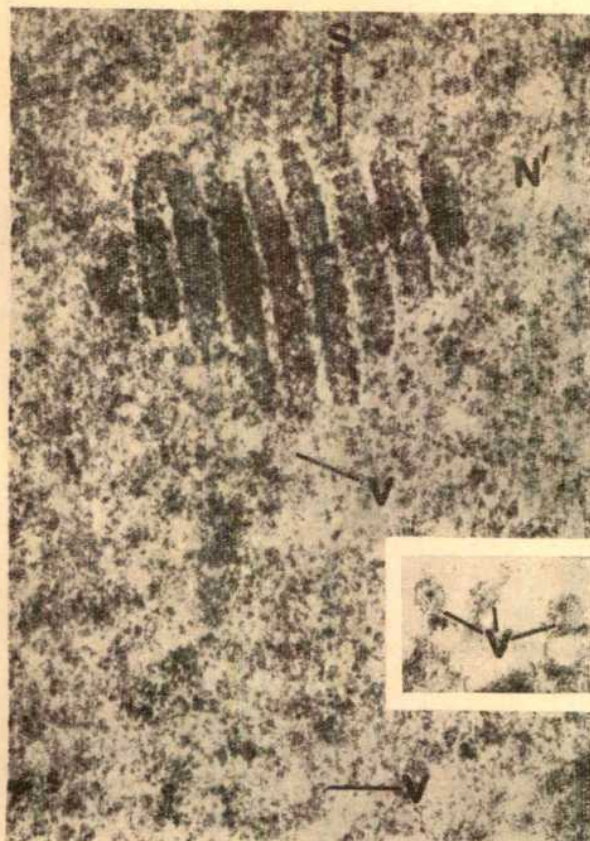


Fig. 5. Extruded nucleolus (N') in 24-h chicken embryo cell. Note folded stack(s) of disks or vesicles (V) and double string of disks extending from base of centre section (V) suggesting that the stacks consist of two coils of such disks ( $\times 40,900$ ). Insert ( $\times 64,900$ ) reveals a central granule in some disks.

matrix of an extruded nucleolus in a somatic cell of a 24-h-old chicken embryo. Several 'stacks' of small (300–400 Å) flattened disks or vesicles, apparently in helical array, can be detected in it. (It is probable that the several stacks represent one or more longer stacks, folded.) From the base of the centre 'stack' two 'strings' of such vesicles appear to emerge and pursue a winding course through the bulk of the nucleolar material (which contains many such vesicles in random orientation). In some cases the presence of a small central granule can be detected (Fig. 5, insert). Brown and Ris<sup>14</sup> have described such 'threads' in nucleoli and have also indicated that the structural units may be hollow spheres (or disks) rather than granules. The significance of the 'stacks' can only be guessed at, as can the presence of organizer material (chromosomal DNA). It is possible that extruded nucleoli, such as this one, are more rapidly fixed due to the absence of a nuclear membrane.

Although a variety of structures may be observed in osmium-fixed nuclei, including (a) dense granules (about 150 Å in diameter) centred in larger vesicles (Fig. 3), and (b) what appear to be clusters of ribosomes (poor fixation?), (c) disks more similar in size to those which comprise the above described nucleolar threads can often be observed close to the nuclear membrane and sometimes appear to be passing through it as in Fig. 4 (note central granules). It is not clear whether or not the nuclear structures other than the small disks of the nucleolus or, indeed, the small disks themselves are variations of—combinations of—artefacts of—the true nature(s) of those substances, but it is recognized that unpredictable swelling of vesicles may take place and that portions of vesicles may condense into granules.

As illustrated in Figs. 1, 6 and 7, passage of vesicular materials from nucleus to cytoplasm appears: (a) to nearly always take place at the site of an annular 'pore', (b) to

involve reduction of the original vesicles into smaller units (we have seen small vesicles), and (c) on the cytoplasmic side of the 'pore' to involve the combination of smaller units (microvesicles) into larger vesicles.

We are not willing to commit ourselves with respect to whether the smaller units pass through the tympanum of the 'pore', whether they traverse the possibly hollow 'cores' (Fig. 2, insert) or whether they 'add' to the cores on the nuclear side provoking the release of a unit from the cytoplasmic side. The pictures of the process in Figs. 1, 6 and 7 suggest that the cores may somehow be involved, at least in some cases.

**Nature of the vesicles.** Many of the vesicles being assembled at the nuclear-cytoplasmic interface assume the morphology depicted in Fig. 8, consisting superficially of a bristled surface and a centrally located rod or sphere. Although the latter are reminiscent of the centrally located granules in the annular 'pores', they do not appear to derive directly from them. In the vesicle that is newly forming (from smaller vesicles elaborating at the 'pore' sites) there is little evidence of a centrally located streak (Fig. 6, SV). The eventual streak is either a dense collection of smaller vesicles or the site of overlapping of the peripheries of larger ones. Especially during assembly stages, the limiting membranes of the emerging vesicles appear to be comprised of at least 2 rows of microvesicles. The 'bristles' of the mature vesicle are only apparent, being manifested by the meeting of the sides of adjacent microvesicles. Although they vary considerably in size and shape, the smallest multi-vesicles, when spherical, measure about 1000 Å in diameter. They appear closely to resemble the 'kinetosomes' described in unicellular organisms by many workers<sup>15</sup>. They possess an inherent

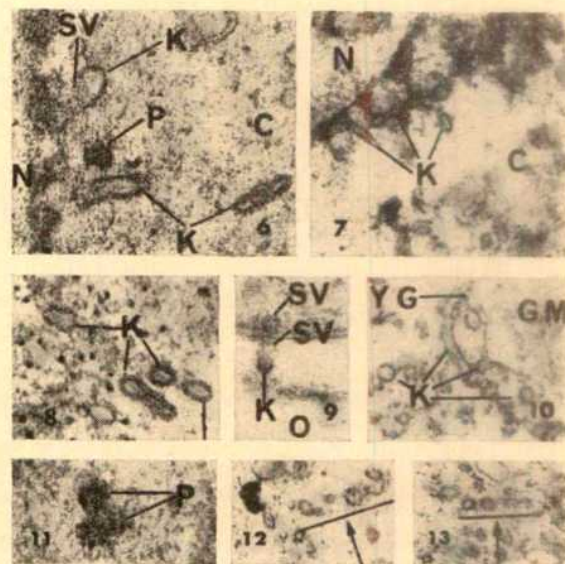


Fig. 6. Oocyte nucleocytoplasmic interface showing elaboration of vesicles (K) from pore sites. Top vesicle appears to be forming from a combination of smaller vesicles each with a central granule. Organelle labelled P may be a polyribosome being formed from a vesicle. Note dark aspect and the presence of a central granule. ( $\times 35,475$ )

Fig. 7. Vesicles (K) being elaborated from 'pore' site at nuclear-cytoplasmic interface in oocyte. ( $\times 18,075$ )

Fig. 8. Nucleic acid-containing multi-vesicles ('kinetosomes'). Note streaks extending along the long axis of the vesicles and the fine surface bristles (probably representing sites where adjacent microvesicles meet). ( $\times 24,525$ )

Fig. 9. This 'kinetosome' (K) in the oocyte (O) appears to be assembling from sub-vesicles (SV) some of which appear to be present at the surface of the follicular cell opposite. There may be connections in the form of narrow tubules between the two cells. ( $\times 24,525$ )

Fig. 10. Vesicles (K) captured in process of being incorporated into yolk granule (YG). Note how vesicles appear to contribute to yolk granule membrane. The vesicles may be both from the surface of the oocyte and the nuclear 'pores'. GM, granular material. ( $\times 16,200$ )

Figs. 11, 12 and 13. Clusters of ribosomes (P) in hen oocyte and chicken embryo cytoplasm (C) which appear to be in a stage of conversion from vesicles. Note centrally located granules (and the darkened interior portions of the polyribosome complexes in Fig. 11). Fig. 11,  $\times 48,675$ . Figs. 12 and 13,  $\times 21,000$



density that is characteristic of nucleic acid (they fail to de-stain markedly following bleaching with  $H_2O_2$ ). They also stand out in contrast to the background following application of periodic acid and silver methenamine. In the oocyte, they probably comprise the substance of the RNA of the yolk nucleus (a large basophilic mass in the cytoplasm where yolk formation first takes place). Similarly appearing vesicles may pass from cell to cell, for example, from follicular cell to oocyte as indicated in Fig. 9. However, even parts of the surface membrane that are quite distant from the surfaces of other membranes may invaginate and give rise to 'pinocytosis vesicles' that closely resemble the nuclear vesicles.

Ehret and de Haller<sup>15</sup> and others have provided circumstantial evidence that 'kinetosomes' act as precursors for cilia of the gullet in *Paramecium*. The presence of 'kinetosome'-like vesicles in the vacuolated mitochondria of this organism suggests that they may play some part in the formation of these organelles as well.

**Vesicles and ribosomes.** Vesicles resembling kinetosomes in morphology contribute to both the internal content and bounding membranes of developing yolk granules in the oocyte of the chicken (Fig. 10). In the chicken, also, similarly appearing vesicles may be involved in the formation of other intracellular organelles<sup>16</sup>.

Although it has been found that the base ratios of the nucleic acids in the nucleolus are similar to the base ratios of the ribosomal and granular materials in the cytoplasm<sup>17</sup>, there exists no evidence that in chicken cells ribosomes pass from the nucleus in the form that they assume in the fixed condition in the cytoplasm. Complexes resembling clusters of ribosomes are detectable in nuclei; however, their increased occurrence therein as a function of what appears to be poor fixation leads to the suspicion that they do not occur naturally in this locus. These considerations suggest that ribosomes (or centres for their formation) are discharged from the nucleus in vesicular form, several thus disguised ribosomes being contained in a given package.

More definitive evidence is available to support this idea. (a) Many of the clusters of ribosomes present in the cytoplasm of chicken cells exhibit centrally located granules which are reminiscent of the central granules seen in 'kinetosomes', and the spacing of the peripheral granules in relation to the central granules often matches the distance observed between granule and 'kinetosome membrane' in the smaller 'kinetosomes' (Fig. 11). (b) Apparent conversion steps of small vesicles to 'ribosome clusters' can be seen in abundance in egg and embryo cells (Figs. 12 and 13).

Recent magnifications of this type of collection of micro-vesicles (which we prefer to term 'kinetosomes' or 'kinetosome-like structures' because these well-known designations summarize our information and speculations regarding their essential character) reveal fibres extending some 500–600 Å from their surfaces which terminate as dense or osmophilic granules (about 200 Å).

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## ESTERASES IN SPIDER MITES HYDROLYSING $\alpha$ -NAPHTHYLACETATE

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**M**ANY populations of the spider mite, *Tetranychus urticae*, have developed resistance to organophosphates. In house-flies it has been found that a 'mutant ali-esterase', capable of organophosphate-detoxification, is a common mechanism of resistance<sup>1</sup>. These mutant ali-esterases have little or no hydrolytic activity to  $\alpha$ -naphthylacetate (NA), if compared with the wild type ali-esterase of susceptible flies<sup>2</sup>. If a similar mechanism of resistance were to occur in mites, this would probably also be reflected by a difference in the rate of NA-hydrolysis. Therefore, the activities of *S*- and *R*-homogenates to this substrate were compared.

The strains used were obtained from and described by Helle<sup>3,4</sup>. He showed the parathion resistance to be mainly dependent on a single genetical factor. Further, Helle applied a procedure of repeated back-crosses between the susceptible (*S*)-strain and the hybrid of *S* and the resistant (*R*)-strain combined with parathion selections after each generation. This procedure tends to eliminate small resistance factors and incidental differences between the *R*- and *S*-strains. The *S*-strain ('Leverkusen normal') and the original *R*-strain ('Systox' or 'Leverkusen RR') are identical with those used by Voss<sup>5</sup> and Voss and Matsu-mura<sup>6,7</sup>. In our work, however, mainly the back-crossed homozygous *R*-strain was used, and in what follows the symbol *R* will be used exclusively for this resistant strain.

Homogenates were made in the cold in all-glass Potter-Elvehjem homogenizers or in smaller ones made of 'Perspex'. The NA-hydrolysis was determined according to the method described by van Asperen<sup>8</sup>. Final concentrations in a typical reaction were:  $3 \times 10^{-4}$  M NA; 0.05 M phosphate buffer pH 7.0; 1 per cent acetone and a single homogenized female per 2 ml. ( $\sim 10$   $\mu$ g/ml.). After a reaction period of 30 min at 27° C, 0.15 ml. of 0.3 per cent tetrazotized di-*o*-anisidine in 3.6 per cent sodium lauryl-sulphate was added per ml. of the reaction mixture. Optical density was measured in a Beckman DU spectrophotometer at 600 m $\mu$ , using a 1-cm cell. A final concentration of  $10^{-5}$  M  $\alpha$ -naphthol gave an optical density of 0.38 after subtraction of the appropriate blanks. Under these conditions the average rate of hydrolysis by homogenates of strain *S* is roughly 3  $\mu$ moles/mg mite-tissue/h. The amount of NA hydrolysed is proportional to the homogenate concentration and the reaction period up to 1 h. In the experiments on the effect of substrate concentration 3.0 per cent acetone had to be used to keep NA in solution at the higher concentrations. Inhibition experiments with the organophosphate diazoxon were performed by pre-incubating the homogenate at 27° C with an excess of inhibitor to realize pseudo first-order kinetics. After a pre-incubation period of 1–5 min, the mixture was diluted at least six times with the substrate solution. The rate of hydrolysis was found to be prac-

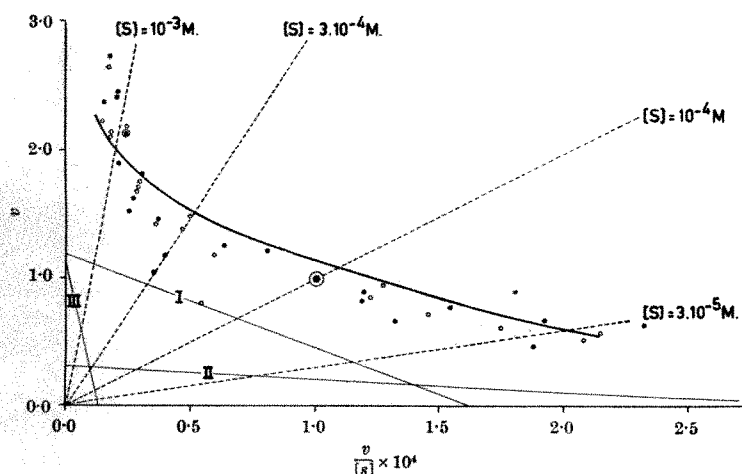


Fig. 1. Effect of NA-concentration (Polar co-ordinates) on activity of a total homogenate. For easy comparison of  $S$  and  $R$  ( $\circ$  and  $\bullet$ ) the activities obtained with a particular homogenate were divided by the activity at  $10^{-4}$  M ( $v$ ). The curve drawn is constructed by summation of the lines representing enzymes I, II and III

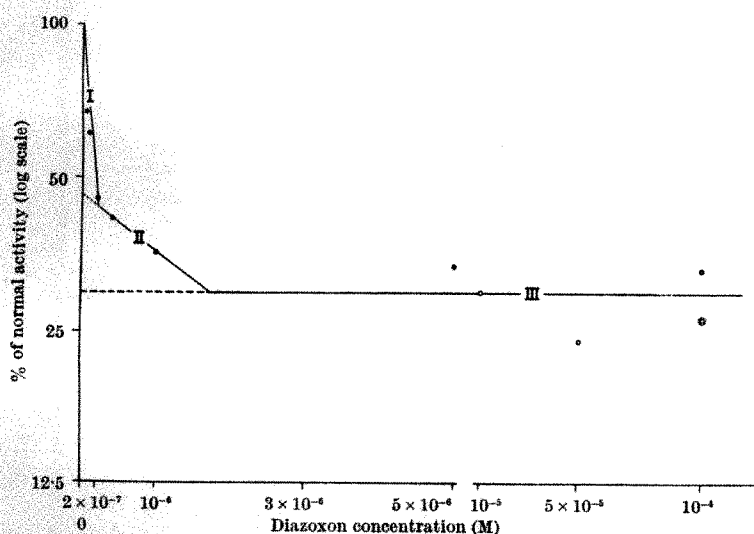


Fig. 2. Effect of diazoxon concentration (M) on percentage activity (log scale) of a total homogenate after pre-incubation for 1 min at  $27^{\circ}\text{C}$  (note the different scales of the abscissa  $\bullet$  and  $\circ$ )  $\circ$  = 10 min pre-incubation. NA conc. =  $10^{-3}$  M

tically constant, indicating that there was no progress of inhibition during the assay.

A small but highly significant ( $P < 0.001$ ) difference was found between the rates of NA-hydrolysis by homogenates of  $S$ - and  $R$ -females in coupled activity determinations. Similar results were obtained with males and with females in the diapause stage of  $S$  and  $R$  and with females of  $S$  and strain 'Systox'. The activities of  $R$ -homogenates were about 20 per cent lower than those of  $S$ -homogenates. A systematic error was excluded in a series of experiments in which 15 single  $S$ - and  $R$ -females were alternately homogenized and immediately assayed.

This result prompted a further analysis of the enzymes hydrolysing NA under the hypothesis of a causal connexion between resistance and the difference in hydrolytic activity found. Experiments on the influence of NA-concentration on the activity of total homogenates (Fig. 1) revealed the presence of at least two enzymes with different affinities ( $1/K_m$ ) to NA. The effect of

diazoxon concentration on activity demonstrated the occurrence of at least three enzymes (I, II, III) with bimolecular rate constants ( $k$ ) in the order of  $10^7$ ,  $10^6$ , and not more than  $10^3 \text{ M}^{-1} \text{ min}^{-1}$ , respectively (Fig. 2). Further experiments were performed in which the activities of one or two of these enzymes were isolated by irreversible inhibition or heat inactivation. In this way, the  $K_m$  values for NA and the bimolecular rate constants for diazoxon inhibition were determined for three enzymes responsible for at least 90 per cent of the total activity at substrate concentrations between  $3 \times 10^{-5}$  and  $1.5 \times 10^{-3}$  M (Table I). After heat denaturation at  $62^{\circ}\text{C}$ , a single enzyme remained active, with  $K_m \sim 8 \times 10^{-5}$  M and  $k \sim 10^7 \text{ M}^{-1} \text{ min}^{-1}$ . The  $K_m$  value of the least diazoxon sensitive enzyme (III) was determined after irreversible inhibition of enzymes I and II, which was achieved by pre-incubation for 1 min with  $10^{-3}$  M diazoxon. According to the influence of substrate concentration a single enzyme remained active with  $K_m \sim 10^{-3}$  M. The  $K_m$  value of the enzyme with the intermediate diazoxon sensitivity (II) was determined in the following way. First, enzyme I was irreversibly inhibited by 1 min pre-incubation with  $2 \times 10^{-7}$  M diazoxon. Thereafter, the remaining activity, due to enzymes II and III, was determined at different substrate concentrations and the activities of enzyme III at these concentrations subtracted according to the method of Hofstee<sup>8</sup>. The subtractions are made along the polar co-ordinates, which represent constant substrate concentrations, in a  $v$  versus  $v/[S]$  plot (Fig. 1). The line found after subtraction indicated a single enzyme with a  $K_m \sim 10^{-3}$  M. The line drawn in Fig. 1 is obtained by summation of the three lines representing the enzymes with the  $K_m$  values found, while the contributions to total activity at  $10^{-3}$  M NA are estimated from the intercepts with the ordinate in Fig. 2. The deviation of the line from the points might be explained by a small error in one of the values of Table I. However, another

explanation would be a small contribution of a fourth enzyme with a  $K_m$  value even higher than  $10^{-3}$  M.

Surprisingly, apart from the difference in total activity, no significant differences between  $S$  and  $R$  were found for any of the characteristics examined. Therefore, it was not possible to hold either one of the three enzymes responsible for the difference found. It should be noted, however, that the accuracy of these experiments is somewhat smaller than that of those simply comparing normal activities of  $S$ - and  $R$ -homogenates. The experiments on diazoxon inhibition make it unlikely that any of the  $R$ - or  $S$ -esterases examined has hydrolytic activity to this compound. Considering the results, there is little to support the hypothesis that the small difference in total activity between  $S$ - and  $R$ -homogenates is directly connected with the mechanism of resistance.

More recent work showed that the cholinesterase of  $R$  mites is considerably less sensitive to diazoxon and paraoxon inhibition than that of  $S$  mites<sup>9</sup>. For paraoxon inhibition, bimolecular rate constants were about  $10^3$  and  $10^4 \text{ M}^{-1} \text{ min}^{-1}$  for  $S$ - and  $R$ -cholinesterase, respectively. Voss and Matsumura<sup>6</sup> essentially confirmed these results.

This work led to the conclusion that the insensitivity of the cholinesterase is the major factor for resistance. Since it is likely that there are no other resistance factors

Table 1. ENZYMES HYDROLYSING  $\alpha$ -NAPHTHYLACETATE

Symbol	% of total activity at $10^{-3}$ M	$K_m$ (M)	$k$ Diazoxon inhibition ( $\text{M}^{-1} \text{ min}^{-1}$ )
I	55	$8 \times 10^{-5}$	$10^7$
II	15	$10^{-3}$	$10^6$
III	30	$10^{-3}$	$\leq 10^3$

in our back-crossed *R*-strain, it is difficult to explain the significant difference in NA hydrolysis between the two strains. One could think of three possibilities. In the first place, the difference in NA hydrolysis may be caused by a higher activity of the cholinesterase of the *S* strain for this substrate. This possibility, however, is excluded by the fact that eserine concentrations up to  $10^{-6}$  M did not inhibit the rate of NA hydrolysis by *S* homogenates. Moreover, an excess of NA did not inhibit the cholinesterase activity to acetylcholine in these homogenates. Secondly, it could be that the lack of effect of the back-cross procedure is caused by incidental linkage of the NA hydrolysis factor(s) with the cholinesterase factor by the occurrence of, for example, inversions disturbing normal cross-over. However, nothing is known about such chromosomal abnormalities because practically no chromosome investigations have been made on mites. In the third place, there is the interesting possibility of

pleiotropism. This would mean that the alteration of the organophosphate sensitivity of the cholinesterase is caused by mutation of a gene which controls a common factor in the production of this enzyme and the esterase(s) hydrolysing NA. Such a common factor is merely hypothetical but perhaps not unlikely. So far we cannot discriminate between the two possibilities left.

I thank Dr. W. Helle for supplying the strains and for his advice, and Miss L. Herreweynen for her assistance.

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## A THEORETICAL BASIS OF ICE NUCLEATION BY ORGANIC CRYSTALS

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A CONSIDERABLE number of organic compounds have now been shown to act as ice nuclei<sup>1-5</sup>, the most promising materials being those which can be sublimed without decomposition to form large numbers of particles active at temperatures near 0° C. The activities of  $\alpha$ -phenazine, phloroglucinol dihydrate and metaldehyde lie within the practically useful range for cloud-seeding purposes but activity is not the only criterion of usefulness; it is also necessary to consider photolytic stability and toxicity. The present article is concerned mainly with the possibility of predicting activity, and its effective assessment in the laboratory.

While some workers regard onset temperature to be a measure of activity, it can be seen from Fig. 1 that there is no obvious relation between this and *N*, the number of nuclei/g, active over an extended temperature range. This is because *N* at any temperature depends on the size-frequency distribution of the seed crystals, and on the distribution of active sites. Nucleative power is ultimately a function of the density of active sites; a large particle may thus contain many sites, the most active of which determines the temperature at which the particle will act, whereas small particles may exist that are inactive over the useful temperature range. Although large particles nucleate with less supercooling than small ones, *N* depends essentially on the number of small particles.

Fig. 1 shows the activity of smokes of the important organic nuclei in comparison with silver iodide as measured in the cloud chamber developed by Edwards and Evans<sup>6</sup>. For most organic nuclei the onset temperature is less sharply marked than for inorganic crystals; this may be related to the lower energy of the organic lattice which allows greater freedom of molecular movement at the surface.

The definition of onset temperature is largely a matter of convention; the value depends on the method of preparation and the particle size range. Thus, some particles of phloroglucinol dihydrate have been said to nucleate ice at -2° C, and Fukuta<sup>5</sup> has claimed  $10^4$  nuclei/g for metaldehyde at -0.6° C.

Whether nucleation can be observed at a given temperature will depend on the time one is prepared to wait. If, however, the temperature is lowered at approximately 2° C/min until a point is reached where the rate of nucleation is about one nucleus per sec per  $10^3$ - $10^4$  particles (which is conveniently observed on the microscope cold stage) the following figures are obtained for large particles: (1) phloroglucinol dihydrate, -4.5° C; (2)  $\alpha$ -phen-

azine, -3.5° C; (3) metaldehyde, -5° to -6° C; (4) silver iodide, -4° C.

These figures are the results of many experiments in these laboratories. The samples were prepared by gently crushing recrystallized materials so as not to destroy all the large particles.

The second laboratory method of assessing nucleative activity (*N*), which is done in the cloud chamber, is complicated by many factors which must be rigidly controlled. To obtain reproducible results from aerosols prepared by sublimation, low concentrations of vapour (for example, 100  $\mu$ g/l.) at constant temperature should be swept at standard speeds through a standard orifice by preheated gas into a large reservoir from which aliquot parts may be taken. It is also important to ensure the presence of a sufficient number of small particles by controlled shock cooling. Owing to the low melting-point of organic compounds, particle sizes of up to 2-5 $\mu$  are

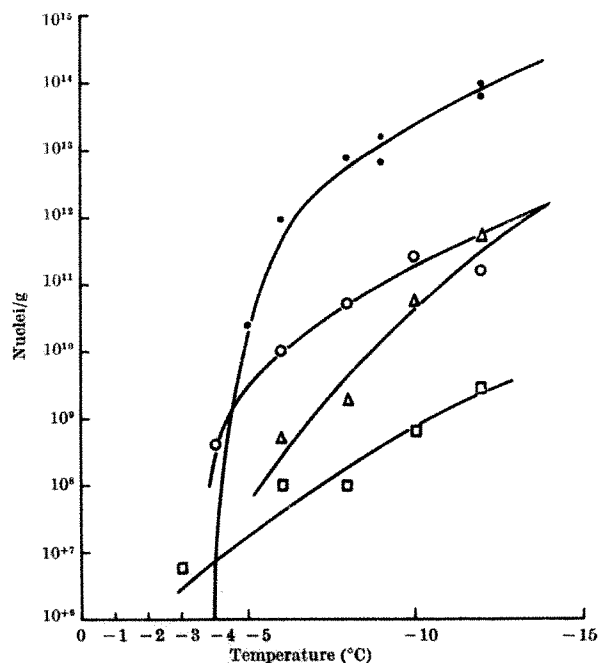


Fig. 1. Nucleative activity of some organic smokes compared with AgI. (Each point is the mean of four determinations.) ●, AgI; ○,  $\alpha$ -phenazine; △, phloroglucinol dihydrate; □, metaldehyde

Table 1. CRYSTALLOGRAPHIC AND NUCLEATIVE CHARACTERISTICS OF SOME ORGANIC ICE NUCLEI

Compound	Type	Space group	Unit cell dimensions Å			No. HBGs per 100 Å <sup>2</sup>	Onset temp. °C	Cloud chamber	
			<i>a</i>	<i>b</i>	<i>c</i>			No. nuclei/gram (smokes)	No. nuclei/gram (powders)
Ice			4.51	—	7.35	5.68-6.97	—	—	—
$\alpha$ -Phenazine	1	$P2_1/a$	13.72	5.061	7.088	All planes $\approx 3.0$	-3.5	$10^{11}$ - $10^{12}$	$10^9$
Phenazine- <i>N</i> -oxide	1	$P2_1/a$	14.34	4.6	7.37		-6.0	$10^{11}$ - $10^{12}$	$10^9$
Phenazine-di- <i>N</i> -oxide	5	$P2_1/a$	15.5	3.95	7.83		Below -12.0	Decomp.	$10^7$
Anthraquinone	5	$P2_1/a$	15.85	3.98	7.92		Below -15.0	$10^6$	$10^6$
Phloroglucinol dihydrate	2	$Pn2a$	6.73	13.58	8.09	3.64 (100)	-4.5	$10^{11}$ - $10^{12}$	$10^9$
$\alpha$ -Phloroglucitol dihydrate	2	$R3c$	8.71	$\alpha = 56^\circ$	10'	6.85 (111)	—	$10^{11}$	$10^9$
Metalddehyde	3	$I4$	10.4	—	4.11	3.69 (001)	-5 to 6	$3 \times 10^9$	$2 \times 10^4$
Fluorenone I	1	$Pbca$	16.0	12.5	18.6	3.02 (010)	-4.0	—	—
Steroids	1	—	—	—	—	1-2 (edge of stack)	> -3.0	—	—
Graphite	4	$C6mc$	2.456	—	0.696	3.47 (1210)	-5 to -7	—	$3 \times 10^4$
Dicyandiamide	7	$C2/c$	15.00	4.44	13.12	9.0 (001), 6.86 (100)	Inactive	—	—
Urea	8	$P4_2/m$	5.87	—	4.726	9.3 (001), 7.5 (100)	Inactive	—	—
Hexamethylene tetramine	9	$I43m$	7.02	—	—	8.1 (001), 8.7 (011)	Inactive	—	—
Isatin	9	$P2_1/c$	6.19	14.46	7.17	6.7 (001), 4.85 (100)	Inactive	—	—
Theophylline HCl	6	$P2_1/a$	13.3	15.3	4.5	11.8 (001)	below -17°	—	—
Melamine	8	$P2_1/a$	10.54	7.45	7.25	10.2 (001), 11.8 (100)	-12°	$4 \times 10^7$	$10^3$

usually obtained under laboratory conditions as compared with  $0.1\mu$  for AgI.

A comparison of activities in the cloud chamber at  $-12^\circ\text{C}$  shows that AgI can produce  $10^{13}$  nuclei/g. This is about ten times greater than the number produced by field experiments using smoke generators. Similarly, results obtained with organic crystals in the cloud chamber may not always reflect the activity observed in field tests which depends on the method of generating the smoke. The least effective of the organics shown in Fig. 1 is metalddehyde, for which *N* is four orders less than for AgI, and two or three orders less than for  $\alpha$ -phenazine.

It is now agreed that efficient ice nucleators need not belong to the same crystal system as ice, but it seems unlikely that ice nucleators can be predicted solely from considerations of molecular structure<sup>7</sup>. While for simple organic crystals it is found that good parametric fit (correspondence between unit cell dimensions or their simple multiples) is necessary for high activity, it is not always sufficient. Head<sup>2</sup> was the first to show that hydrogen-bonding is essential for ice nucleation by organic crystals, and the presence of the bonds implies a registry between the compound and the ice. The present concept of fit is thus based on the coincidence of hydrogen-bonding groups (HBGs) at the interface between the ice and the organic crystal. For more complex structures, however, this coincidence is not always immediately obvious from the parameters of the unit cell.

Typical situations leading to activity are thus: (1) single HBGs exposed at the same depth, repeating at multiples (4:1 to 1:1) or sub-multiples of multiples (for example, 3:2) of the ice parameters with a disregistry of < 7 per cent ( $\alpha$ -phenazine, fluorenone I); (2) structures such as phloroglucinol dihydrate, in which the HBG pattern is hexagonal and provides a close fit to the 0001 ice plane; (3) small clusters of HBGs in which the individual members of the cluster have approximately the same spacing as the oxygens in a close-packed ice plane; simple parametric fit is replaced by the probability with which some members of each cluster will coincide with oxygen groups in ice planes (metalddehyde); (4) random HBGs present in optimal concentrations (graphite<sup>8</sup>, chars, polymers).

Arrangements imparting little or no activity are: (5) single HBGs exposed at the same depth with a disregistry of such a value as to allow little coincidence with those of the ice (anthraquinone); (6) HBGs exposed at different levels although parametric fit may be good (theophylline hydrochloride); (7) structures in which parametric fit is satisfactory but in which the number of HBGs presented at the same level is greater than that in ice (dicyandiamide); (8) structures in which disregistry is large and the density of HBGs is too high (melamine); (9) structures in which suitable HBGs are either enclosed

or shielded (hexamethylene tetramine), or blocked by esterification as in cholesteryl acetate, or where they are lacking as in hydrocarbons.

Several examples, demonstrating the different types, are given in Table 1. (References to crystal structures can be found in previous papers.)

Of the good nucleators, type 1 shows an obvious relation between lattice parameters and activity based on a simple pattern of widely separated single HBGs as in  $\alpha$ -phenazine and phenazine-*N*-oxide. The importance of parametric fit is evident from a comparison of these two with the less active phenazine di-*N*-oxide and the almost inactive anthraquinone because disregistry is gradually increasing.  $\alpha$ -Phenazine and anthraquinone, in addition to possessing the same space group, have precisely the same molecular symmetry, showing the same pattern of HBGs in the various planes. The disregistries of ice with respect to  $\alpha$ -phenazine along the *a*, *b* and *c* axes are respectively 2.29, 3.16 and 3.09 per cent, whereas for anthraquinone corresponding values are 14.6, 23.1 and 7.2 per cent.

In phloroglucinol dihydrate, there is an obvious fit of ice to the 100 plane, although it is not evident from the parameters that the HBG pattern is hexagonal (Fig. 2c). While all the hydrate oxygens of the 100 plane of phloroglucinol dihydrate take part in hydrogen bonding, only half the phenolic hydroxyl groups can be so used, since they are presented above the plane of the hydrate rings at such a height that they can take part in a three-dimensional bonding pattern with ice. The other half of them is hidden below the general plane. The structure of phloroglucinol dihydrate is thus unique, and is very different from those of other trimers such as melamine considered by Langer and Rosinski<sup>7</sup>.

An analysis of the HBG pattern of the 111 plane (Fig. 2d) of the  $\alpha$ -isomer of 1:3:5-cyclohexanetriol dihydrate ( $\alpha$ -phloroglucitol dihydrate)<sup>9</sup> suggested high ice nucleative activity, and cloud chamber tests proved this to be correct. This is the first instance of a new highly active organic ice nucleator that has been predicted from theory<sup>10</sup>. Up to  $10^{11}$  nuclei/g were counted at  $-12^\circ\text{C}$ , showing it to possess the same order of activity as phloroglucinol dihydrate and  $\alpha$ -phenazine. The oxygen atoms in the 111 plane show a 1:1 correspondence with those in the 0001 plane of ice, the disregistry being 4.8 per cent. The density of HBGs is thus about 50 per cent greater than in the 100 plane of phloroglucinol dihydrate.

The activity of metalddehyde may be a consequence of parametric fit as suggested by Fukuta<sup>5</sup>. However, a more plausible explanation can be based on the random fit of clusters of four oxygen atoms, which, because of the zig-zag molecular configuration, are exposed freely at the same level. Since the members of the clusters are spaced at about the same distance apart as the oxygen atoms in the 0001 plane of ice, a high degree of coincidence



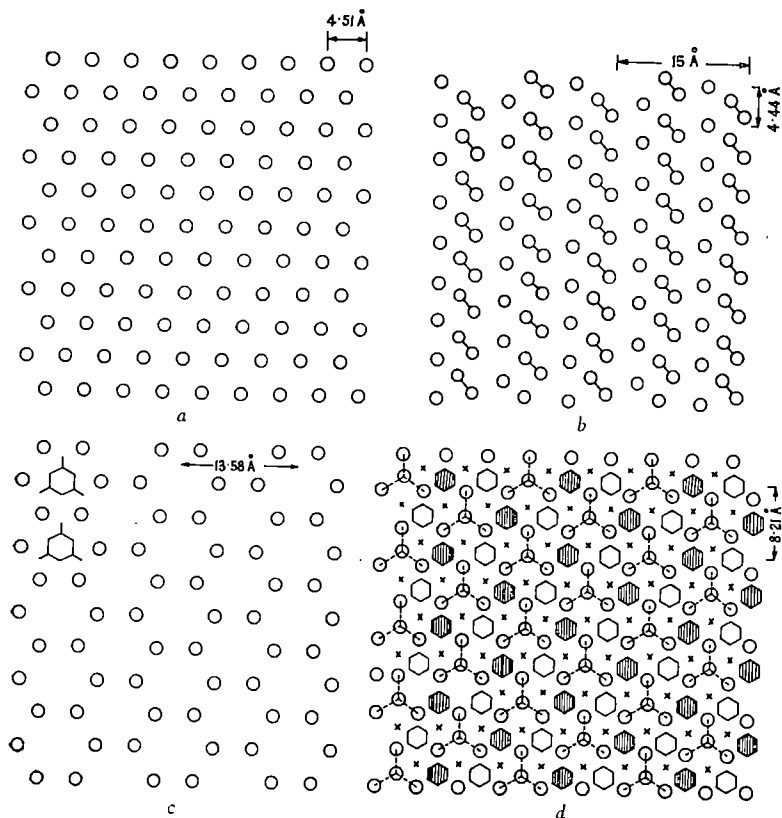


Fig. 2. Hydrogen bonding patterns of: a, 0001 plane of ice; b, 001 plane of dicyandiamide; c, 100 plane of phloroglucinol dihydrate; d, 111 plane of  $\alpha$ -phloroglucitol dihydrate

occurs at any orientation; thus lattice parameters are of little direct importance here.

In all the active compounds so far discussed, the densities of HBGs (3–4 per 100 Å<sup>2</sup>) are less than those of ice (5–7 per 100 Å<sup>2</sup>). When the density on any plane exceeds this latter figure, nucleative ability disappears and the substance becomes hydrophilic. Dicyandiamide, despite good parametric fit, is thus totally inactive although the HBGs are presented at almost the same level on at least one plane. The pattern is shown in Fig. 2b. The effect of the excess HBGs is evidently to stabilize the denser structure of liquid water rather than that of ice. Similar arguments apply to urea, melamine and hexamethylenetetramine. In isatin, the structure of ice cannot be stabilized because, in addition to high disregistry, the HBGs are presented at various levels. Two Canadian groups<sup>11,12</sup> have recently examined the onset temperatures of amino-acid crystals both as freezing and sublimation nuclei. However, an examination of HBG patterns cannot be made yet since the crystal structures of many of the acids are not known.

It has been shown that slightly oxidized carbons can be made which are highly active, but that it is difficult to keep the number of HBGs within useful limits<sup>8</sup>. Nucleative power has also been claimed for silica gel particles in which the number of hydrophilic sites has been reduced by heat treatment<sup>13</sup>. Whether the efficiency of such materials is due to randomly dispersed HBGs some of which may provide good registry over small areas, or, alternatively, might have to do with microcrystallinity, is still an open question.

In the past, the suggestion has been made that the absence of a molecular dipole moment is required for good nucleative activity<sup>7</sup>. It is seen, however, that when there is good registry between a nucleator and ice, nucleative power is independent of the presence or absence of a resultant dipole moment (compare phenazine-N-oxide with  $\alpha$ -phenazine). Furthermore, melamine with no resultant dipole moment is, in fact, a very poor ice

nucleator. Nor can the high activity of phloroglucinol be attributed to the lack of a resultant dipole moment in the molecule, since it is the dihydrate crystal that shows activity.

There has also been a consideration of an entropy effect<sup>14</sup> which may play an inhibiting part on certain inorganic surfaces, but no good example is as yet known for organic crystals. Inhibition could result from the presence of closely spaced keto, ether oxygen, or tertiary amino groups and thus prevent the formation of the alternating donor-acceptor ice structure in the interface. If, however, such groups are more widely spaced, the ice structure can compensate by arranging its internal bonds in the opposite sense in the gaps so as to preserve the alternating donor-acceptor sequence.

However much the present theory may help in predicting new organic nucleators, the selection of practically useful cloud seeding agents must necessarily also take into account the toxicity and stability of the compounds that have proved efficient in laboratory tests. While the loss of the activity of AgI nuclei is the result of photo-reduction, organic crystals are invariably deactivated by photo-oxidation, the small particles being more prone to attack in both processes. However, neither activity (*N*) nor photolytic stability of organic nuclei appears to be reflected in molecular structure. Thus we have found, by absorption spectroscopy in solution and electron spin resonance investigations in frozen solutions, that the

phloroglucinol molecule is destroyed by a free radical mechanism 3–4 times faster than phenazine. The actual sites of attack in a crystal are decided rather by the ease of accommodation of extra oxygen atoms, which is found to be greater for  $\alpha$ -phenazine than for phloroglucinol dihydrate.

To avoid undesirable pollution of water and food, any new ice-nucleating substances must be tested for acute and chronic pathological effects. Although organic materials may be present in minute quantities only, hazards can arise from selective accumulation of the original materials or their photolytic products, unless biological degradation is complete. Phenazine belongs to a family of bacteriostatic substances to which phenothiazine and amino-acridine are related; its use would be hazardous, since French workers<sup>15</sup> have reported the occurrence of bladder tumours in rats after implantation of pellets containing phenazine. While large doses of metaldehyde produce strychnine-like symptoms<sup>16</sup>, the danger of pollution would be very small because of depolymerization and oxidation to acetic acid. Very little is known about the toxicity of phloroglucinol, but it can be expected to be similar to that of resorcinol, which is a skin irritant.  $\alpha$ -Phloroglucitol, owing to its alicyclic nature and polyalcoholic character, is probably non-toxic.

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## LETTERS TO THE EDITOR

## ASTROPHYSICS

## Abundance Analysis of a Supergiant in the Large Magellanic Cloud

THE Magellanic Clouds are the nearest extragalactic systems and differ considerably in structure and stellar content from our own galaxy. The chemical compositions of individual objects in the clouds are consequently of great interest. So far some gaseous nebulae have been examined by Aller and Faulkner<sup>1</sup> and found to differ but slightly from corresponding galactic nebulae, but stars have so far been treated by rather general arguments and none has yet been analysed in detail.

Even the brightest stars in the clouds are normally beyond the reach of the Coudé spectrograph of Mount Stromlo Observatory. However, in the hope that a reasonably good spectrum of the brightest member of the Large Cloud, HD 33579 ( $\alpha = 5^h 6^m 1^s$ ,  $\delta = 67^\circ 57'$ , 1950-0, 9.4 mg), could be obtained in exceptionally good atmospheric conditions, an attempt to observe this star was made on December 24, 1963. In order to minimize light losses the slit-width of the spectrograph was increased to  $400\mu$  corresponding to a projected slit-width of  $57\mu$ , which is equal to double the average size of the photographic grain. The resulting loss in resolution is compensated by the high micro-turbulent velocity of 6.3 km/sec.

The attempt was successful and quite a good spectrum of 0.4 mm width and of 10.6 Å/mm dispersion was obtained on a baked Kodak II aO plate. The exposure time was 5 h. The useful range extends from 3700 Å to 4830 Å.

The effective temperature of HD 33579 is not well known. Six-colour photometry by G. E. Kron (private communication) shows that this star is at least 400 degrees cooler than the galactic supergiant  $\alpha$  Cygni unless it is heavily reddened by interstellar absorption, which is unlikely. Assuming  $T_e = 9,170^\circ$  for the effective temperature of  $\alpha$  Cygni (H.-G. Groth<sup>2</sup>) we obtain  $8,765^\circ$  as the highest possible effective temperature of HD 33579. On the other hand, from a consideration of the energy distribution, bearing in mind the effects of electron scattering, it is unlikely that the effective temperature is less than  $7,750^\circ$ , even in the absence of reddening. Unfortunately it was not possible to determine the temperature more precisely.

In view of these difficulties a coarse analysis of the spectrum of HD 33579 was carried out using Wrubel's curve of growth for four different reciprocal temperatures  $\theta = 5040/T = 0.575, 0.600, 0.625, 0.650$ . In all cases the electron pressure  $p_e$  was chosen to satisfy Saha's ionization equation for iron, the only element for which reliable line strengths could be found in two stages of ionization (FeI and FeII). From this, the continuous absorption coefficient (due to Thompson scattering by free electrons and bound-free neutral hydrogen transitions) was calculated. Abundances were determined for the three most important elements of the iron peak group, iron, chromium and titanium.

The  $gf$  values were taken from Corliss and Warner's<sup>3</sup> critical survey for neutral iron and from Corliss and Bozman's<sup>4</sup> monograph for ionized titanium and a few lines of neutral iron. Stellar values derived by Groth<sup>2</sup> in his analysis of  $\alpha$  Cygni were used for ionized iron and chromium.

The results are shown in Table 1. They are normalized to the logarithm of the abundance of hydrogen  $\log N(H) = 23.52$  corresponding to Unsöld's mixture. Likely errors due to the uncertainties in curve of growth fitting are of the order of  $\pm 0.15$  in the logarithm.

Table 1

	0	0.575	0.600	0.625	0.650
$T$		8,765-0°	8,400-0°	8,064-0°	7,754-0°
$\log p_e$		+0.44	0.00	-0.27	-0.42
$\log N(H)$		23.52	23.52	23.52	23.52
$\log N(Fe)$		18.64	18.63	18.61	18.59
$\log N(Cr)$		16.80	16.80	16.79	16.72
$\log N(Ti)$		16.31	16.33	16.13	16.08

As Table 1 shows, the abundances of three elements of the iron peak depend very little on the adopted temperature. This is due to the fact that the metals are predominantly in the singly ionized state and the variation in the continuous absorption coefficient is small.

In view of the fact that the results obtained in an analysis are affected by errors in  $gf$  values, it was deemed advantageous to repeat exactly the same procedure for the galactic supergiant  $\alpha$  Cygni. The equivalent widths of spectral lines were taken from Groth's<sup>2</sup> publication. The results are shown in Table 2 together with Groth's results and Suess and Urey's<sup>5</sup> cosmic abundances, all normalized to  $\log N(H) = 23.52$ .

Table 2

	$\alpha$ Cygni A.P.	Groth's analysis Coarse	Fine	Suess-Urey
$T$	9,163-0	9,163-0		
$\log p_e$	+0.39	+0.76		
$\log N(H)$	23.52	23.52	23.52	23.52
$\log N(Fe)$	18.88	19.10	19.14	18.79
$\log N(Cr)$	16.93	16.10		16.90
$\log N(Ti)$	16.53	16.89	16.65	16.40

A comparison of results shows that Groth's abundances for iron and titanium are higher roughly by a factor of two. There is a large discrepancy for chromium in spite of the fact that Groth's stellar  $gf$  values were used for this element in my analysis.

Although two independent absolute analyses were made for HD 33579 and  $\alpha$  Cygni, the adopted procedure and the  $gf$  values were exactly the same for both stars. The differences in the abundances derived for both stars should therefore be quite reliable. A comparison between Tables 1 and 2 shows that the metal content of the iron peak group is lower in HD 33579 by a factor not exceeding 2. In view of possible systematic differences in equivalent width measurement between the present investigation and that of Groth, one may conclude that the abundance ratio of the metals iron, chromium and titanium to hydrogen in HD 33579 differs from that in  $\alpha$  Cygni by:

$$-0.2 \pm 0.2$$

in the logarithm. This means that the rate of formation of stars and the closely related nucleogenesis may be slightly lower in the Large Magellanic Cloud than in our own galaxy.

The computations on which the foregoing results are based were made partly at Mount Stromlo Observatory and partly at the Royal Greenwich Observatory. I thank the Astronomer Royal for his hospitality. I also thank Dr. B. E. J. Pagel, Dr. A. W. Rogers and Dr. L. Searle for their advice.

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## GEOPHYSICS

## A Typical Taurid Meteor Spectrum

INTEREST in spectrophotography of meteors has been fast increasing in recent times<sup>1</sup>. About 500 meteor spectra<sup>2</sup> became available from different countries by 1962. This communication describes the second meteor spectrum from India taken at Waltair at 03 h, 24 min I.S.T. on the night of November 18–19, 1963. Its visual magnitude has been estimated about zero. From the plot of its path on a star map it was found to proceed from the Taurid radiant. The spectrum of this meteor has been photographed on an Ilford 'HP3' film (400 ASA) using a conventional meteor spectrograph with the rotating shutter arrangement. The Contax 35-mm camera used for this purpose carried before its lens ( $f = 50$  mm) a Bausch and Lomb plane replica transmission grating with 200 lines/mm and blazed for 5000 Å. From the duration of the segments produced by the rotating shutter and the plot of the meteor on the star chart, the velocity of the meteor has roughly been estimated at 30 km/sec, which is in close agreement with the mean velocity for the Taurid shower. This appears to be the second best Taurid spectrum in the world, the first one being that secured by Ridley<sup>3</sup>. Dr. Millman mentions (private communication) that there are very few Taurid spectra available in the world.

Table 1. WAVE-LENGTHS AND IDENTIFICATIONS OF THE LINES IN THE SECOND AND THIRD SEGMENTS OF THE SPECTRUM

Wave-lengths	Multiplets identified
3,924.6	FeI (4), CaII (1)
3,968.6	CaII (1)
4,232.4	FeI (3), FeI (152), FeII (27)
4,358.0	CaI (37), MgI (14)
4,461.3	FeI (2), CaI (4), FeI (350)
4,633.1	FeI (554), FeI (822)
5,172.8	MgI (2), FeI (36)
5,253.5	FeI (1), FeI (553)
5,529.5	MgI (9), BaI (2), FeII (55)
5,894.0	NaI (1)
6,349.0	SiII (2)

Nineteen lines have been measured from the present Taurid spectrum (Fig. 1) and eleven wave-lengths have been identified from them. The spectrum can be classified as the 'cY' type, following Millman<sup>4</sup>, the ionized calcium *H* and *K* lines being the strongest features. The lines of MgI, NaI and SiII are also bright, although to a lesser extent than those of CaII in this spectrum. The other lines in the spectrum are very faint and cannot easily be recognized in Fig. 1. From an analysis of Ridley's<sup>3</sup> spectrum, Millman (private communication) found the lines of CaII, MgI and NaI to be the strongest features. This is in close agreement with the general features of the present spectrum. The wave-lengths of the various lines and their identifications are presented in Table 1. The identifications in Table 1 compare well with those of Millman (private communication) for the other Taurid spectrum secured by Ridley<sup>3</sup>, to the extent that both contain different multiplets of CaII, SiII, CaI, MgI, NaI

and FeI, although disagreements do exist in the multiplets identified. The observations recorded here support the already established idea, that meteor spectra from each shower are remarkably similar to each other, having the same general features within the limitations of photographic resolution and emulsion sensitivity<sup>5</sup>.

The excitation potential of the SiII (2) line, equal to 10.03 eV, is the largest recorded in the spectrum. The chance of Taurid spectra being recorded is normally low because of the low geocentric velocity of Taurid meteors. Yet the present spectrum is a specially bright one indicating a large level of excitation. The particular meteoroid producing this spectrum might have had considerable mass to supply the necessary excitation. This is consistent with the suggestion of the two-parameter dependence of ionization and excitation in meteor spectra by Cook<sup>6</sup>, the parameters being velocity and ratio of characteristic dimension to mean free path of an evaporated atom.

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### Low-velocity Layer as a Source of the Anomalous Vertical Component of Geomagnetic Variations near the Coast

WITH the development of new techniques and a growing interest in the electrical conductivity of the Earth, the examination of electromagnetic induction in certain types of environment on the Earth's surface has been considerable. Of particular interest is the anomalously large ratio of the variations in the vertical and horizontal geomagnetic components observed near the coast during micropulsation and bay type disturbances. The region of enhancement extends to the landward side of the coast a distance of the order of 100 km, but the extent seaward is at present very poorly known.

The cause of the anomaly at micropulsation frequencies is almost certainly the conductivity contrast found at the land-sea interface.

The source of the bay anomaly is more obscure. In explaining the anomaly found in California, Schmucker<sup>1</sup> has suggested a conductivity distribution that varies

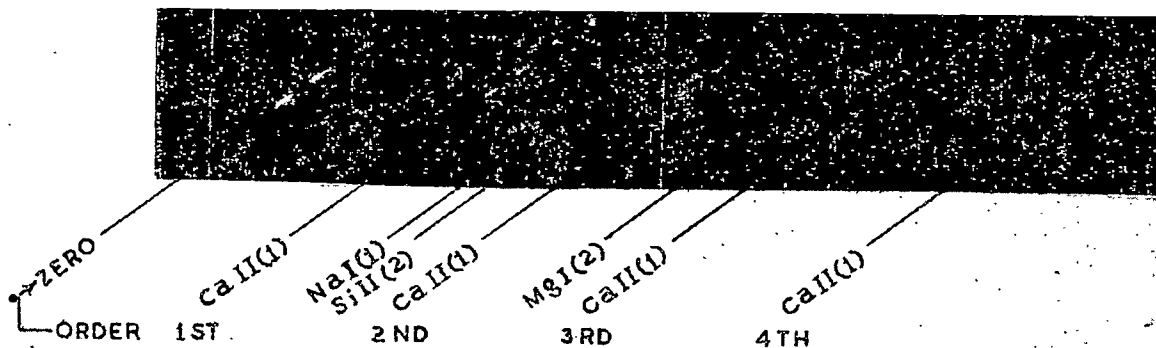


Fig. 1. Spectrum of the Taurid meteor observed at 03 h 24 min I.S.T. on the night of November 18–19, 1963

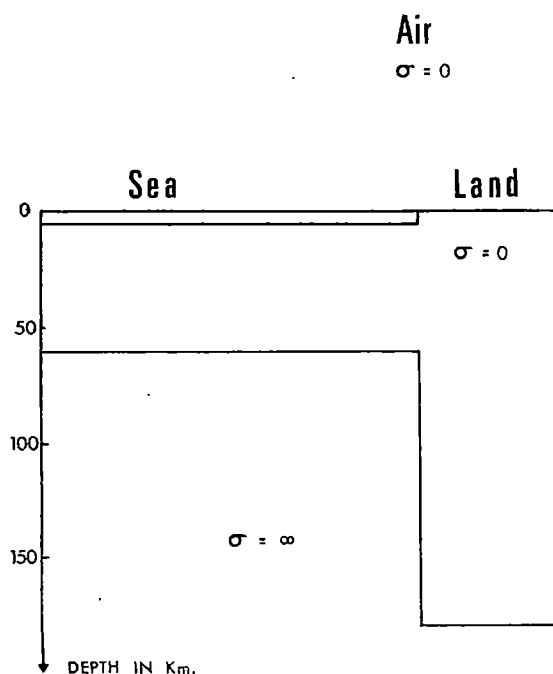


Fig. 1. Model of the electrical conductivity ( $\sigma$ ) distribution below the coast (after Schmucker)

both with depth and laterally (Fig. 1). Parkinson<sup>2</sup> has independently suggested that the cause of the coastal anomaly lies partly in the upper mantle and cannot be totally generated by the land-sea interface.

In recent years seismology has also found such a lateral inhomogeneity in the mantle. Brune and Dorman<sup>3</sup> have indicated that the average depth to the low-velocity layer below young continents, that is, continental areas other than shield areas (115–310 km), differs from that found below oceans (60–215 km). Differences in the depth of the low-velocity layer are probably caused as much by chemical inhomogeneities as by lateral temperature differences. Allowing for the difference in the temperature distribution under oceans and continents, and ignoring differences of phase and composition on a level surface, the ratio of sub-oceanic conductivity to sub-continental conductivity at a depth of, say, 120 km would be of the order of 1:10, and in the sense required in Schmucker's conductivity model (Fig. 1). With chemical or phase differences as well, it is quite possible that the ratio would be substantially changed, but it is not known, at present, in what sense.

It is important to realize that the bay-type disturbances are affected to an appreciable extent by both the land-sea contact and the mantle differences referred to above. It is to be expected that the position of the mantle conductivity step (Fig. 1) is correlated with the position of the continental slopes, so that where the continental shelf is broad the variations will be of distinctly different character to places where the shelf is narrow. This would account to some extent for the observations of Parkinson<sup>4</sup> that the disturbance vectors bear some relationship to the direction of the nearest deep water.

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<sup>1</sup> Schmucker, U., reported at the Newcastle Upon Tyne meeting of the Roy. Astro. Soc., by Filloux, J., to be published in *Quart. J. Roy. Astro. Soc.*

<sup>2</sup> Parkinson, W. D., *Geomagnetism and Geoelectricity*, 15, 4 (1964).

<sup>3</sup> Brune, J., and Dorman, J., *Bull. Seis. Soc. Amer.*, 53, 1 (1963).

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## OCEANOGRAPHY

### Hot Salty Water at the Bottom of the Red Sea

WATER at a temperature of more than 44° C and salinity exceeding 270 parts per thousand (close to saturation) has been found by the R.R.S. *Discovery* in a small depression below 2,000 m depth in the Red Sea near 21° 17' N., 38° 02' E. Three previous expeditions<sup>1-3</sup> have reported abnormal water in this neighbourhood, but nothing so extreme.

This unusual water was found at *Discovery* station 5580, on September 11, 1964, on the way back from the Indian Ocean, in attempting to re-occupy the *Atlantis II* station 42 (ref. 3). The deep parts of the Red Sea are very irregular, with depth changes of several hundred metres frequently occurring within a few miles. There may be many small isolated basins, though not all of them contain abnormal water.

In order to find the most likely place for sampling, a dan buoy was anchored and a sounding survey made relative to it, within about 5 miles radius (Fig. 1). This revealed two small depressions; one of them, about 1.5 miles across and 200 m deeper than its immediate surroundings, was chosen. Water samples were collected from it, using a pinger as a guide in putting the deepest

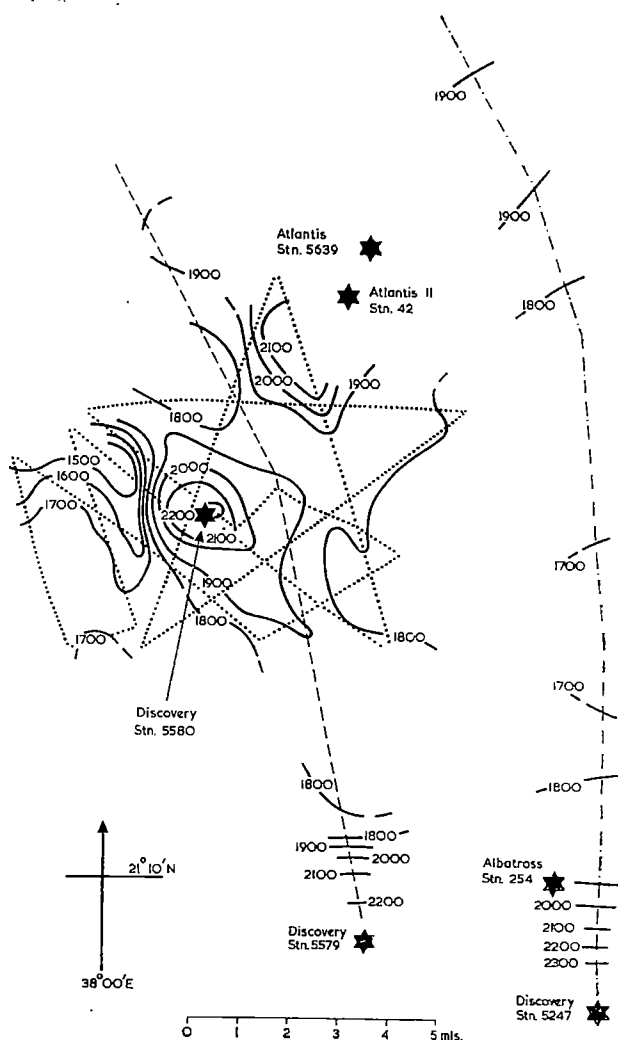


Fig. 1. Bathymetry and station positions where unusually hot salty water has been found. Contours are in metres, corrected according to Matthews's table for Area 51 (ref. 5). —, R.R.S. *Discovery* sounding track, Mar. 1, 1964; —, sounding track, Sept. 11, 1964; . . . . ., sounding track, Sept. 11, 1964, with positions determined by radar from an anchored buoy



bottle close to the bottom. Two casts were made, the first one consisting of 12 bottles spread over 400 m of depth range. The temperatures observed were normal for Red Sea deep water (about 22° C) down to 200 m off the bottom, followed by a sharp increase to well over 40° C in the lowest 150 m. The second cast was arranged to provide more detail in the transition zone and to collect larger samples of the abnormal deep water. Above 35° C we could use only 60° C unprotected thermometers of low-pressure sensitivity since all other available thermometers, protected and unprotected, were off scale.

Temperatures within the hot water were obtained from the unprotected thermometers by applying a pressure correction appropriate to the sampling depth. The latter could be determined by extrapolation from known shallower sampling depths, combined with evidence from the pinger reflexions and the known depth of water.

The vertical profiles of temperature and salinity are shown in Fig. 2. The 'salinities' were measured on a conductivity bridge, after dilution where necessary. The highest salinities required dilution to about one-eighth concentration by weight to bring them on scale. The depths, and hence temperatures, marked † are less certain ( $\pm 15$  m,  $\pm 0.2^\circ$  C) due to greater wire angle and unknown effect of sea floor slope.

The bathymetric contours of Fig. 1, based only on *Discovery* soundings, suggest that the *Atlantis* stations may well have been in a different small basin, and indeed they show a different sill depth and temperature-salinity curve from *Discovery* station 5580. The position of the *Albatross* station 254, shown in Fig. 1, seems inconsistent with the *Discovery* positions, since at *Discovery* station 5247 only a trace of the abnormal water was found<sup>4</sup>.

Preliminary estimates of sulphate, magnesium and calcium, and of the chlorinity: conductivity ratio, suggest that this water is not just concentrated sea-water. Further chemical work is proceeding at the University of Liverpool and at the National Institute of Oceanography.

Speculating on the origin of the abnormal water, it seems unlikely that it can have been formed by evaporation in a shallow sea, as suggested by Charnock<sup>4</sup> for the previously reported abnormal water. More probably it may be due to solution of salt deposits exposed on the sea floor.

The increased density of the concentrated solution would inhibit convection through the transition layer due to heating from the interior of the Earth, and would permit some increase of temperature. The observed temperature gradient in the transition region (nearly  $0.5^\circ$  C  $m^{-1}$ ) is such that the heat loss upwards, with no more than molecular conduction, must be approximately 6 microcalories per square centimetre per second, comparable to the heat flow found coming through the sea

floor itself in regions of high tectonic activity, such as the central part of the Red Sea.

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<sup>2</sup> Neumann, A. C., and Densmore, C. D., unpublished manuscript, *Ref. 60-2*, Woods Hole Oceanographic Institution (1959).

<sup>3</sup> Miller, A. R., *Nature*, 203, 590 (1964).

<sup>4</sup> Charnock, H., *Nature*, 203, 591 (1964).

<sup>5</sup> Matthews, D. J., *Tables of the Velocity of Sound in Pure Water and Sea-water for Use in Echo-sounding and Sound-ranging* (Hydrographic Department, Admiralty, London, 1939).

## METEOROLOGY

### Relation between Wind and Pressure at Low Latitudes

INVESTIGATIONS of the relation between wind and pressure in the extra-tropical latitudes show that in the case of large-scale flow the actual wind does not deviate much from the geostrophic wind. Similar investigations at low latitudes have been few. It is generally realized that on account of the small value of the Coriolis parameter at low latitudes one should be cautious in extending considerations based on quasi-geostrophic balance to such latitudes.

Jordan<sup>1</sup> made a comparison between the observed and geostrophic winds from latitude  $13^\circ$  to  $32^\circ$  N. over certain parts of the Pacific and the Atlantic, and found that more than 75 per cent of the observed winds were appreciably sub-geostrophic south of latitude  $20^\circ$  N. Palmer *et al.*<sup>2</sup> found that the monthly mean mid-tropospheric flow in the Central Pacific during a period of steady easterlies was quasi-geostrophic. Worthley<sup>3</sup> examined the relation between observed and geostrophic winds at 500 mb-level over the Pacific based on aircraft reconnaissance reports and rawinsonde data. He found that the median percentage deviation between the observed and geostrophic winds increased from 35 per cent at  $30^\circ$  N. to 87 per cent at  $8.5^\circ$  N. From their investigations over the Equatorial African region, Johnson and Mörth<sup>4</sup> have suggested that with certain types of pressure distributions quasi-geostrophic flow can obtain within a few degrees of the equator. Ramage<sup>5</sup> and Lockwood<sup>6</sup> have recently commented on the subject.

The comparatively weak winds and feeble pressure gradients at low latitudes pose difficulties for a proper observational examination of the relationship between them. In this respect the Indian area of the tropics offers more favourable conditions, since both winds and pressure gradients over this area are stronger compared with the rest of the tropics in certain seasons. We have examined the relationship between the zonal components of the actual and geostrophic winds over the latitude interval  $8^\circ$  N. to  $28^\circ$  N. utilizing the daily radiosonde/rawin data of the following stations which lie approximately along the meridian of  $77^\circ$  E.:

(i) Trivandrum	$08^\circ 28' N.$	$76^\circ 57' E.$
(ii) Bangalore	$12^\circ 58' N.$	$77^\circ 35' E.$
(iii) Madras	$13^\circ 00' N.$	$80^\circ 11' E.$
(iv) Nagpur	$21^\circ 08' N.$	$79^\circ 03' E.$
(v) New Delhi	$28^\circ 35' N.$	$77^\circ 12' E.$

The investigation was made for three lower tropospheric levels (850, 800 and 700 mb) and three upper tropospheric levels (300, 250 and

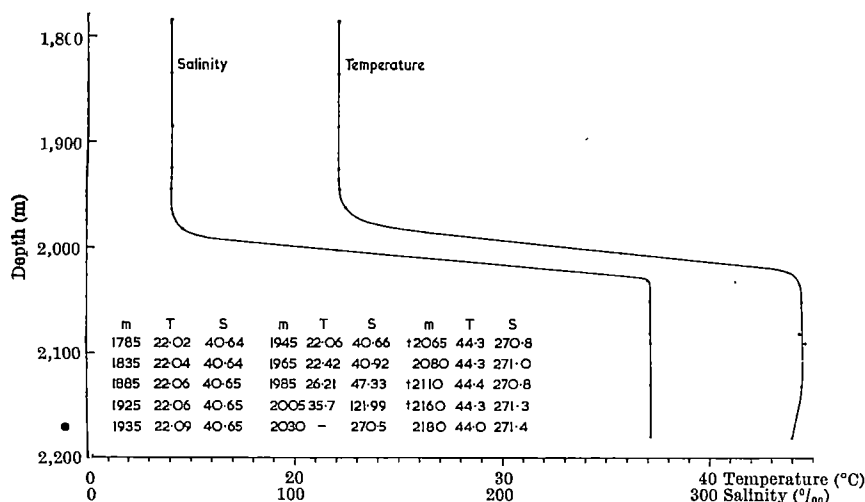


Fig. 2. Provisional values of temperature and salinity for *Discovery* station 5580

200 mb) separately for the south-west monsoon season (July, August, September) and for winter season (January, February, March) utilizing the data for the period 1961-64. The geostrophic winds ( $u_g$ ) computed from the observed contour height differences for adjacent pairs of stations were compared with the corresponding mean zonal winds ( $u$ ) between the stations, and the ratios  $u/u_g$  were determined for different groups of wind speeds.

The results of this investigation are briefly as follows.

(a) *South-west monsoon season.* (i) Over the Trivandrum-Bangalore sector, the zonal westerlies in the lower troposphere are super-geostrophic ( $u/u_g \sim 1.5$ ); the zonal easterlies in the upper troposphere are sub-geostrophic ( $u/u_g \sim 0.6$ ).

(ii) Over the Madras-Nagpur sector the zonal westerlies of the lower troposphere are nearly geostrophic; the zonal easterlies of the upper troposphere are sub-geostrophic ( $u/u_g \sim 0.6$ ).

(b) *Winter season.* (i) Over the Trivandrum-Bangalore sector, the zonal easterlies are sub-geostrophic, the value of  $u/u_g$  increasing from 0.3 to 0.6 with increase of wind speed from 10 to 25 knots.

(ii) The upper tropospheric zonal westerlies practically geostrophic over the Madras-Nagpur and Nagpur-New Delhi sectors.

Systematic diurnal variations of  $u/u_g$  are also noticed south of lat.  $20^\circ$  N. in both the seasons.

From the equation of motion (neglecting friction):

$$\frac{dv}{dt} = -f(u - u_g)$$

it is readily seen that the nature of the departure of the observed zonal wind from the corresponding geostrophic wind south of latitude  $13^\circ$  N. is such as to give rise to a southward directed acceleration of an air parcel in the lower troposphere in both the seasons. The sub-geostrophic easterlies in the upper troposphere south of  $20^\circ$  N. in the monsoon season will also lead to the same result. These findings are in conformity with the observed<sup>7</sup> meridional circulation.

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<sup>1</sup> Jordan, C. L., *Quart. J. Roy. Met. Soc.*, **79**, 153 (1953).

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<sup>4</sup> Johnson, D. H., and Mörth, H. T., *Mem. East African Met. Dept.*, **3**, 8 (1961).

<sup>5</sup> Ramage, C. S., *Nature*, **201**, 1208 (1964).

<sup>6</sup> Lockwood, J. G., *Nature*, **202**, 1324 (1964).

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## Influence of Solar Flare Particles on the General Circulation of the Atmosphere

In the past a few attempts have been made to establish an influence of solar flares on atmospheric pressure, all attempts being limited to a rather small region or even a single station<sup>1</sup>. In the work recorded here I have computed the change in height of the 500-mb level during the first 24 h after a flare for 54 grid-points covering a large portion of the northern hemisphere. The period of investigation is the International Geophysical Year, for which 53 flares well distributed over the whole period have been selected

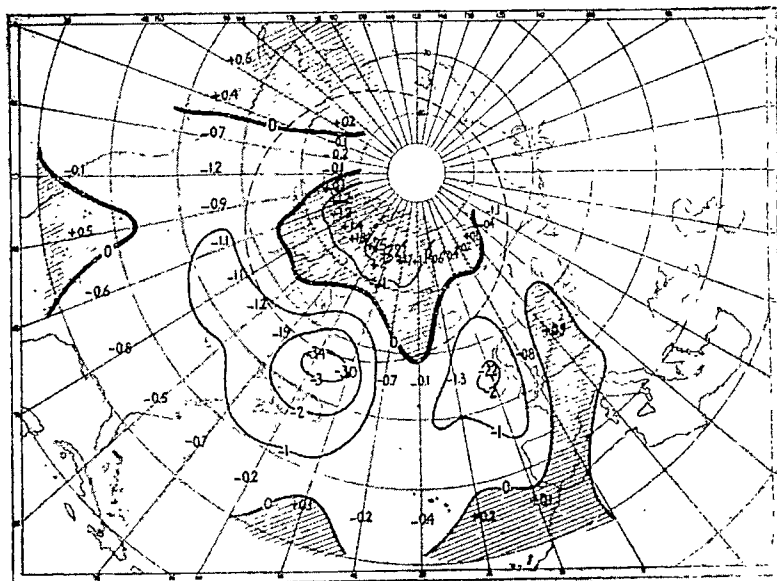


Fig. 1. Change in height of the 500-mbar level during the first 24 h after the occurrence of a solar flare. Mean of 53 cases. Units: geopotential decametres/day. The dashed circle indicates the zone of maximum auroral frequency

from the Boulder Reports, all flares being of importance  $2^+$  or higher. For flare outbursts which occurred between 12 U.T. and 24 U.T. the next day was taken as 'zero-day'; this was a consequence of the fact that only height data of 00 U.T. were used.

Thus I computed the mean change in height of the 53 cases and plotted the results for the 54 grid-points in Fig. 1. For only five grid-points the mean values are significant beyond the 5 per cent level. Nevertheless, the pattern shows a remarkable regularity with a symmetry with respect to the geomagnetic rather than to the geographic pole.

The main features suggested by the picture are a rise in height of about 2.5 geopotential decametres/day in the region of the geomagnetic axis-pole and a wide region of fall in height (max. 3.5 geopotential decametres/day) coinciding quite well with the auroral zone. Towards the equator of this ring-shaped region again a rise in height seems to occur.

The idea of the auroral zone as the most obvious region of tropospheric reaction to solar particles has already been put forward by Riehl<sup>2</sup>.

More recently Berkofsky and Shapiro<sup>3,4</sup> treated the problem in a theoretical way by assuming a particle-induced heat source having the shape of the auroral zone.

On a similar assumption, but without specifying the height of the heat source (which is actually unknown), I tried to compute the pressure variations in the atmosphere due to such a source of heat.

To perform the calculations, for convenience I have chosen geomagnetic co-ordinates ( $x, y, z$ ). Starting with the formula:

$$\frac{\partial p}{\partial t} = g \int_z^{\infty} \frac{\partial \rho}{\partial t} dz \quad (1)$$

thus neglecting compensating vertical motions, I take into account only the density variations due to isobaric divergence, which according to Schmidt<sup>5</sup> can be written as:

$$\frac{\partial \rho}{\partial t} = -\frac{1}{f^2} \left( \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) \frac{\partial p}{\partial t} \quad (2)$$

where  $f$  is the Coriolis parameter.

Further, let us assume that the heat source  $Q(\vec{x}, y, z, t)$  causes a heating which can be expressed as  $dT B(x, y) F(z)$ . This heating will initiate a density variation at constant pressure:

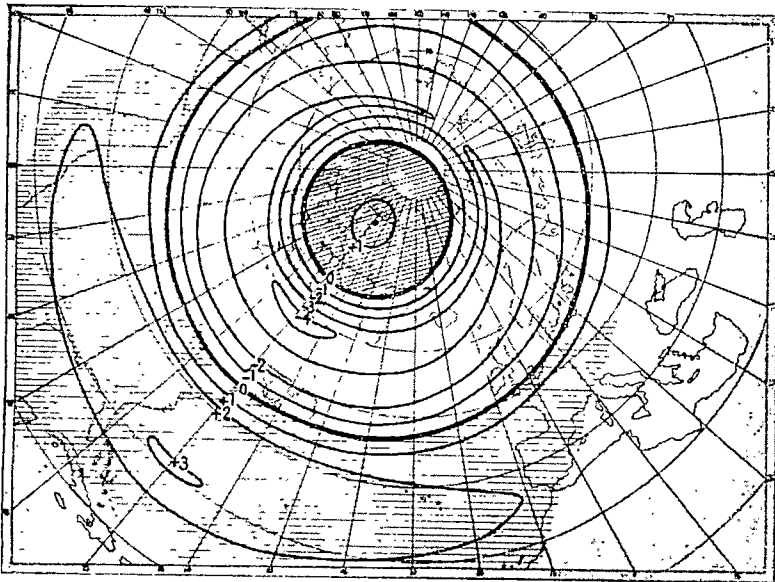


Fig. 2. Computed pressure change at sea-level (in arbitrary units) due to an atmospheric heat source having the shape of the auroral zone

$$\frac{\partial p}{\partial t} = -\frac{p}{T} \frac{\partial T}{\partial t} = -\frac{p}{T} dT B(x, y) F(z) \quad (3)$$

After integration of equation (3) with respect to  $z$  in (1) and substitution of the result in (2), we obtain a second

approximation for  $\frac{\partial p}{\partial t}$ . By integration of the latter expres-

sion again with respect to  $z$ , but this time from zero to infinity because of the unknown vertical displacements, we arrive at the tendency equation:

$$\frac{\partial p}{\partial t}(x, y)_0 = \frac{g^2 dT F_0}{f^2} \left( \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) B(x, y) \quad (4)$$

Since the auroral zone is almost circular about the geomagnetic pole, we take  $B(x, y)$  independent of  $x$  and assume it normally distributed in  $y$ , so that:

$$B(x, y) = \exp \left( -\frac{(y - y_m)^2}{b^2} \right) \quad (5)$$

where  $y_m$  is the geomagnetic latitude of maximum auroral frequency and  $b$  is the width of the distribution function. Carrying out the differentiation in (4), we find:

$$\frac{\partial p}{\partial t}(x, y)_0 = \frac{2 g^2 dT F_0}{b^2 f^2} \exp \left( -\frac{(y - y_m)^2}{b^2} \right) \left\{ \frac{2(y - y_m)^2}{b^2} - 1 \right\} \quad (6)$$

Now we have to transform  $f = 2\Omega \sin \varphi$  into  $(x, y)$  coordinates. From a simple calculation in a spherical triangle follows:

$$\sin \varphi = \cos \Delta \sin y + \sin \Delta \cos y \cos x \quad (7)$$

with  $\Delta$  is the angular distance between the geographic and the geomagnetic pole ( $\Delta = 11^\circ 30'$ ).

Combining (6) and (7), we obtain:

$$\frac{\partial p}{\partial t}(x, y)_0 = \frac{g^2 dT F_0}{2 b^2 \Omega^2} \exp \left( -\frac{(y - y_m)^2}{b^2} \right) \left\{ \frac{2(y - y_m)^2}{b^2} - 1 \right\} \quad (8)$$

$$\sin^2 y \cos^2 \Delta + \cos^2 y \sin^2 \Delta \cos^2 x + \frac{1}{2} \sin 2y \sin 2\Delta \cos x$$

From equation (8) I have drawn a tendency map (Fig. 2) in arbitrary units (without knowing the height and vertical stratification of the heat source it is impossible to

evaluate  $F_0$ ), for  $y_m = 67.5^\circ$  N. (geomagnetic latitude) and  $b = 15^\circ (= 1.67 \times 10^8$  cm). Compared with Fig. 1 it may be concluded that probably this manner of theoretical approach is able to explain the principal features of the observed pattern.

In order to obtain a better insight into the mechanism involved, this study is now extended to other levels than 500 mb, while the duration of the influence is also examined. Special attention is given to stratospheric temperatures in order to establish the height of the diabatic heat source. From the patterns shown (Figs. 1 and 2), one may conclude that one of the possible consequences of the particle-induced disturbance for the general circulation is the development of wave numbers one and two. From their dynamical study, Berkofsky and Shapiro came to the same conclusion<sup>4</sup>.

After a solar particle invasion the pressure gradient over the east Atlantic changes in such a way as to stimulate a south-westerly current. Indeed, during the recent period of high flare frequency (1946–1960) south-westerlies have been much

more frequent than usual over this area.

Finally, as a rather speculative comment, the phenomena described in this communication may be of great importance for the establishment of solar-climate relationships.

I thank Dr. F. H. Schmidt for his stimulating interest and the many valuable discussions.

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<sup>1</sup> Nordø, J., *Pub. Norwegian Acad. Sci.*, No. 2 (1953).

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<sup>4</sup> Berkofsky, L., and Shapiro, R., *Planet. and Space Sci.*, 12, 219 (1964).

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## Tattering of Flags under Controlled Conditions

THE rate of tatter of standardized textile flags has been used as a cheap and convenient index of exposure to wind<sup>1-3</sup>, but no attempt has been made to determine whether, or to what extent, water in the flag affects the rate of tatter. This effect has been measured to determine whether loss in dry matter may be substituted for loss in area as a measure of tatter, and whether it is acceptable to remove the protective fray from a used flag in order to re-expose it on its original site for further periods.

Flags with initial free-flying area  $9 \times 14$  in. were prepared by placing 2 oz. madapollam (D.T.D. 343) between sheets of glass illuminated from below, and by tracing outlines parallel to the weft and warp. Threads were then shredded and cut from the material until only the appropriate outline remained. Air-flow of known velocity was generated by controlling the size of outlet and speed of centrifugal fans; flags were mounted in the air-flow and measurements made of losses in area and dry matter over periods of  $1\frac{1}{2}$ –12 h. The outline of solid weave was recorded after the protective fray had been removed and the residue weighed. Flags were thus exposed for successive periods, the loss in area and dry matter being recorded each time as a percentage of the area and weight of free-flying material in the initial flag. The mean of percentage reduction in area and percentage weight loss of dry flag was adopted as the most accurate measure of tatter, and for each flag, means over successive periods were averaged to give a long-term measure.

Some flags were exposed dry; others were kept saturated throughout. Fig. 1 shows that for dry flags a linear

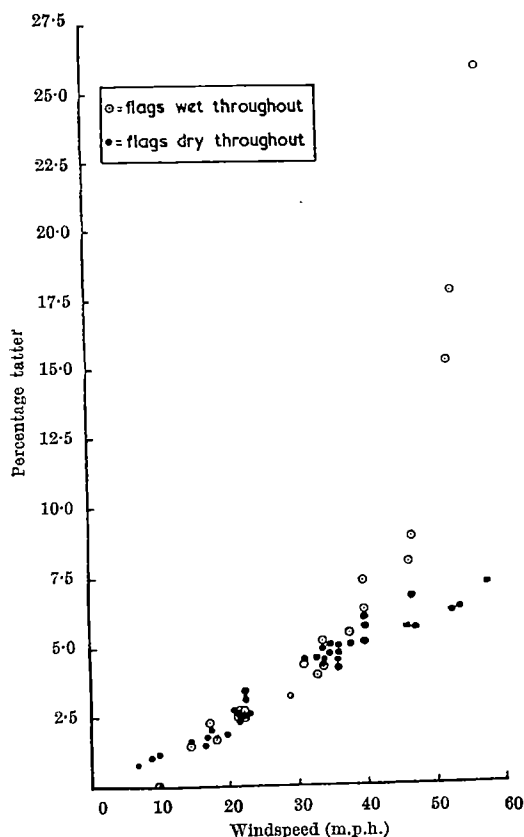


Fig. 1. Flags were exposed over eight successive 90-min periods. For each period a mean was calculated of losses in dry-weight and area as percentages of free flying material in the initial flag. Readings were averaged over successive periods and the resultant means are shown in relation to windspeed

relationship holds between percentage tatter ( $y$ ) and windspeed ( $x$ ), the regression line being given by

$$y = -0.256 + 0.136x$$

This linear regression contrasts with an exponential relationship for saturated flags (a linear relationship  $\log y = -0.134 + 0.024x$  between the logarithm of percentage tatter ( $y$ ) and windspeed ( $x$ ) shows that this relation is exponential). When percentage tatter is plotted against run of wind, linear and exponential relationships are again evident.

The regression equation  $y = -0.256 \pm 0.136x$  indicates that, theoretically, winds down to 1.882 m.p.h. cause tattering when flags are dry. In practice flags must fly freely before tattering can occur, and in the case of wet flags of  $9 \times 14$  in., winds of 10 m.p.h. are necessary to allow them to fly freely. Once flags are supported by the air flow, however, they tatter in accordance with the relationships  $y = -0.256 + 0.136x$ , and  $\log y = -0.134 + 0.024x$ , for dry and wet flags respectively.

These results indicate that under dry conditions the madapollam flag gives a reasonable measure of run of wind and of mean windspeed. Unfortunately, unless continuous records of rainfall and windspeed allow elimination of excess tatter due to water in the flag at high windspeeds, it is evident that the use of flags where high winds accompany rain must exaggerate variation in exposure to wind. If no correction factor for rainfall can be applied to field measurements, no valid comparison of run of wind or mean windspeed can be made between sites or between non-contemporaneous exposures.

Water in the flag is unlikely to exaggerate exposure to wind at windspeeds below 30–35 m.p.h., but if windspeeds exceed this level during periods of rain, and if no correction

factor for rainfall can be established, exposure will be exaggerated. Consequently, the use of flags as indicators of exposure in biological studies will be acceptable only where a measure of integrated effects of wind and rain is sought, and where single and integrated biological effects may be described by regression lines similar to those for wet and dry flags. The madapollam flag cannot be regarded as a physical anemometric instrument; it gives a measure of exposure, but only as a function of the interaction of wind and rain with the particular fabric used.

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## CHEMISTRY

### Preparation and Melting Point of Di-2-phenylethylmercury

THE preparation of di-2-phenylethylmercury (I) has been reported by Criegee, Dimroth and Schempf<sup>1</sup>, and Nerdel and Makower<sup>2</sup>. Criegee *et al.*<sup>1</sup> prepared (I) by the reaction of 2-phenylethylmagnesium chloride (II) with mercuric chloride and obtained (I) as an oily product, the identity of which was confirmed by its reaction with mercuric chloride to form 2-phenylethylmercuric chloride (III) (85 per cent) and with mercuric acetate to form 2-phenylethylmercuric acetate (35 per cent). The melting-point of (I) was not recorded. Nerdel and Makower<sup>2</sup>, using a similar method (reaction of 2-phenylethylmagnesium bromide (IV) with mercuric chloride), obtained a sample of (I) which crystallized at  $-40^\circ$ .

In an attempt to clarify these results, the preparation of (I) was carried out by three methods. The first method was a two-stage synthesis in which (II) (0.140 mole) reacted with mercuric chloride (0.154 mole) to form (III), m.p.  $164^\circ$  (Criegee *et al.*<sup>1</sup>: m.p.  $165^\circ$ – $168^\circ$ ), and then (III) was caused to react with (II) to form (I). The second method was also a two-stage synthesis in which (IV) (0.11 mole) reacted with mercuric bromide (0.12 mole) to form a 2-phenylethylmercuric bromide (V), m.p.  $169^\circ$  (Hill<sup>3</sup>: m.p.  $169^\circ$ ), and then (V) (0.05 mole) reacted with (IV) (0.10 mole) to form (I). The third method was a one-stage synthesis in which (IV) (0.12 mole) reacted with mercuric bromide (0.04 mole) to form (I). The symmetrical mercurial, (I), was obtained from all three syntheses as an oily product with approximate m.p.  $-44^\circ$ , which is comparable to the melting-point obtained by Nerdel and Makower<sup>2</sup>.

I thank E. J. Young for assistance with the experimental work.

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### Assignment of the Infra-red and Raman Bands of Liquid Water

THE assignment of the fundamental vibrational frequencies of water vapour is well established<sup>1</sup>: 3,651.7 cm<sup>-1</sup>  $\nu_1$  symmetric stretch, 1,595.0 cm<sup>-1</sup>  $\nu_2$  bending and 3,755.8 cm<sup>-1</sup>  $\nu_3$  antisymmetric stretch, of symmetry species  $a_1$ ,  $a_1$  and  $b_1$  respectively for molecular symmetry  $C_{2v}$ .

The assignment in the liquid phase is still not properly understood; the bands become very broad in both infra-red (IR) and Raman (R) spectroscopy.

Table 1 shows how the frequencies change from gas phase to liquid phase, and the assignments for liquid water made by previous authors.

	$\nu_1$	$\nu_2$	$\nu_3$
Gas	3652	1595	3756
Liquid	3450	1640	3615

Table 1

The dotted arrows in Table 1 represent the conclusions of Fox and Martin<sup>2</sup> and the solid arrows the assignments of Schultz and Hornig<sup>3</sup> and Walrafen<sup>4</sup>.

If Fox and Martin (Table 1) are correct, the origin of the 3,615 cm<sup>-1</sup> Raman band must be determined; if Schultz and Hornig are correct, the absence of a band corresponding to  $\nu_3$  near 3,615 cm<sup>-1</sup> in the infra-red spectrum must be explained. For a molecule without a centre of symmetry the rule of mutual exclusion is not applicable and there should be a maximum of correspondence between infra-red and Raman spectra.

The assignments of the Raman bands given by Walrafen, and Schultz and Hornig, are based on polarization data. Schultz and Hornig show that the depolarization ratio,  $\rho$ , for the bands at 1,640, 3,225 and 3,450 cm<sup>-1</sup> respectively is considerably less than 6/7, and, therefore, the major portion of the intensity of these bands must arise from symmetric vibrations. For the 3,615 cm<sup>-1</sup> band,  $\rho = 0.60$ , which is still rather less than  $\rho = 6/7$  expected for  $\nu_3$ . Schultz and Hornig attribute the discrepancy between 0.60 and 6/7 to overlap by the strong 3,450 cm<sup>-1</sup> band. However, if  $\rho$  were 6/7 at 3,615 cm<sup>-1</sup>, the band would not necessarily arise from an asymmetric vibration, since even symmetric vibrations may give  $\rho = 6/7$ . In our opinion, this evidence in favour of the assignment of the 3,615 cm<sup>-1</sup> band to  $\nu_3$  is extremely weak.

In general, in infra-red and Raman spectroscopy, the absolute band intensity arising from a symmetric vibration is a minimum and a maximum, respectively. The converse is true for antisymmetric vibrations. In the gas phase, the infra-red spectrum shows  $\nu_1$  very weak relative to  $\nu_3$ , and in the Raman spectrum the converse is true. It is, therefore, reasonable to suppose that in the liquid the 3,450 cm<sup>-1</sup> infra-red band is mainly  $\nu_3$  and that the 3,450 cm<sup>-1</sup> Raman band is mainly  $\nu_1$ . The 1,640 cm<sup>-1</sup> band (IR and R) is undoubtedly  $\nu_2$  from considerations of both frequency and polarization data. Assignment of the 3,225 cm<sup>-1</sup> band to  $2\nu_2$  in Fermi resonance with  $\nu_1$  is well supported by its polarization, and variation of intensity with temperature<sup>5</sup>.

We prefer the overall assignment of Fox and Martin (Table 1) and give our full assignment in Table 2. We

believe that the best assignment for the 3,615 cm<sup>-1</sup> band is a combination, 3,450 +  $\nu_H$ , where  $\nu_H$  is the stretching vibration of a hydrogen bond. The value of  $\nu_H$  is about 180 cm<sup>-1</sup> (there is some doubt about the exact value, but it is between 160 and 200 cm<sup>-1</sup>). This combination band would be expected to have a low intensity and, therefore, we postulate a second Fermi resonance between  $\nu_1$  and this band; that is,  $\nu_H$  must have the same symmetry as the 3,450 cm<sup>-1</sup> component of the combination. The result is an  $a$ -type band. This assignment explains the absence of the 3,615 cm<sup>-1</sup> band in the infra-red spectrum, because the main band at 3,450 cm<sup>-1</sup> is now  $\nu_3$ , symmetry  $b_1$ , so that Fermi resonance is not allowed. The same reasoning applies to the 3,225 cm<sup>-1</sup> ( $2\nu_2$ ) band, and consequently the 3,450 cm<sup>-1</sup> infra-red band appears with only a very slight asymmetry on the low-frequency side because of a residual intensity from  $2\nu_2$ .

The assignment we propose for liquid water is supported by several other pieces of experimental evidence. Various authors (see ref. 5) have investigated the infra-red spectrum of water in different solvents. The separation  $\Delta\nu$  between  $\nu_1$  and  $\nu_3$  is found to vary with the dielectric properties of the solvent. In carbon tetrachloride  $\Delta\nu$  is 95 cm<sup>-1</sup> while in dioxan it is only about 70 cm<sup>-1</sup>. In pyridine only one band is observed, and this has been interpreted as  $\nu_1$  and  $\nu_3$  having the same frequency. However, no polarization investigations have been made on these solutions and assignment of the two bands to more than one hydrogen-bonded species of water molecule is not excluded. Walrafen<sup>4</sup> has examined the variation of intensity of the 175 cm<sup>-1</sup> Raman band as a function of temperature and of electrolyte addition. Our results and those of Schultz and Hornig<sup>3</sup> indicate that as electrolyte is added the 3,615 cm<sup>-1</sup> and 175 cm<sup>-1</sup> bands decrease in intensity. When the temperature is raised the 3,615 cm<sup>-1</sup> band intensity appears to increase slightly<sup>3</sup> while that of the 175 cm<sup>-1</sup> band decreases<sup>4</sup>. However, this anomaly in the temperature effect is not necessarily in disagreement with our assignment, because a change in the Fermi resonance conditions with temperature could more than compensate for the temperature coefficient of the 175 cm<sup>-1</sup> band.

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### Mechanisms of Reactions in the Acenaphthene Series. Migration of *t*-Butyl, and Disproportionation

By a variety of Friedel-Crafts reactions, using *t*-butyl chloride and acenaphthene, we have synthesized and orientated 1-, 2- and 3-*t*-butylacenaphthene<sup>1-3</sup>. A mechanism is now proposed to explain the *t*-butylation of acenaphthene and the novel migrations of the *t*-butyl group observed when *t*-butylacenaphthenes were treated with 0.15 mol. of aluminium chloride at 45°:

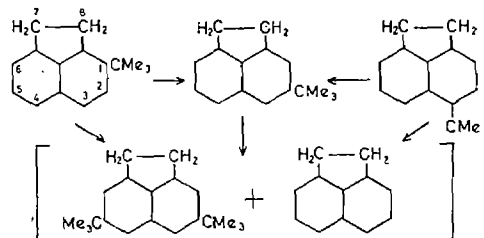


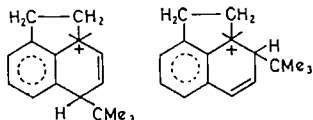
Table 2

Phase	cm <sup>-1</sup>	Symmetry species	Assignment	Observed in IR or R
Gas	1,595	$a_1$	$\nu_2$	IR
	3,652	$a_1$	$\nu_1$	R
	3,756	$b_1$	$\nu_3$	IR
Liquid	1,640	$a_1$	$\nu_2$	IR, R
	3,450	$a_1$	$\nu_1$	R
	3,450	$b_1$	$\nu_3$	IR
	3,225	$a_1$	$2\nu_2$	R
	3,615	$a_1$	$\nu_1 + \nu_H$ or $\nu_3 + \nu_H$	R

*t*-Butyl chloride is very reactive in Friedel-Crafts reactions with acenaphthene, only a small amount of aluminium chloride being required. It is probable that ionization of *t*-butyl chloride is induced by the catalyst, and that *t*-butylation of acenaphthene follows the procedure of electrophilic aromatic substitution, with attack by the relatively stable *t*-butyl carbonium ion. Reaction mixtures tend towards equilibrium, the thermodynamically most stable 2-*t*-butyl derivative, with least free energy, being formed at the expense of the less stable 1- and 3-*t*-butyl compounds. It was surprising that the main product, using aluminium chloride, was 2-*t*-butylacenaphthene, even at reaction temperatures of  $-10^{\circ}$  to  $-5^{\circ}$ . The greater thermodynamic stability of the 2-isomer could be partly due to the less sterically hindered nature of this position in acenaphthene.

Unlike aluminium chloride, ferric chloride does not cause migration of the *t*-butyl group in the acenaphthene nucleus, and a controlling factor is probably the rate of reaction. 1-*t*-Butylacenaphthene predominates at reaction temperatures of  $10^{\circ}$ – $15^{\circ}$ , whereas at  $45^{\circ}$  the 3-isomer is the main product formed. In the presence of ferric chloride, further *t*-butylation of the 1-isomer at  $10^{\circ}$ – $15^{\circ}$  gave, by ortho substitution, 1,6-di-*t*-butylacenaphthene, and thence 1,3,6-tri-*t*-butylacenaphthene, also derived directly from 1- or 3-*t*-butylacenaphthene.

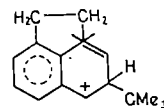
Regarding electron-availability, acenaphthene is a more reactive nucleus than benzene<sup>4</sup>. In electrophilic attack on acenaphthene, the electron-releasing inductive effect of the ace-bridge would activate both benzene rings, but in considering monosubstitution, it is convenient to represent attack to give the more stable intermediate carbonium ions of the type shown, where the positive charge is located at the carbon atom nearest the activating group:



The above represent only contributory structures of the complex hybrid carbonium ions formed by attack at positions 1 and 3, with the positive charge developing and being dispersed in the ring. The analogous carbonium ion formulated by attack at the 2-position comprises less-stable configurations. In the case of acenaphthene, any stabilization of carbonium ions by contributory hyperconjugated structures is probably a minor factor. Thus, at low temperatures and with a catalyst of moderate strength, the Friedel-Crafts *t*-butylation of acenaphthene follows the normal orientation, without cleavage of *t*-butyl, and loss of a proton from the respective hybrid carbonium ions affords the *t*-butylacenaphthene.

The literature contains no reference to group migration in the acenaphthene series. Baddeley<sup>5</sup> suggested that in alkylbenzenes, an intramolecular rearrangement occurred, the group migrating without leaving the sphere of influence of the molecule. The relative merits of intramolecular and intermolecular migration of radicals in cymenes were discussed by Allen, Alfrey and Yats<sup>6</sup>. While we have an open mind on intramolecular rearrangement mechanisms concerning migration of methyl groups, in our case, we favour a de-alkylation-re-alkylation mechanism, with formation of a *t*-butyl carbonium ion.

Formation of the carbonium ions derived by 1- and 3-attack on acenaphthene involves a reversible reaction; in the presence of aluminium chloride, the liberated *t*-butyl carbonium ion yields 2-*t*-butylacenaphthene, probably by loss of a proton from a very unstable carbonium ion, of which one contributory structure of the hybrid could be represented as:



The thermodynamic stability of 2-*t*-butylacenaphthene would cause it to persist.

A similar mechanism for the disproportionation reactions, which occur when any of the three mono-*t*-butylacenaphthenes is heated with a little aluminium chloride at  $45^{\circ}$ , would involve prior addition of a proton, probably arising from the presence of a little hydrochloric acid: a chain reaction would then ensue. Even 2-*t*-butylacenaphthene accepts a proton, and the *t*-butyl carbonium ion is liberated; this then attacks 2-*t*-butylacenaphthene at the other meta position, and subsequent loss of a proton gives 2,5-di-*t*-butylacenaphthene: thus a continuous interchange of proton and *t*-butyl carbonium ion occurs. 2,5-Di-*t*-butylacenaphthene forms a complex with aluminium chloride, and it may be the most basic, and is certainly the most stable, of the mono- and di-*t*-butylacenaphthenes. It is isolated readily from 1-, 2- and 3-*t*-butyl- and 1,6-di-*t*-butyl-acenaphthene, by treatment with 0.15 mol. of aluminium chloride in boiling carbon disulphide.

The work represents pioneer orientation in the acenaphthene series. Interest in acenaphthene, a 'cinderella' among coal-tar hydrocarbons, has been aroused by the recent development of commercial dyes for polyester fibres, of excellent fastness properties.

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## BIOCHEMISTRY

### Effects of Antibiotics on Chromosomes of Cultured Human Leucocytes

PENICILLIN and streptomycin have been commonly added to growth media used in the culture of human leucocytes for cytogenetic analysis. Since the literature on human cytogenetics is increasing rapidly, largely through the use of these techniques, it seemed appropriate to examine any possible cytological effects of antibiotics *per se* on cultured human leucocyte chromosomes or on the mitotic index.

The antibiotics used were buffered penicillin G potassium for injection, streptomycin sulphate for injection, chloramphenicol powder and crystalline tetracycline. Venous blood was drawn from a healthy male of blood type O, Rh-positive and previously known to have a normal karyotype. A modified version of the Moorhead technique for leucocyte culture was adapted for use with smaller amounts of blood, and the blood sample was divided into six parts of 2 ml. each. Phytohemagglutinin (Difco, form M) was used for the separation of red cells, and the plasma obtained was added to the culture medium (TC 199) so as to give final antibiotic concentrations of 0, 50, 250, 500, 1,000 and 2,000  $\mu\text{g/ml}$ . The fixative used was glacial acetic acid and methanol (1:3). Slides were flamed over an alcohol lamp. The higher antibiotic concentrations used were well above those which have been found in the blood serum of individuals after the administration of antibiotics in therapeutic amounts<sup>1</sup>. Each antibiotic was tested twice at all six concentrations.

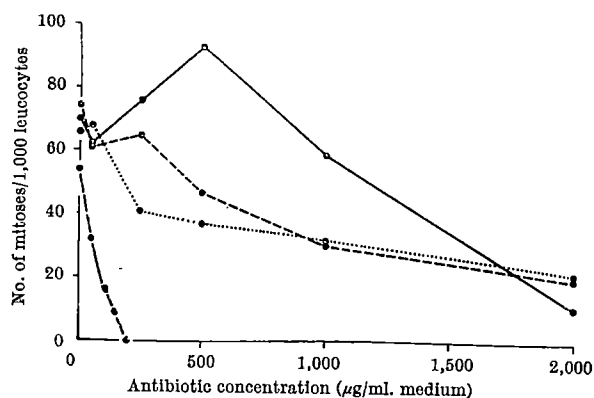


Fig. 1. — — — —, Penicillin; —, streptomycin; ·····, chloramphenicol; — · — ·, tetracycline

When possible, the number of chromosomes in 50 cells was counted as well as the number of mitoses per 1,000 leucocyte cells at each concentration. Any abnormalities, such as an atypical number of chromosomes per metaphase or alterations in chromosome morphology, were noted.

Analysis of the metaphases revealed few chromosomal aberrations in any of the cultures. With occasional exceptions, there were 46 chromosomes in each metaphase examined, and the number of chromosomal breaks and gaps was within normal limits of 2–5 per cent, as observed in control groups by Nichols *et al.*<sup>2</sup>. The occasional metaphases with 45 chromosomes were evidently products of broken cells. The findings mentioned here do not, of course, exclude chromosomal alteration at the molecular level. The chromosomes had a tendency to clump together in the highest concentration of streptomycin, chloramphenicol or tetracycline, and good spreading of the metaphases on the slide for examination was difficult to achieve. It was not possible, therefore, to count 50 metaphases at the 2,000-μg concentrations, or to determine whether or not many breaks or gaps were present.

The effects of a given antibiotic on the mitotic index varied according to its concentration, as shown in Fig. 1. The most striking effect is seen to be that of tetracycline, which inhibited mitoses completely at concentrations of more than 150 μg/ml. The tetracycline cultures were made at antibiotic concentrations of 0, 50, 100, 150, 200 and 250 μg/ml. of medium to bracket more accurately the toxic concentrations. This was done after the first series failed to produce mitoses at concentrations of more than 50 μg/ml. Although the graph indicates zero mitoses at the 200 μg/ml. level, a scanning of all the slides revealed two metaphases which were too clumped together for analysis. A small amount of precipitate was formed in the tetracycline cultures after a few hours in the incubator, a phenomenon also encountered by Hu *et al.*<sup>3</sup>. The amount of precipitate was negligible in the second series run with lower concentrations.

In the range of 500 μg/ml., streptomycin apparently stimulated mitoses. There were very slight increases in mitotic activity with lower concentrations of penicillin and chloramphenicol; but these peaks were not as convincing as in the case of streptomycin. Wilson has found that certain concentrations of penicillin had a slightly stimulatory effect on mitosis in root tips of the onion (*Allium cepa*)<sup>4</sup>.

The concentrations at which the four antibiotics tested were found to be inhibitory to mitosis in the leucocyte cultures of the present experiments were, for the most part, in agreement with those levels found to inhibit mitoses in human skin cultures<sup>5</sup>. The high concentrations of antibiotics used in the cultures are not known to be reached in blood serum when given in therapeutic amounts, except perhaps transiently at the site of

administration. Higher levels might also be found at sites of elimination, degradation or deposition. For example, 14.9 μg/ml. is one of the highest concentrations of tetracycline which has been reported for blood serum, whereas up to 320 μg/ml. of tetracycline has been measured in urine<sup>1</sup>.

Tetracycline is known to interfere with normal bone growth in chicks, rats and human infants<sup>6,7</sup>. It is also of interest that serum concentrations of penicillin, streptomycin, chloramphenicol and terramycin in the therapeutic range have been shown to traverse the placenta<sup>7</sup>. Some clinical investigations have suggested a relationship between antibiotic therapy of mothers and congenital abnormalities in their children<sup>8,9</sup>.

It would seem at least a possibility that the *in vitro* effects of these antibiotics on leucocyte mitosis as noted in the present investigation have some relevance to their effects in the intact, growing organism.

Samples of penicillin and streptomycin were kindly provided by E. R. Squibb and Sons; chloramphenicol by Parke, Davis and Co.; and tetracycline by Bristol Laboratories.

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### Photochemical Oxidation and Reduction of Plastoquinone sensitized by Isolated Chlorophylls

SINCE the discovery of plastoquinone in chloroplasts by Crane<sup>1,2</sup>, many investigations have been reported on the role of the quinone in photosynthesis. Bishop<sup>3</sup> showed that the quinone participated in the photolysis of water. Krogmann<sup>4</sup> and Krogmann and Olivero<sup>5</sup> demonstrated the indispensability of the quinone as a co-factor for photophosphorylation. Crane *et al.*<sup>6</sup> and Redfearn and Friend<sup>7</sup> observed photo-induced reduction and dark reoxidation of the quinone in chloroplasts. The localization of plastoquinone in the electron transport system of photosynthesis was verified by Witt's school<sup>8,9</sup>. They indicated that the quinone acted as a primary electron acceptor from photochemically reduced chlorophyll *a*. Since all these investigations were carried out with chloroplasts, no evidence has been presented showing the direct interaction of plastoquinone with chlorophylls. Tollin and Green<sup>10</sup> observed electron paramagnetic resonance signals, showing the light-induced single electron transfer reactions between chlorophyll *a* and various kinds of quinones, but plastoquinone was not included among the quinones they used. In this communication, we present evidence indicating that plastoquinone can be

oxidized or reduced photochemically in the presence of chlorophyll *a* or *b*.

Chromatographically pure chlorophyll *a* and *b* were prepared according to the method of Perkins and Roberts<sup>11</sup>. Crystalline plastoquinone and its reduced form were prepared as described by Crane<sup>2</sup>. Photo-reduction of plastoquinone was carried out in a small Thunberg-type tube. An ethanolic solution contained 0.056  $\mu$ mole of chlorophyll *a*, 0.145  $\mu$ mole of plastoquinone and 50  $\mu$ moles of ascorbic acid in a total volume of 2 ml. The tube containing the above reaction mixture was evacuated for 5 min with a vacuum pump. After standing for a further 10 min in the dark, the mixture was illuminated for 15 min with a 500-W white incandescent tungsten lamp through a 10-cm water layer, which gave a light intensity of approximately 40,000 lux. The reaction was run at room temperature, about 20° C. After illumination, 0.5 ml. of 0.25 N HCl and 4 ml. of iso-octane were added. The mixture was transferred into a small glass-stoppered centrifuge tube. After shaking vigorously, the mixture was centrifuged at a low speed. The upper iso-octane layer contained plastoquinone, while ascorbic acid remained in an aqueous ethanolic layer. Then the absorption spectrum of the iso-octane layer was measured with the Hitachi recording spectrophotometer, type 'EPS-2'. Fig. 1*a* shows the spectrum of the illuminated sample (curve 1). After standing for 3 h in the dark, the spectrum of the same sample was measured (curve 2). Fig. 1*b* also shows the spectrum of the sample wrapped with aluminium foil to protect from light (curve 1), and that measured after reduction with sodium borohydride (curve 2).

These spectra indicate that plastoquinone could be photoreduced in the system described here (Fig. 1*a*, curve 1) and reoxidized slowly in the dark (Fig. 1*a*, curve 2), and that thermal or dark reduction of plastoquinone by ascorbic acid was negligible (Fig. 1*b*, curve 1).

The reaction velocity of the photoreduction was determined as follows; ethanolic solution containing 0.02  $\mu$ mole of chlorophyll *a*, 0.75  $\mu$ mole of plastoquinone and 250  $\mu$ moles of ascorbic acid in a total volume of 10 ml. was placed in a small 'lollipop' and bubbled throughout the experiment by a stream of nitrogen. Light from a 200-W projection lamp was passed through a red filter (which cut off the wave-lengths shorter than 600 m $\mu$ ) and a 10-cm water layer. The light intensity was approximately 7,000 lux. An aliquot (2 ml.) was taken from the mixture at various time intervals, and each aliquot was treated as described here. The photoreduction was found to be of second order and the initial velocity of the reaction was 12.3  $\mu$ moles plastoquinone photoreduced/min/mg chlorophyll *a*. On an equivalent electron basis, the rate was about ten times faster than that of the Hill reaction by isolated spinach chloroplasts, since 1.38–1.86  $\mu$ moles 2,6-dichlorophenol indophenol was found to be photoreduced/min/mg chlorophyll under the same experimental conditions.

On the other hand, the dye was reduced instantaneously with reduced plastoquinone. Therefore the Hill reaction using the dye as an oxidant may be drawn in the following scheme in which plastoquinone acts as a primary electron acceptor from chlorophyll *a*.

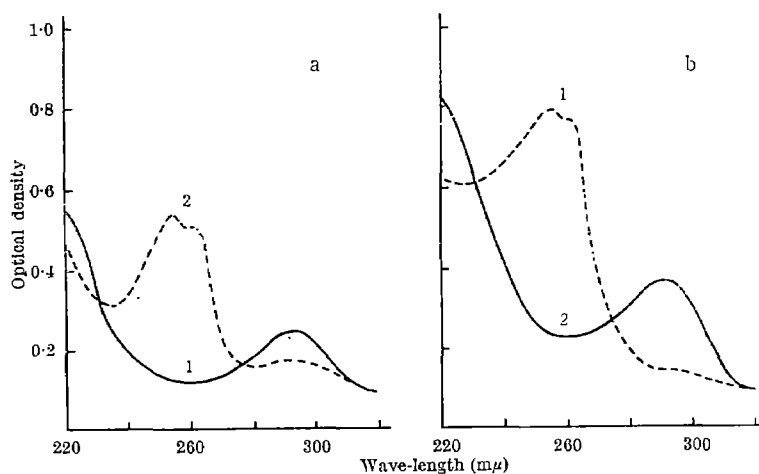
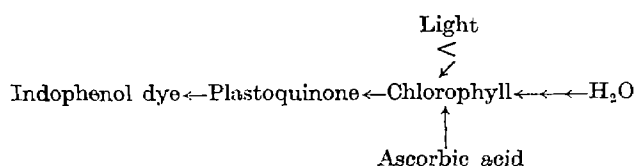


Fig. 1. Chlorophyll *a*-sensitized photoreduction of plastoquinone by ascorbic acid. *a*, Curve 1, spectrum measured immediately after illumination; curve 2, spectrum measured 3 h after illumination; *b*, curve 1, spectrum of non-illuminated sample; curve 2, spectrum measured after reduction with a few grains of borohydride

Table 1 shows that the presence of chlorophyll *a* or *b*, and ascorbic acid as an electron donor, was necessary for the photochemical reduction of plastoquinone, and that both chlorophyll *a* and *b* were almost equally effective.

Table 1. PHOTOREDUCTION OF PLASTOQUINONE: REQUIREMENTS OF A SENSITIZER AND AN ELECTRON DONOR\*

Sensitizer	Reductant	Oxidant	$\mu$ mole plastoquinone photoreduced per 15 min
Chlorophyll <i>a</i>	Ascorbic acid	Plastoquinone	0.145
Chlorophyll <i>b</i>	Ascorbic acid	Plastoquinone	0.145
—	Ascorbic acid	Plastoquinone	0.018
Chlorophyll <i>a</i>	—	Plastoquinone	0.027

\* A complete reaction mixture contained 0.056  $\mu$ mole of chlorophyll *a* or 0.002  $\mu$ mole of chlorophyll *b*, 50  $\mu$ moles of ascorbic acid and 0.145  $\mu$ mole of plastoquinone.

Auto-oxidation of reduced plastoquinone was also sensitized by chlorophyll *a* or *b*. The reaction was followed in 3 ml. quartz cell having a light path of 1.0 cm. Ethanolic solution containing either 0.056  $\mu$ mole of chlorophyll *a* or 0.062  $\mu$ mole of chlorophyll *b* and 0.054  $\mu$ mole of reduced plastoquinone in a total volume of 3 ml. was illuminated with a white incandescent lamp (40,000 lux). As a dark control, the cell was wrapped with aluminium foil. The spectrum taken after reaching a steady-state level showed the typical absorption peak of oxidized plastoquinone at 255 m $\mu$ . The time course of the oxidation is shown in Fig. 2. Here also the sensitizing activity of chlorophyll *a* and *b* was essentially the same.

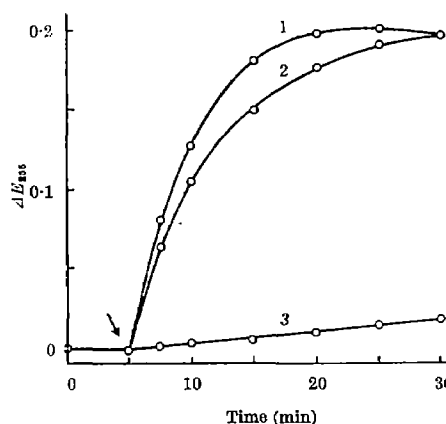


Fig. 2. Time course of the photochemical oxidation of reduced plastoquinone sensitized by chlorophylls. Curve 1, with chlorophyll *b* as a sensitizer. Curve 2, with chlorophyll *a*. Curve 3, dark control with chlorophyll *a*. Arrow indicates time of turning on the light



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### Chromatography of Soluble Macromolecular Components of HeLa Cells

ONLY limited success has been obtained in the electrophoretic analysis of soluble macromolecular components from HeLa human cancer cells or other cells grown *in vitro*. The separation is poor, and the fractions overlap; in the case of HeLa cells, the relatively best results have been obtained with starch gel<sup>1</sup>. Macromolecules can be efficiently fractionated on cellulose ion exchangers, and under strictly defined conditions their chromatographic properties may be as characteristic for them as electrophoretic mobility and behaviour in sedimentation. In the work recorded here, the chromatography on DEAE-cellulose columns of soluble components of HeLa cells has been studied. In view of the small amount of material available, micromethods have been applied, and both proteins and nucleic acids have been estimated sensitively by their absorption at 210 m $\mu$ . Amounts of the order of 1–3 mg protein could still be analysed.

HeLa cells were grown in bottles (40 cm<sup>2</sup> surface) containing 10 ml. of a salt-lactalbumin hydrolysate medium with 10 per cent human cord serum. Usually the material of 5–15 bottles, amounting to  $(50-150) \times 10^6$  cells, was used for an experiment. The cells were collected, washed, suspended in 1–2 ml. of 0.01 M phosphate buffer (pH 7.3–7.4) containing 0.003 M MgCl<sub>2</sub>, and treated in an all-glass Potter-Elvehjem homogenizer. Unbroken cells, nuclei and mitochondria were sedimented by centrifugation at 10,000*g* for 10 min. The supernatant was further fractionated into the microsomal and the 105,000*g* supernatant fraction in a Spinco preparative ultracentrifuge. The microsomal pellet suspended in 0.01 M phosphate buffer and the supernatant fraction were dialysed against the starting buffer before chromatography.

Elution was carried out with a series of phosphate buffers (KH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub>) of constant pH (7.2) and increasing molarity from 0.01 up to 0.3 M. Two additional eluants were 0.3 M KH<sub>2</sub>PO<sub>4</sub> and 0.3 M KH<sub>2</sub>PO<sub>4</sub> + 1 M NaCl. The absorption was determined at 210 m $\mu$  against a blank of the corresponding buffer<sup>2</sup>. Occasional readings were taken also at 260 m $\mu$ . The content of nucleic acids is reflected by the 210/260 ratio. It was higher than 10 for the majority of fractions. It was, however, only 2–3 for the 0.3 M KH<sub>2</sub>PO<sub>4</sub> + 1 M NaCl fraction; clearly nucleic acid is present here.

The separation is efficient and well reproducible. The chromatographic profile obtained appears to be characteristic of soluble cell components and differs decidedly from that obtained with serum proteins. Soluble macromolecular components from other cells grown *in vitro* (human fibroblasts) as well as from freshly isolated spleen and liver cells give similar patterns. General features are

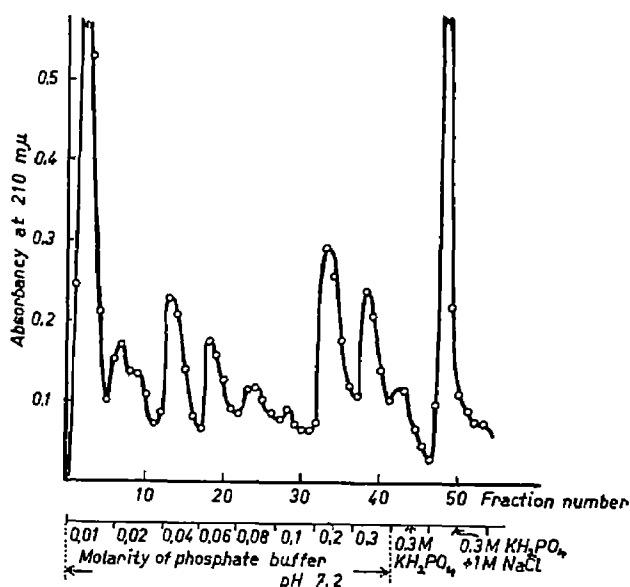


Fig. 1. Chromatogram of 105,000*g* supernatant after dialysis overnight against the starting buffer. 0.5 ml. of the solution with an absorbancy 150 at 210 m $\mu$  were placed on a 15 cm  $\times$  0.7 cm column loaded to a height of 10 cm; the flow rate was 5 min per fraction (2.2–2.4 ml. each).

the large amount of material eluted with buffers in the 0.1–0.3 M range, and the nucleic acid content of the last fraction.

Differential ultracentrifugation and subsequent analysis of the microsomes and the 105,000*g* supernatant showed that the components eluted in the 0.01–0.1 M range occur predominantly in the latter fraction (Fig. 1). Components emerging from the column at higher buffer molarity are present both in the microsomal and supernatant fraction (Figs. 1 and 2). This distribution probably reflects differences in chemical nature as well as in molecular size. Heterogeneity in molecular weight was confirmed by gel filtration on 'Sephadex G-200' of microsomes and supernatant<sup>3</sup>.

In the elution of serum proteins, at 0.08 M mainly albumins appear. In the case of HeLa, little material is present in this range. Similarly, only little protein with the electrophoretic mobility of albumins had been found in HeLa<sup>1</sup>. Fractions obtained at <0.08 M in HeLa probably correspond to components with the mobility of  $\alpha$ - and  $\beta$ -globulins<sup>1</sup>. One fraction is of special interest, namely, that eluted at 0.01 M and containing material not adsorbed

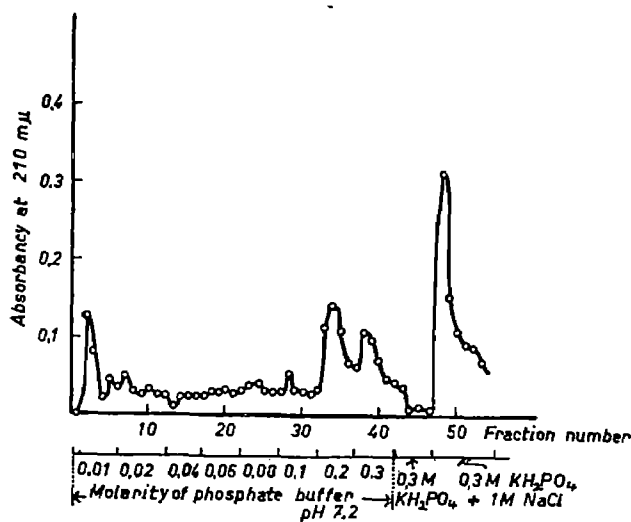


Fig. 2. Chromatogram of microsomal fraction. The pellet was suspended in 0.01 M phosphate buffer and dialysed overnight against this buffer. 0.5 ml. of the solution with an absorbancy 70 at 210 m $\mu$  were placed on the column and separated as described under Fig. 1.

on the ion exchanger. In the serum protein system, this fraction has been shown to consist of  $\gamma$ -globulins. An electrophoretic fraction with the mobility of  $\gamma$ -globulin has been demonstrated in HeLa proteins<sup>4</sup>, and at least part of the 0.01 M fraction appears to be identical with it.

In the presence of iron, HeLa cells produce ferritin<sup>5</sup>. We have grown HeLa with 10  $\mu$ g FeSO<sub>4</sub> and 0.3–1  $\mu$ c. <sup>59</sup>Fe per ml. The radio-iron was found in three peaks after chromatography of the homogenate, two of them emerging from the column with the 0.2 and 0.3 M phosphate buffers, and the third with 0.3 M KH<sub>2</sub>PO<sub>4</sub> + 1 M NaCl. Purified horse spleen ferritin<sup>6</sup> gave a chromatogram much like that of the component containing radio-iron from HeLa. Further evidence that the radioactivity is due to ferritin has been obtained by gel filtration on 'Sephadex G-200' (ref. 3). In paper electrophoresis, the mobility of the radioactive component of HeLa was identical with that of purified ferritin from horse spleen, but a little greater than that of ferritin from normal human liver. This is consistent with results from polyacrylamide gel electrophoresis<sup>7</sup>.

It appears, then, that DEAE-cellulose chromatography in conjunction with the measurement of ultra-violet absorption at 210 m $\mu$  and of radioactivity is an efficient method for the separation and identification of soluble macromolecular cell components.

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### Identity of Cow $\beta$ -Lactoglobulin 'B' and Buffalo $\beta$ -Lactoglobulin

SINCE the demonstration by Aschaffenburg and Drewry<sup>1,2</sup> that  $\beta$ -lactoglobulins of cow's milk occur in two different forms, 'A' and 'B', many attempts have been made to find the differences in the primary structure of these two protein types. The differences between the two genetic species have been shown<sup>3,4</sup> to lie in the contents of four amino-acids, namely, aspartic acid, valine, glycine and alanine,  $\beta$ -lactoglobulin A having one more residue each of aspartic acid and valine and one less residue each of glycine and alanine than  $\beta$ -lactoglobulin B per half molecule. Likewise, chymotryptic peptide pattern analyses of sulphonated  $\beta$ -lactoglobulins A and B have indicated similarity of peptide pattern except for a single pair in which one aspartic acid residue in 'A' is replaced by one glycine residue in the corresponding 'B' peptide<sup>5</sup>. Information regarding the buffalo  $\beta$ -lactoglobulin, on the other hand, is scanty. It has been reported<sup>6,7</sup> that cow  $\beta$ -lactoglobulin B and buffalo  $\beta$ -lactoglobulin have approximately the same molecular weight and are practically indistinguishable in crystal form, electrophoresis, sedimentation and ultra-violet absorption.

The present report deals with the structural characterization of cow  $\beta$ -lactoglobulin B and buffalo  $\beta$ -lactoglobulin by means of amino-acid composition and peptide pattern analysis.

Table 1. AMINO-ACID COMPOSITION OF COW  $\beta$ -LACTOGLOBULIN B AND BUFFALO  $\beta$ -LACTOGLOBULIN

Amino-acid	Ratio amino-acid to protein (g/100 g):	
	In cow $\beta$ -lactoglobulin B	In buffalo $\beta$ -lactoglobulin
Aspartic acid	11.02 (15)	10.73 (15)
Threonine	5.25 (8)	5.06 (8)
Serine	3.89 (7)	3.78 (7)
Glutamic acid	19.95 (25)	19.79 (25)
Proline	5.27 (8)	5.20 (8)
Glycine	1.55 (4)	1.60 (4)
Alanine	7.23 (15)	7.23 (15)
Valine	6.00 (9)	5.70 (9)
Methionine	3.09 (4)	2.02 (4)
Isoleucine	7.22 (10)	7.43 (10)
Leucine	15.58 (22)	15.86 (22)
Tyrosine	3.75 (4)	4.24 (4)
Phenyl alanine	3.60 (4)	3.42 (4)
Lysine	11.79 (15)	12.27 (15)
Histidine	1.44 (2)	1.61 (2)
Arginine	2.66 (3)	2.66 (3)
Tryptophan	2.00 (2)	2.11 (2)

The values represent averages of three determinations. Figures in parentheses represent nearest integers of the number of residues per half mole of protein of molecular weight 18,300. Tryptophan was determined on separate samples by ultra-violet spectroscopy (ref. 13).

Non-casein protein fractions were prepared by the method of Aschaffenburg<sup>2</sup>. Paper electrophoresis for the survey of whey proteins was run at 110–150 V on Whatman No. 1 paper for 15 h using barbitone-HCl buffer, pH 8.6 ( $I = 0.05$ ). Suitable samples of cow and buffalo  $\beta$ -lactoglobulins were crystallized according to the method of Larson *et al.*<sup>8</sup>, and were recrystallized four times before use. The three  $\beta$ -lactoglobulins were subjected to column chromatography on DEAE cellulose (0.65 m.equiv./g; Bio-Rad Lab., U.S.A.) according to the method of Piez *et al.*<sup>4</sup> with certain modifications. The elution of  $\beta$ -lactoglobulins was observed to occur between 0.04 M and 0.08 M NaCl in 0.05 M phosphate buffer, pH 5.8 (column 10 cm.  $\times$  0.9 cm; speed 30–40 ml./h). Appropriate fractions were dialysed and freeze-dried. The amino-acid composition was determined according to the method of Moore and Stein as adapted by Van der Helm<sup>9</sup>. Sulphonated derivatives were prepared by the method of Weil *et al.*<sup>10</sup>. Tryptic digests and 'fingerprints' of the native and *S*-sulphonated derivatives were prepared in the usual manner<sup>11</sup> with pyridine-butanolic acetic acid-water as the chromatographic solvent<sup>12</sup>.

A survey of the occurrence of cow  $\beta$ -lactoglobulins in the 'Gir' breed indicated that there is a more or less equal content of  $\beta$ -AB and  $\beta$ -B types, while  $\beta$ -A was not detected in any of 65 samples of milk examined. No polymorphism, however, was observed in the  $\beta$ -lactoglobulins in 20 milk samples each from 'Jaffarabadi' and 'Surti' breeds of buffaloes, which showed only one protein type. The evaluation by electrophoretic and chromatographic procedures indicated that cow  $\beta$ -lactoglobulin B was identical in its behaviour to buffalo  $\beta$ -lactoglobulin. The amino-acid composition of cow  $\beta$ -lactoglobulin B was also found to be similar to that of buffalo  $\beta$ -lactoglobulin (Table 1). The 'fingerprints' of the tryptic peptides of these two protein types were also similar (Fig. 1). No difference was found between cow  $\beta$ -B obtained from  $\beta$ -B

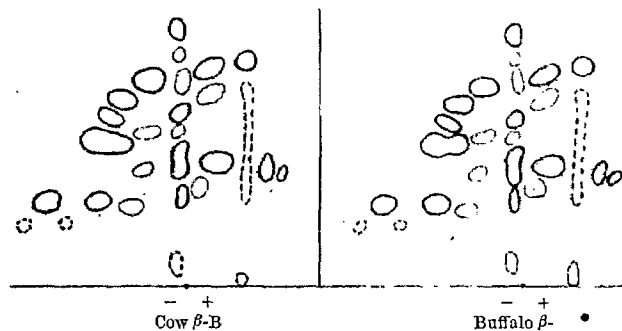


Fig. 1. Tracings of the 'fingerprints' of tryptic digests of cow  $\beta$ -lactoglobulin B and buffalo  $\beta$ -lactoglobulin. Electrophoretic buffer: Mcll's buffer pH 6.4. Chromatographic solvent: butanol-pyridine-acetic acid-water (30:20:8:24). Major peptide spots are marked boldly.

homozygotes and that isolated from cow  $\beta$ -AB through DEAE cellulose columns. These results suggest that cow  $\beta$ -B and buffalo  $\beta$ -lactoglobulin may be identical. Differences, if any, must be very small, involving uncharged groups.

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### Inhibition of Plasma Monoamine Oxidase by Cysteamine

CYSTEAMINE has been shown to cause a transient inhibition of diamine oxidase<sup>1</sup>, which was explained by its coupling with pyridoxal phosphate in a thiazolidine ring. Even when linked to pyridoxal phosphate thiols may undergo oxidation to the corresponding disulphides<sup>2,3</sup> in the case of cysteamine the non-enzymatic oxidation to cystamine liberates pyridoxal phosphate, and the diamine oxidase activity is restored. Because the enzyme oxidizes cystamine<sup>4</sup>, we have an interesting example of an inhibitor which is changed into a substrate during the reaction.

To test the suggestion that cysteamine acts as inhibitor by binding pyridoxal phosphate, we have examined its activity towards another pyridoxal dependent enzyme, plasma monoamine oxidase. This was obtained from beef plasma according to Yamada and Yasunobu<sup>5</sup>. The action of cysteamine on the oxidation of spermine or spermidine was examined with enzyme preparations purified up to the steps 3 and 4 of the above authors, with similar results. The enzymatic activity was determined by following the oxygen uptake in the Warburg apparatus.

Fig. 1 shows that in the presence of cysteamine the oxidation of spermidine is inhibited until all the cysteamine has been non-enzymically oxidized to the corresponding disulphide. This is clearly indicated by the biphasic behaviour of the curves of the oxygen uptake (Fig. 1, curves 3 and 4). After the oxygen consumption has reached the theoretical value for oxidation of the thiol to the disulphide, the enzyme starts to oxidize its own substrate. The complete oxidation of cysteamine at this point has been checked by the nitroprusside test. The non-enzymatic character of the cysteamine oxidation was demonstrated by incubation with a boiled enzyme (Fig. 1, curve 7). The inhibitory effect of cysteamine has also been obtained using spermine as substrate for the enzyme. The transient inhibition of monoamine oxidase by cysteamine, which is very similar to the inhibition already demonstrated for diamine oxidase, also supports the suggestion that this particular activity of cysteamine involves pyridoxal phosphate. In this respect cysteamine

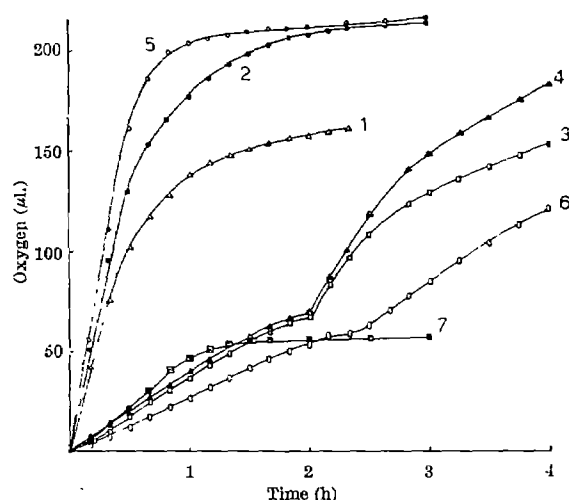


Fig. 1. 2 mg of beef plasma monoamine oxidase purified up to the step 4 of ref. 5 were incubated in 0.1 M phosphate buffer pH 7, with the following compounds: curve 1, spermidine 10  $\mu$ moles; curve 2, spermidine 20  $\mu$ moles; curve 3, spermidine 10  $\mu$ moles + cysteamine 10  $\mu$ moles; curve 4, spermidine 20  $\mu$ moles + cysteamine 10  $\mu$ moles; curve 5, spermidine 10  $\mu$ moles + cysteamine 10  $\mu$ moles; curve 6, cysteamine 10  $\mu$ moles. Temp.: 38°. Final volume, 3 ml. Gas phase: air. 2 mg of boiled enzyme were incubated in the same conditions with 10  $\mu$ moles of cysteamine (curve 7).

shows a noticeable specificity: another thiol tested, cysteine, which is unable to affect diamine oxidase<sup>1</sup>, is also ineffective on monoamine oxidase (Fig. 1, curve 5).

In the presence of cysteamine alone, an oxygen uptake greater than the theoretical amount for oxidation to the disulphide (Fig. 1, curve 6) indicates that cystamine is oxidized by the enzyme. Preliminary experiments in fact have shown that the purified plasma monoamine oxidase oxidizes cystamine, in agreement with results obtained with ox plasma by Bergeret and Blaschko<sup>6</sup>.

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### Conversion of Cyano- and Hydroxo-cobalamin *in vivo* into Co-enzyme Form of Vitamin B<sub>12</sub> in the Rat

SINCE 5,6-dimethylbenzimidazolyl cobamide co-enzyme (DBCC) was shown to be one of the active forms of vitamin B<sub>12</sub> by Barker *et al.* in 1959 (ref. 1), its biochemical co-enzymatic activity (conversion of glutamate to methyl aspartate, methyl malonyl-CoA to succinyl-CoA, and 1,2-diols to aldehydes) and its physiological metabolism (tissue distribution, excretion and absorption) have been investigated<sup>2-8</sup>. It is now believed that vitamin B<sub>12</sub> exists as a co-enzyme form in the liver, and takes part in the transformation of methyl malonyl-CoA to succinyl-CoA (ref. 9). On the other hand, enzymatic synthesis of DBCC from B<sub>12</sub> derivatives has been confirmed by several workers at the bacterial enzymatic level. It has been reported that the liver and kidney homogenate of rat could convert cyanocobalamin (CN-B<sub>12</sub>) to co-enzyme B<sub>12</sub> *in vitro*<sup>10</sup>. But the only report indicating that CN-B<sub>12</sub> or hydroxocobalamin (OH-B<sub>12</sub>) can be converted to co-enzyme form *in vivo*, on the quantitative base, is Fenrych's short communication reporting the conversion of CN-B<sub>12</sub> into the

Table 1. CONVERSION OF CN-B<sub>12</sub> AND OH-B<sub>12</sub> TO DBCC IN RAT LIVER 3, 12 AND 24 h AFTER ADMINISTRATION

	Administration of			
	CN-B <sub>12</sub>		OH-B <sub>12</sub>	
	Alone	With H.I.F.	Alone	With H.I.F.
After 3 h:				
Uptake by liver (μg)	5.3	46.5	10.6	50.3
Conversion rate to DBCC (%)	11.9	8.7	15.3	10.6
Absolute value of DBCC (μg)	0.63	4.04	1.62	5.23
After 12 h:				
Uptake by liver (μg)	4.9	21.5	11.2	45.4
Conversion rate to DBCC (%)	19.6	9.5	24.8	16.8
Absolute value of DBCC (μg)	0.96	2.04	2.78	7.63
After 24 h:				
Uptake by liver (μg)	7.8	15.2	13.4	31.8
Conversion rate to DBCC (%)	26.3	20.0	46.9	35.8
Absolute value of DBCC (μg)	2.05	3.04	6.28	11.4

Table 2. CONVERSION OF CN-B<sub>12</sub> AND OH-B<sub>12</sub> TO DBCC IN RAT LIVER 3 h AFTER ADMINISTRATION (CCl<sub>4</sub> INJURY)

	CN-B <sub>12</sub> administration			
	Normal		CCl <sub>4</sub> 6 h	
	Alone	With H.I.F.	Alone	With H.I.F.
Uptake by liver (μg)	5.3	46.5	2.4	17.2
Conversion rate to DBCC (%)	11.9	8.7	9.9	9.6
Absolute value of DBCC (μg)	0.63	4.04	0.23	1.65
	OH-B <sub>12</sub> administration			
	Normal		CCl <sub>4</sub> 12 h	
	Alone	With H.I.F.	Alone	With H.I.F.
Uptake by liver (μg)	10.6	50.3	9.8	26.6
Conversion rate to DBCC (%)	15.3	10.6	10.5	9.5
Absolute value of DBCC (μg)	1.62	5.23	1.03	2.66

co-enzyme form in rabbit<sup>11</sup>. Our preliminary report concerning this conversion, which was obtained by <sup>57</sup>Co-labelling of the DBCC fraction in rat liver following the intravenous administration of <sup>57</sup>Co-labelled CN-B<sub>12</sub> and <sup>57</sup>Co-labelled OH-B<sub>12</sub> in rat, will be described here.

Male albino rats of Wistar strain weighing 150–200 g received, by intravenous injection, 100 μg of <sup>57</sup>Co-labelled CN-B<sub>12</sub> or <sup>57</sup>Co-OH-B<sub>12</sub> with or without 5 mg of hog intrinsic factor (H.I.F.) (Bifactor) and killed 3, 12 and 24 h afterwards. The rat liver was removed, homogenized in acetone and filtered, and then the residue was dried. The acetone powder was extracted with ethanol and the filtrate was again dried at 40°C *in vacuo* and then dissolved in water, according to Barker's method<sup>1</sup>. An aliquot of this solution was placed on 10 × 1 cm P-cellulose column (Serva, Entwicklungslabor, Heidelberg) buffered with 0.1 N acetate buffer of pH 3.5. CN-B<sub>12</sub> was eluted with distilled water, OH-B<sub>12</sub> with 0.05 M CH<sub>3</sub>COOH at pH 3 and DBCC with 0.1 M CH<sub>3</sub>COONH<sub>4</sub> of pH 6.9, respectively. Identification of B<sub>12</sub> derivatives was made by absorption spectra with a Beckman-type spectrophotometer as well as by isotopic counting of a paper-strip-electrophoretogram which was done with pH 2.7 acetate at 16 V/cm for 3.5 h. The chromatographic separation and identification of CN-B<sub>12</sub>, OH-B<sub>12</sub> and DBCC was based on the method by Kato *et al.*<sup>12</sup> and will be reported elsewhere in detail. The counting of the DBCC fraction was done with a well-type scintillation counter with a Type SP-1 spectrometer (Shimadzu Co., Ltd., Japan), and the conversion rates and amounts were calculated.

The results obtained in normal rats are shown in Table 1. The amounts of DBCC converted from the administered CN-B<sub>12</sub> 3 h after administration, with and without H.I.F., were 4.04 and 0.63 μg, respectively. The conversion rates increased with time, and the converted DBCC amounted to 0.96 and 2.05 μg, 12 and 24 h after the administration without H.I.F., and 2.04 and 3.04 μg when given with H.I.F. It is interesting that the intravenous co-administration of H.I.F. enhanced not only the hepatic uptake but also amounts of DBCC converted from CN-B<sub>12</sub>, although the conversion rates were rather low, as compared to those without H.I.F. OH-B<sub>12</sub>, which perhaps occupies a more important position in B<sub>12</sub> metabolism than CN-B<sub>12</sub> (refs. 13 and 14), showed higher conversion to DBCC as compared to CN-B<sub>12</sub>. The amount of DBCC converted from <sup>57</sup>Co-OH-B<sub>12</sub> was approximately three times that from <sup>57</sup>Co-CN-B<sub>12</sub>, 3, 12 and 24 h after the administration without H.I.F. On the other hand, although a little more

DBCC was converted from OH-B<sub>12</sub> than from CN-B<sub>12</sub> 3 h after the administration when given with H.I.F., almost three times as much was obtained after 12 and 24 h.

The conversion of CN-B<sub>12</sub> or OH-B<sub>12</sub> to DBCC in rat liver pre-treated with 10 per cent carbon tetrachloride is shown in Table 2. One hundred μg of <sup>57</sup>Co CN-B<sub>12</sub> or <sup>57</sup>Co-OH-B<sub>12</sub>, with or without H.I.F., was intravenously injected 6 or 12 h after the intraperitoneal administration of CCl<sub>4</sub>, and the liver was removed 3 h after B<sub>12</sub> injection. As the conversion is thought to occur in the liver, and a significant decrease of DBCC content was found in CCl<sub>4</sub>-injured liver<sup>15</sup>, it is reasonable to presume that the conversion is decreased in the injured liver. It was found that the hepatic uptake of CN-B<sub>12</sub> and OH-B<sub>12</sub> was decreased 6 h after CCl<sub>4</sub> administration and again increased after 12 h. But conversion to DBCC did not recover even after 12 h; and more OH-B<sub>12</sub> than CN-B<sub>12</sub> was converted to DBCC.

It can be concluded that CN-B<sub>12</sub> or OH-B<sub>12</sub> are converted into the co-enzyme form of B<sub>12</sub> in the normal rat liver. Though 100 μg of <sup>57</sup>Co-CN-B<sub>12</sub> or <sup>57</sup>Co OH-B<sub>12</sub> is thought to be beyond the physiological dose, this dose was used to obtain enough counts for measurement. These results also suggest that the disturbance of CN-B<sub>12</sub> utilization, generally used in the clinical field, could occur in some conditions with liver injury and, furthermore, therapeutic application of OH-B<sub>12</sub> has an advantage over that of CN-B<sub>12</sub> in patients with liver diseases<sup>16</sup>, not only because of its reported retention for a long time in the blood stream but also because of its easy convertibility to co-enzyme form. On the other hand, the results of CCl<sub>4</sub> injury indicated a more significant disturbance of the conversion of CN-B<sub>12</sub> than of OH-B<sub>12</sub> to DBCC. This appears to suggest that CN-B<sub>12</sub> will be transformed to DBCC through OH-B<sub>12</sub> and CCl<sub>4</sub> injury primarily disturbs the pathway from CN-B<sub>12</sub> to OH-B<sub>12</sub>. Work on the effect of ATP or FAD administration on this conversion reaction *in vivo*, and possible increase of conversion in the B<sub>12</sub>-deficient rat, is now in progress.

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## PHYSIOLOGY

Circulatory and Metabolic Processes in Adipose Tissue *in vivo*

To permit the study *in vivo* of circulatory and metabolic processes in adipose tissue, a part of the dog's subcutaneous tissue was prepared with intact circulation and innervation. The preparation was found to be suitable for quantitative investigations on the nervous control of the blood flow and of the release of free fatty acids. We considered it to be of general interest to describe the method and some of its applications.

The experiments were performed on female mongrel dogs (weight 10–15 kg) anaesthetized with sodium pentobarbital (30 mg/kg with supplement as necessary). The animals were fasted for about 24 h. The subcutaneous adipose tissue in the lower abdominal region was exposed by a midline incision from the pubis to the level of the pubic mammary glands. The left or right part of the subcutaneous adipose tissue was then isolated from the overlying skin and the underlying abdominal muscles by cautery and blunt dissection. The adipose tissue was tied both caudally and cranially with ligatures. The cranial ligatures isolated the adipose tissue from the mammary glands. Great care was taken to leave the nerves and blood vessels supplying the adipose tissue undamaged during the preparation. One or sometimes two nerves (n. iliohypogastricus, n. ilioinguinalis) accompanied the artery and the vein (a. et v. epigastrica superficialis) from the external hiatus of the inguinal canal to the adipose tissue.

When the foregoing nerves and vessels had been prepared, all other vascular connexions between the adipose tissue preparation and the rest of the dog were ligated. The skin was replaced to maintain warmth and moistness. The adipose tissue prepared in this way weighed between 23 and 97 g (mean 44 g,  $n = 17$ ). In 5 dogs, blood flow was measured by cannulating the vein and directing the blood through a drop chamber filled with silicone oil. Heparin was administered (intravenously) to prevent clotting. In 12 dogs, the tissue was perfused at a constant flow rate with defibrinated blood from the same dog via the superficial epigastric artery, by means of a technique described by Renkin and Rosell<sup>2</sup>. Heparin was not used when the release of free fatty acids was examined, as it is known to release the clearing factor lipase. The ipsilateral sympathetic chain was isolated via the anterior approach and transected. The peripheral ends of the cut sympathetic chain or the cut nerves to the adipose tissue were stimulated with a Grass *S 4D* stimulator. For the study of release of free fatty acids, arterial samples were taken from the blood reservoir in the perfusion apparatus. Venous blood samples were collected during 1- to 5-min periods. Free fatty acids were determined in each sample according to Dole<sup>3</sup> as modified by Trout, Estes and Friedberg<sup>4</sup>. The net uptake or release was calculated as the arteriovenous free fatty acid difference times plasma flow.

Acute denervation did not change flow of blood significantly. Blood flow in the acutely denervated preparation was found to vary between 3.4 and 9.3 ml./min.100 g tissue (mean value = 6.7 ml./min.100 g,  $n = 5$ ). A pronounced vasoconstriction occurred following stimulation of the sympathetic chain (2–5 V) at the level of  $L_1$ – $L_3$  or stimulation of the appropriate peripheral nerves. When stimulation had ceased the vasoconstriction was sometimes followed by a vasodilatation for several minutes. The vasoconstriction was blocked by dihydroergotamine, indicating that it was due to the stimulation of adrenergic vasoconstrictor nerves. Stimulation following the administration of dihydroergotamine induced a vasodilatation. This could not be blocked by atropine, which indicates that it was not due to the stimulation of cholinergic vasodilator nerves similar to those innervating

skeletal muscle vessels<sup>5</sup>. No further attempts have so far been made to analyse the mechanism for this vasodilatation.

In another series of experiments, the adipose tissue was perfused with defibrinated blood at a constant level of flow and the net release of free fatty acids determined. The level of flow was set at a value giving approximately the same perfusion pressure as the prevailing arterial blood pressure. Such an experiment is illustrated in Fig. 1. The perfusion pressure was about 100 mm Hg at a constant blood flow of 8.1 ml./min.100 g. Before the nerves to the preparation were stimulated there was a small net uptake of free fatty acids. Electrical stimulation of the appropriate nerves (3 i.p.s.) resulted in an immediate and pronounced increase in perfusion pressure. The net outflow of free fatty acids did not change appreciably during the 10-min stimulation period. When stimulation was discontinued the perfusion pressure returned towards resting level. A net release of free fatty acids occurred, with a maximum of about 0.9  $\mu$ equiv./min.100 g adipose tissue. By about 15 min after stimulation had stopped the net release of free fatty acids was back to prestimulation level and there followed a period of net uptake. Similar results were obtained by stimulating the sympathetic chain at the level of  $L_1$ – $L_3$ .

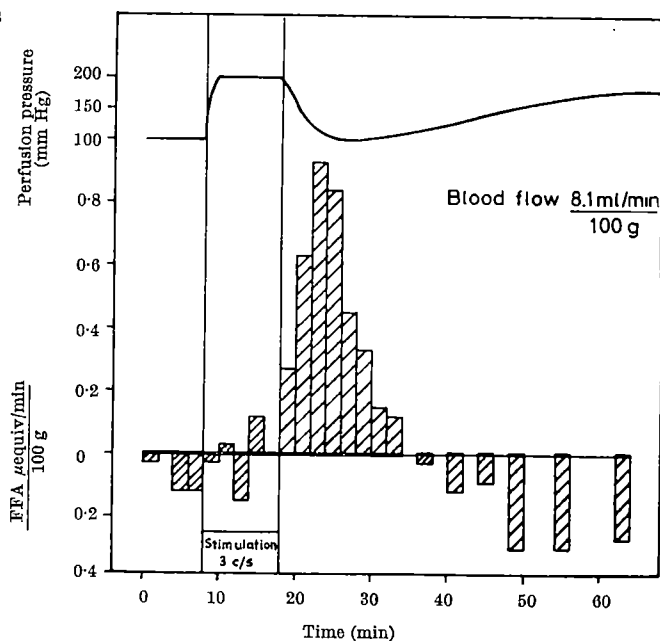


Fig. 1. Perfused subcutaneous adipose tissue from dog. Changes in perfusion pressure and in the net release of free fatty acid (FFA) with electrical stimulation of the nervous supply (5 V, 10 msec, 3 i.p.s.)

Spitzer and Hohenleitner<sup>6</sup> determined the change in plasma free fatty acids on passage through the abdominal subcutaneous tissue in dogs. Their technique, however, did not permit any quantitative determinations of the release of free fatty acids, mainly due to a lack of information on blood flow in the tissue. Our results indicate that there is an appreciable flow of blood in the subcutaneous adipose tissue in dog under resting conditions. A mean value of about 7 ml./min.100 g is in the upper range for resting blood flow in, for example, the skeletal muscles<sup>7</sup>. Moreover, it is directly shown that electrical stimulation of the nervous supply to subcutaneous adipose tissue stimulates the release of free fatty acids, which is in accordance with the results obtained by Correll<sup>8</sup> from *in vitro* experiments. Furthermore, it is demonstrated that there is a pronounced net release of free fatty acids during nerve stimulation at frequencies considered to be within the physiological range (3 i.p.s.).



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## Free and Bound Noradrenaline in the Rabbit Heart

SUB-CELLULAR storage particles containing noradrenaline (NA) have been demonstrated in noradrenergic nerves and in organs supplied with such nerves<sup>1,2</sup>. The nerve vesicles, which have a diameter of 0.03–0.1  $\mu$  (ref. 3), have been subjected to a number of investigations regarding stability in various media, release and uptake of NA and other amines, and action of drugs<sup>4,5</sup>.

The NA in the vesicles represents on an average  $28 \pm 3$  (S.D.) per cent of the total NA present in the press juice obtained from the nerves<sup>4</sup>. Higher proportions were obtained with the same technique applied to the suprarenal medulla, chromaffin cell tumours, and organs containing chromaffin cells. These results indicate that the major part of the noradrenaline in the nerve axons is free or loosely bound.

The NA-containing particles in the terminal portions of the adrenergic nerves, previously demonstrated in homogenates of organs<sup>1,6</sup>, show many properties in common with the nerve vesicles<sup>7,8</sup>.

Electron microscopic pictures<sup>9</sup> suggest that the proportion of vesicle-bound NA is considerably higher in the nerve terminals than in the pre-terminal axons. In the work recorded here NA was determined in the high-speed sediment (vesicle-bound) and in the soluble fractions of the homogenized rabbit heart.

Rabbits of both sexes, weighing 2–3 kg. were killed by air injection and the hearts immediately removed, washed in ice-cold phosphate buffer, blotted dry, and homogenized in 10 ml. buffer per g organ in an Ultra Turrax apparatus. The homogenate was subjected to differential centrifugation in order to separate the particulate fraction containing NA from the coarse fraction and the soluble NA. Previous experience from separation of the different fractions in press juice of bovine splenic nerves has shown that centrifugation at 9,000g for 10 min yielded a relatively pure particle suspension as shown by electron microscopic pictures<sup>3</sup>.

The supernatant after treatment of the rabbit heart homogenate in this way was later centrifuged at 100,000g for 45 min. The sediment contained a high proportion of vesicles of the same type as found in the nerve (Fig. 1).

The amounts of NA/g of rabbit heart and the proportion of NA in the different fractions are given in Table 1. All fractions were extracted with trichloroacetic acid and adsorbed on alumina. NA was determined fluorimetrically and the values are given as the base (uncorrected).

If the NA in the coarse fraction, representing mostly incompletely homogenized tissue, is subtracted from the total amount, the proportion of particle-bound NA to the sum of the bound and free NA was  $52 \pm 2.4$  per cent.



Fig. 1. Suspension of high-speed sediment from homogenate of rabbit heart. Negative staining with phosphotungstate. Inserted mark 0.1  $\mu$  ( $\times c. 36,000$ ).

Since the recovery of added NA to the homogenate was about 80 per cent, it was assumed that the loss by enzymatic breakdown was negligible.

The present results thus indicate that the proportion of vesicle-bound NA relative to the sum of the free and vesicle-bound NA is significantly higher than that in bovine splenic nerves, the difference being  $24 \pm 2.7$  per cent ( $P < 0.001$ ).

The relative as well as the absolute values obtained for the vesicle-bound NA in the rabbit heart may well be too low, since a portion of the vesicles will probably sediment with the coarse fraction. Moreover, it appears likely that the proportion of vesicle-bound NA is still higher in the varicosities proper which have been shown by histochemical<sup>10</sup> and electron microscopic technique<sup>9</sup> to have a high concentration of NA and vesicles.

Hertting and Hess<sup>11</sup> have reported that the specific activity of the NA in guinea-pig heart, after previous injection of tritiated NA in animals treated with reserpine, is unchanged at different degrees of depletion, suggesting that the intra- and extra-granular NA are in equilibrium. This has been shown with direct measurements for the rabbit heart in our laboratory<sup>5</sup>. In a series of experiments

Table 1. NA IN FRACTIONS OF HOMOGENIZED RABBIT HEART (Mean  $\pm$  S.E.M.)

	NA ( $\mu$ g/g)	% of total NA	% of sum of vesicle bound and soluble
Vesicle bound (high-speed sediment)	$0.66 \pm 0.091$	$39 \pm 1.5$	$52 \pm 2.4$
Soluble fraction	$0.66 \pm 0.053$	$36 \pm 1.7$	$48 \pm 2.4$
Coarse fraction (sediment from 9,000g)	$0.43 \pm 0.058$	$25 \pm 0.65$	—
Total	$1.7 \pm 0.18$	—	—

Table 2. RELATIONSHIPS BETWEEN TOTAL NA AND NA IN HIGH-SPEED SEDIMENT (VESICLE BOUND) AND IN THE SOLUBLE FRACTION OF HOMOGENIZED RABBIT HEART

	Decaborane treated				Controls $M \pm S.E.M.$
Total NA ( $\mu$ g/g)	0.21	0.60	0.71	0.82	$1.7 \pm 0.18$
Vesicle bound in % of total NA	33	36	35	34	$39 \pm 1.5$
Vesicle bound in % of soluble NA	80	105	86	93	$109 \pm 10.4$
Vesicle bound in % of sum of vesicle bound and soluble NA	45	51	46	43	$52 \pm 2.4$



on the rabbit heart partially depleted with decaborane we have also observed that the proportion of vesicle-bound NA in relation to the sum of vesicle-bound and soluble NA is approximately the same, independently of the total amount of NA (Table 2). If the vesicle-bound and the soluble NA are in dynamic equilibrium, as suggested by our experiments and by those of Michaelson *et al.*<sup>12</sup>, the concentration of the free NA might still be the same in the axons and in the terminal parts under normal conditions although the relative amounts of vesicle-bound and free NA are different.

The results recorded here show that the proportion of vesicle-bound NA in the terminal parts of the axons is considerably higher than that in the pre-terminal parts and that the proportions of NA in the high-speed sediment and in the soluble fractions of the homogenized rabbit heart are approximately constant and independent of the total amount of NA in the organ, suggesting a state of equilibrium between these fractions.

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### Chemical Control of Sensory Ganglia during a Critical Period of Development

Two populations of cells appear during differentiation of sensory ganglia in the chick embryo. These have been designated as ventro-lateral (V-L) and median-dorsal (M-D)<sup>1-3</sup>. Changing the field of innervation, by either implantation or extirpation of limb buds, produces morphologic alterations solely in the V-L cells. The nerve growth-promoting protein (NGF), described and partially characterized by Levi-Montalcini and Cohen<sup>4-6</sup>, produces hyperplasia and hypertrophy of embryonic M-D sensory cells as well as sympathetic nerve cells. Although the growth-promoting effects of NGF on sympathetic cells persist into adulthood in various species, its stimulatory effect on sensory cells is restricted to the embryonic period of development<sup>5</sup>. It was the purpose of this investigation to delineate further the critical period during development when sensory ganglia exhibit a morphological response to purified NGF and to explore some of the mechanisms controlling the reactive sequence.

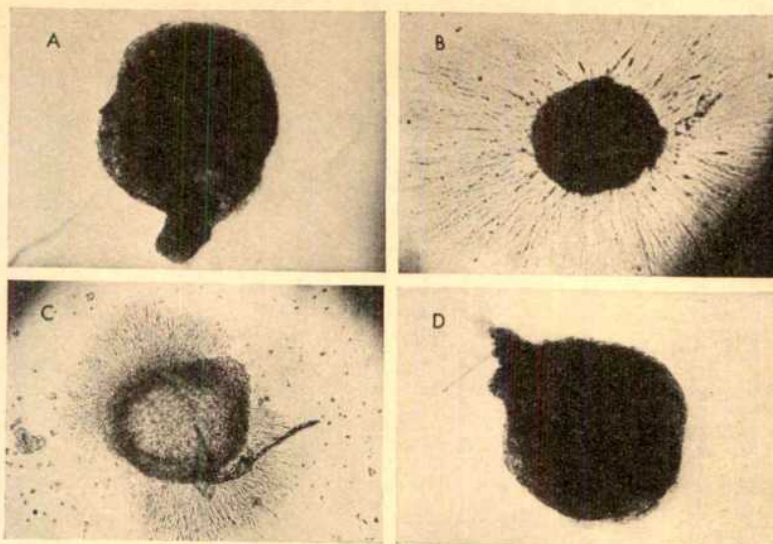


Fig. 1. Response of sensory ganglia of various ages to test concentrations of NGF (16-20 h incubation): (A), 6 day; (B), 7 day; (C), 13 day; (D) 14 day

NGF was purified from adult male mouse salivary glands according to a modification of the method of Cohen<sup>6,7</sup>. Anti-serum was prepared by serial injections of purified NGF with Freund's complete adjuvant into rabbits. A single band of precipitation is demonstrable following agar diffusion and immunoelectrophoresis when purified NGF is reacted against its anti-serum.

Sensory ganglia were removed from chick embryos on the fifth through the 14th day of incubation. *In vitro* demonstration of the growth-promoting effects of NGF was performed by a modification of the method of Levi-Montalcini<sup>8</sup>. The amount of NGF required to produce a 3+ response (arbitrary 1 to 4+ scale) in sensory ganglia from embryos incubated 9-10 days (approximately 0.02 to 0.08 µg/c.c.) was used when culturing ganglia from all embryos. The response to NGF was evaluated in terms of the characteristic appearance of a halo of fibres within 24 h. Ganglia grown *in vitro* prior to bioassay were cultured in depression slides, in a liquid medium of similar composition to that used for bioassay but omitting embryo extract.

Chick sensory ganglia react to NGF only from the seventh through the 13th day of incubation. Before 7 and after 13 days, they exhibit no visible growth when exposed to the nerve growth-promoting factor. Fig. 1 compares the reaction of 6-, 7-, 13- and 14-day ganglia. All these changes in response occur abruptly, since there is no demonstrable growth in 6- and 14-day ganglia even if tested with 100 times the concentration of NGF.

Although both 5- and 6-day ganglia are unresponsive, they exhibit a marked difference in their ability to develop this response in tissue culture. Ganglia removed at six days' incubation and grown for 24 h *in vitro* gain the ability to demonstrate fibre outgrowth when afterwards incubated with NGF. Those removed at five days and cultured for one or two days before testing remain unreactive (Table 1).

The abrupt loss of the capacity to exhibit fibre outgrowth in response to NGF, occurring between the 13th and 14th day of incubation, only occurs *in vivo*. Removal of sensory ganglia at 12 days, followed by two days in tissue culture, results in ganglia still able to respond. If 14-day ganglia, however, are grown for two days *in vitro*, they are still refractory to the effects of NGF.

If small amounts of NGF are added to ganglia from 5-day embryos in tissue culture, they remain unreactive when afterwards assayed with standard amounts of NGF. However, when a similar experiment is conducted with 12-day ganglia, no response to NGF can be demon-



Table 1. RESPONSE OF SENSORY GANGLIA TO NGF, AS DETERMINED BY NERVE FIBRE OUTGROWTH 16-20 h AFTER BEING PLACED IN PLASMA CLOT CULTURE

Culture medium includes: 1 part Eagle's basal medium-chick embryo extract (5:1), 1 part rooster plasma, 1 part NGF in 0.9 per cent saline (0.02-0.08  $\mu\text{g}/\text{c.c.}$  incubation media). *In vitro* refers to tissue culture period prior to bioassay. NGF added to tissue culture in a concentration (0.002-0.004  $\mu\text{g}/\text{c.c.}$  media) too low to effect fibre outgrowth (NGF *in vitro*)

Age of ganglia (days)		No. of experiments *	NGF <i>in vitro</i>	Response
Total	<i>In vivo</i>			
5	5	0	3	Neg.
6	5	1	3	Neg.
6	5	1	2	Neg.
6	6	0	3	Neg.
7	5	2	2	Neg.
7	5	2	2	Neg.
7	6	1	3	Pos.
7	6	1	2	Pos.
7-13	7-13	0	10	Pos.
14	14	0	10	Neg.
14	12	2	4	Pos.
14	12	2	3	Neg.

\* Each experiment involves observations on 6-14 ganglia.

strated after 2 days in tissue culture. This change is age specific, since 10-day ganglia, similarly cultured, continue to respond two days later.

These changes in the response of ganglia at different ages do not reflect permeability factors. For the demonstration of permeability of ganglia to NGF by the fluorescent antibody technique, ganglia were incubated in liquid culture for 24 h at 37°C, washed in 0.01 M phosphate buffer, pH 6.8, and afterwards incubated for 30 min with specific anti-serum or control rabbit serum. The ganglia were re-washed with buffer and stained with goat anti-rabbit  $\gamma$ -globulin which had been previously conjugated with fluorescein (Antibodies, Inc., Davis, California). After washing again in buffer, the ganglia were mounted on slides and examined for specific fluorescence<sup>9</sup>. Using the fluorescent antibody technique, nerve growth-promoting protein is bound to 6- and 14-day ganglia after incubation for 24 h, even though no fibre growth occurs (Fig. 2).

These results indicate that sensory ganglia from chick embryos undergo a sequence of differentiative phases. Between the fifth and sixth day, a change occurs wherein they develop a characteristic response to NGF on the seventh day whether left *in vivo* or cultured *in vitro*. At 13 days, another change occurs, when ganglia lose their capacity to respond to NGF, in the time-sequence examined, only if left *in vivo*. While NGF does not seem to effect the initial change, the loss of reactivity may be under control of the nerve growth factor.

The developmental sequence described in these investigations provides a model system for the correlation of

morphological and biochemical events during various phases of differentiation of sensory ganglia.

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## PHARMACOLOGY

### A General Theory of the Genesis of Drug Dependence by Induction of Receptors\*

DRUG dependence can occur in organisms as far apart as bacteria<sup>1</sup> and man, and even in isolated preparations of cells<sup>2</sup> or muscle<sup>3</sup>. The theory proposed here is intended to explain the genesis in mammals of dependence on addictive drugs, such as morphine; but there seems no reason why it might not apply to drug dependence in other organisms or in isolated preparations and to tolerance towards non-addictive drugs. This explanation is based on the concept of Ehrlich<sup>4</sup> that a chemical substance acts on a living system through its molecules becoming attached to particular sites (receptors) on cells. It uses the following aspects of receptor theory, which are to be found in pharmacological literature in other connexions.

(1) *Silent receptors*. Several authors have proposed that a drug may be taken up on certain receptors without producing an observable response<sup>5</sup>. Such receptors have been called 'silent receptors', 'sites of loss' or 'dead spots'. Silent receptors are conceived as reducing drug activity by taking up molecules which would otherwise reach 'active', 'audible' or 'live' receptors.

(2) *Interaction with endogenous substances*. A drug may act by depriving cells of an endogenous substance which plays a natural part in their working. Drugs are believed to do this by occupying receptors for that substance or by lessening the amount of it produced or released. For example, at the nerve-muscle junction, *d*-tubocurarine is believed to paralyse by blocking access of the natural transmitter (acetylcholine) to the muscle end-plate, and botulinum toxin to paralyse by reducing the amount of acetylcholine released<sup>6</sup>. A drug may also act by the opposite mechanism of producing an excess of endogenous substance, either

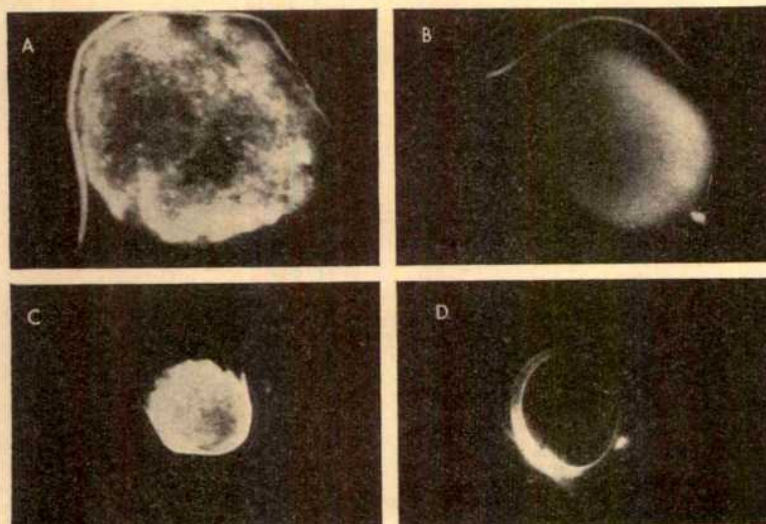


Fig. 2. Immunofluorescence of ganglia when incubated with specific anti-serum or control rabbit serum and tested with goat anti-rabbit  $\gamma$ -globulin coupled with fluorescein. (A), 14-day ganglia plus specific anti-serum; (B), 14-day ganglia plus control rabbit serum; (C), 6-day ganglia plus specific anti-serum; (D), 6-day ganglia plus control rabbit serum

\* I outlined this theory at the Ciba Foundation Study Group on Hashish in London on October 21, 1964.



by releasing it from store or by inhibiting its destruction.

(3) *Induction of receptors.* Ehrlich<sup>4</sup> held that new receptors for an antigen are produced when specific antibodies are formed. Miledi<sup>7</sup> has used the term 'induction of receptors' to describe the increase of sensitivity to acetylcholine which develops on skeletal muscle fibres elsewhere than at the end-plate, after transection of a muscle fibre or motor nerve. If a living system is distorted by excess or deficiency of chemical substance, induction of receptors in the direction lessening that distortion is to be expected. Thus a foreign chemical would tend to induce silent receptors for itself, whereas deprivation of an endogenous substance would tend to induce active receptors for the lacking substance. For example, within one or two weeks of its administration, botulinum toxin induces receptors for acetylcholine over the whole surface of the muscle fibre<sup>6</sup>. Increase in the number of accessible active receptors to an endogenous substance is assumed to increase the ease with which that substance can excite a tissue. A converse concept, that excess of an endogenous substance reduces the number of its receptors and the ease with which it excites a tissue, has also been proposed<sup>6</sup>.

Concepts 1 and 3 provide a mechanism by which tolerance might develop. This would be expected if one dose of drug were to induce new silent receptors, which would in turn take up part of a second dose of drug without giving a pharmacological response. This explanation has the advantage that it does not require an increase in enzyme destroying the drug, as has often been suggested, but has seldom been found sufficient to explain the degree of tolerance reached. To explain the development of tolerance to one action of a drug but not another, or the presence of tolerance in tissue isolated from a treated animal, silent receptors might be supposed to develop alongside active receptors in cells responding to drug. In so far as tolerance arises from induction of silent receptors for drug, it need not be associated with dependence. Tolerance associated with dependence might be expected to develop by the mechanism proposed below for dependence.

Concepts 2 and 3 provide a mechanism by which tolerance, withdrawal effects and craving for drug might arise. If a drug were to lessen the amount available to nerve cells of an endogenous substance concerned with their activity, this deprivation might be expected to induce additional active receptors for the substance. Tolerance to drug would develop as these extra receptors became available. When the blockade of receptors was lifted by withdrawing the drug, the endogenous substance would reach both original and extra receptors and so elicit excessive responses—the withdrawal effects. If the substance concerned had only psychic actions, the withdrawal effects would be psychic; if it also affected bodily systems, physical withdrawal signs might be expected. If the drug were again given, receptors for the endogenous substance would again be deprived and withdrawal effects abate. Drug would therefore be craved to prevent such effects. If the drug were to produce excess, instead of deficiency, of an endogenous substance, this might be expected to reduce the number of active receptors for that substance. From this reduction, tolerance and dependence would arise by a converse mechanism of the above.

An attempt to work out how the foregoing mechanisms would operate in a particular instance may be made with morphine. Some part of tolerance to morphine may arise through its induction of silent receptors; but, inasmuch as tolerance to morphine is associated with dependence, both might be expected to develop together, through morphine changing the availability to receptors of some endogenous substance concerned with central nervous activity.

Several known substances may be considered as candidates for the role of endogenous substance involved in

morphine dependence. Among these, 5-hydroxytryptamine (5-HT) would seem one of the more suitable, for several reasons: (1) morphine antagonizes some of the actions of 5-HT (refs. 8–10); (2) 5-HT has been supposed to be concerned with normal mental activity<sup>11,12</sup>; (3) 5-HT is continuously produced in brain<sup>13</sup>; (4) the effects of raising the brain concentration of 5-HT in dogs<sup>14</sup> resemble in several characteristics those of withdrawing morphine from addicted animals of that species<sup>15</sup>.

If morphine blockades 5-HT receptors on nerve cells, this might be expected to induce extra 5-HT receptors. On withdrawing morphine, endogenous 5-HT would reach the larger number of active receptors now available, eliciting excessive pharmacological reactions, which are the withdrawal effects. This series of events would explain the genesis of withdrawal effects without the need to postulate changes in concentration of 5-HT in the brain during morphine treatment and withdrawal, which do not happen<sup>16,17</sup>.

Since morphine inhibits release of acetylcholine from guinea-pig intestine<sup>3,18</sup>, acetylcholine provides an alternative endogenous substance which might be concerned in morphine dependence. Induction of acetylcholine receptors on nerve cells after deprivation of this substance by morphine might well be comparable to induction of acetylcholine receptors on muscle by botulinum toxin.

After chronic treatment of dogs and rats with morphine, its withdrawal lowers the level of catecholamines in the brain<sup>17,19–21</sup>. This implies that catecholamines may be involved in morphine dependence, but it need not conflict with the general theory here presented.

To summarize, the proposed explanation of the development of tolerance to and dependence on drugs is based on the concept of receptors. It requires five assumptions: (1) that receptors may be of two types—active and silent; (2) that the number of receptors of either type for a substance can increase or decrease at the same rate as tolerance or dependence develops; (3) that a drug causing dependence acts by increasing or decreasing the amount of an endogenous substance available to receptors; (4) that when excess or deficiency of a chemical substance distorts a living system, the numbers of receptors of either type for that substance change in the direction lessening the distortion; (5) that the ease with which a substance excites a tissue depends on the number of accessible receptors for that substance.

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### Effect of Segontin and Reserpine on Isolated Medullary Granules

In a previous paper<sup>1</sup> we have shown that tyramine releases catecholamines but not ATP from isolated medullary granules, and that the released catecholamines are almost quantitatively replaced by the uptake of equimolar amounts of tyramine. Segontin [N-(3'-phenylpropyl-(2')-1,1-diphenylpropyl-(3)-amine], a potent dilator of the coronaries, causes, *in vitro* as well as *in vivo*, a release of catecholamines from the medullary granules<sup>2</sup>. In order to determine whether segontin acts like tyramine by displacing catecholamines from their binding sites we investigated the release of catecholamines and ATP by segontin and its uptake into the chromaffin granules. For comparison, experiments with reserpine were undertaken, as it is known that this compound also releases catecholamines from medullary granules *in vivo* and *in vitro*<sup>3,4</sup>.

The chromaffin granules were prepared from suprarenal medulla of cattle as previously described<sup>5</sup> and suspended in sucrose-phosphate buffer pH 6.8. The samples (7 ml.) were incubated for 60 min at 37° C under shaking. The granules of the incubated samples as well as of the non-incubated controls were sedimented by centrifugation (15 min, 12,000g, in stainless steel tubes), washed once with 3 ml. sucrose-phosphate buffer and sedimented with 12,000g. The granules were then suspended in 5 ml. saline; 4 ml. of this suspension were extracted with 0.15 ml. concentrated perchloric acid and centrifuged. The catecholamines and ATP were determined in the supernatant, the former by the method of v. Euler and Hamberg<sup>6</sup>, the latter by the method of Strehler and Totter<sup>7</sup>. In order to measure the uptake of <sup>14</sup>C-segontin, 1 ml. of the granular suspension was dried at 60° C and the residue dissolved in 1 ml. hyamine hydroxide at 40° C. After cooling, 10 ml. scintillator (4 g PPO and 100 mg POPOP in 1,000 ml. toluene) were added and the radioactivity was determined in a Packard 314X liquid scintillation spectrometer. An internal standard (<sup>14</sup>C-toluene) was used to correct for quenching and efficiency of counting.

Table 1. UPTAKE OF SEGONTIN BY ISOLATED CHROMAFFIN GRANULES AND ITS EFFECT ON THE RELEASE OF CATECHOLAMINES (CA)

Segontin ( $\mu$ M/sample)	Segontin uptake ( $\mu$ M)	Release of catecholamines ( $\mu$ M)	CA release/ Seg. uptake
0.165	0.060	1.39	48
0.280	0.098	2.27	78
0.476	0.124	2.92	100

The samples contain in 7 ml.: 2 ml. granules from 140 mg medullary tissue, 0.1 ml. segontin, 0.4 ml. <sup>14</sup>C-segontin (9.6  $\mu$ M; specific activity 5  $\mu$ Ci/2.4  $\mu$ M) and 4.5 ml. sucrose-phosphate buffer, pH 6.8. Incubation: 60 min at 37° C.

During incubation of isolated chromaffin granules at 37° C, catecholamines and ATP are released spontaneously to the same degree<sup>1,8</sup>. The addition of either 0.3  $\mu$ M segontin or 1  $\mu$ M reserpine per sample (Fig. 1) proportionally increases the release of catecholamines and ATP so that the original molar ratio, amine/ATP of 4 : 1, remains unchanged. Table 1 shows that during incubation with <sup>14</sup>C-segontin the uptake of segontin, as well as the release of catecholamines, depends on the amount of segontin added to the samples. It is interesting to note that the ability of the granules to take up segontin is limited, since after addition of doses of segontin higher than necessary to deplete the granules completely (0.476  $\mu$ M/sample) no more segontin is taken up; consequently the molar ratio, catecholamine release/segontin uptake, remains constant and is about 23. In a further 14 experiments with various doses of segontin, and therefore various releasing effects (20–100 per cent), the molar ratio, catecholamine release/segontin uptake, was 20.5 (standard deviation, 5.26), that is, uptake of segontin is proportional to the release of catecholamines although 20 times smaller. The determination of the uptake of segontin and of the release of catecholamines in the non-incubated samples indicated that segontin is already taken up from the granules during

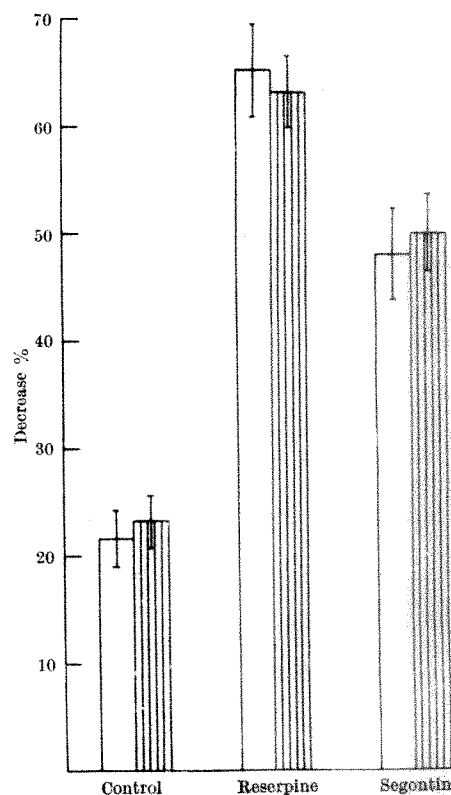


Fig. 1. Release of catecholamines (white) and ATP (hatched) from isolated granules by lyophilized reserpine phosphate (1  $\mu$ M/sample) and segontin lactate (0.3  $\mu$ M/sample). The samples contain in 7 ml.: 2 ml. granules from 200 mg medullary tissue, 0.1 ml. segontin or reserpine and 4.9 ml. sucrose-phosphate buffer pH 6.8. Incubation: 60 min at 37° C. Means of 4 experiments and their standard deviations

centrifugation (15 min, 0° C) while the catecholamines are released only during the following incubation at 37° C. Tyramine, on the other hand, is exclusively taken up at the same time as it releases catecholamines. Therefore, at 0° C no uptake of tyramine and no release of catecholamines take place.

From these results it is obvious that in contrast to tyramine both segontin and reserpine cause, *in vitro*, in addition to the release of catecholamines a proportionate release of ATP. The uptake of segontin, as well as the release of catecholamines, depends on the dose of segontin added. Whereas in the case of tyramine the released catecholamines are replaced by the uptake of equimolar amounts of tyramine, the uptake of 1 mole of segontin is followed by the release of about 20 moles of catecholamines. It can therefore be concluded that the mechanisms of action of tyramine and segontin are different.

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### Metabolism of Acid Mucopolysaccharides of the Rabbit's Dermis following Skin Treatment with Irritating Substances

In the rabbit's skin chronically treated with croton oil a remarkable change is observed in the ratio between hyaluronic acid and chondroitin sulphuric acid, the former largely prevailing over the latter after one month's treatment; the same change occurs when the skin is treated with carcinogenic substances<sup>1</sup>. These data supplement what is already known about the presence of mucopolysaccharides in inflammatory processes (see, for references up to 1959, Delaunay and Bazin<sup>2</sup> and, more recently, Berenson and Dalferes<sup>3</sup>, White *et al.*<sup>4</sup>, Zonta *et al.*<sup>5</sup>). So far as we know, no data are available concerning the changes induced in the metabolism of mucopolysaccharides by those stimuli which elicit inflammation. The present work was concerned with the dynamics of the metabolism of acid mucopolysaccharides of the rabbit's skin treated for short periods of time with croton oil, evaluating the incorporation into them of glucose (as glucosamine and galactosamine).

Albino rabbits weighing 2.5–3 kg were shaved on approximately 300 cm<sup>2</sup> of the back. Half this surface was

Table 1 shows the treatments performed on each single animal and the specific activity of total hexosamines as well as that of glucosamine and galactosamine taken separately. These values were obtained from MPS extracted and hydrolysed separately from both control and treated areas of each animal's skin.

From examination of Table 1 it appears that:

(1) The activity of hexosamines obtained by hydrolysis of MPS extracted from the treated area is always stronger than that of corresponding hexosamines extracted from the control area.

(2) Such increase in activity occurs rapidly; it is, in fact, fairly evident in animals which were killed 3 h after the one treatment received.

(3) The increase in activity of glucosamine from MPS of the treated area (compared to that of the corresponding control area) is more relevant than the increase in activity of galactosamine from MPS of the treated area (still compared to that of the corresponding control area). The only exception is animal No. 1, in which the treatment was notably weaker than the average.

The results of the present work suggest that under a local irritating stimulus there is an accelerated synthesis of acid mucopolysaccharides of the dermis, and that this

Table 1

Rabbit No.	No. treatments with croton oil	Time of glucose injection (h after last treatment with croton oil)	Time of killing (h after glucose injection)	Ratio between activity of hexosamines T and hexosamines C	Activity of glucosamine (c.p.m./mg)	Activity of galactosamine (c.p.m./mg)	Ratio between activity of glucosamine and activity of galactosamine
1*	2, at 24 h distance	0.5	18	2.85	C 55.8 T 161.0	C 18.4 T 56.0	C 3.04 T 2.88
2*	2, at 70 h distance	0.5	18	5.3	C 67.0 T 282.0	C 26.0 T 87.5	C 2.6 T 3.23
3	1	6	18	11.5	C 81.0 T 950.0	C 32.0 T 116.0	C 2.55 T 8.2
4	1	10	18	6.9	C 63.0 T 750.0	C 20.0 T 94.0	C 3.15 T 8.0
5†	1	1	2	4.13	C 84.0 T 372.0	C 23.0 T 51.0	C 3.6 T 7.3
6†	1	1	2	4.06	C 86.0 T 355.0	C 25.0 T 49.0	C 3.4 T 7.2
7‡	2, at 24 h distance	0.5	18	4.3	C 21.5 T 96.0	C 8.0 T 24.0	C 2.7 T 4.0
8	2, at 18 h distance	0.5	18	3.8	C 52.0 T 190.0	C 24.5 T 62.0	C 2.12 T 3.05

C, MPS extracted from the control area.

T, MPS extracted from the skin area treated with croton oil.

\* The amount of croton oil solution administered each time was notably inferior to the average. Also the macroscopic reaction, especially in the first animal, was less severe.

† Injected with 50 µc/kg, instead of 10 µc/kg.

‡ The amount of croton oil solution was larger than that normally used. The macroscopic reaction was quite evident, with a most marked edema of both dermis and subcutaneous tissue. The activity of hexosamines of the control area is much less than average; probably the very strong treatment caused more than merely local effects.

treated, the other half was kept as control. Treatment was carried out with a 2.5 per cent solution of croton oil in acetone, dropped on the skin (about 1.5 ml. solution per 100 cm<sup>2</sup> surface). Each animal was intravenously injected with 10 µc/kg of glucose <sup>14</sup>C (U) (specific activity 3.57 mc./mmole)—except two which, as will be explained, received 50 µc/kg. The times of injection and killing are reported in Table 1. The skin was removed separately from treated and control areas, leaving between the two areas a strip about 2 cm wide. The subcutaneous tissue was mechanically removed. The skins were minced and soaked in acetone, which was replaced three times in a week. The material was then dried, and acid mucopolysaccharides (MPS) were extracted from it after digestion with papain, by the method described by Scott<sup>6</sup>. Part of the MPS extracted was hydrolysed with 5 N HCl for 7 h at 100° C, and the hydrolysate was dried. Column chromatography (0.8 × 45 cm) on 'Dowex 50 WX8', 200–400 mesh, was performed for the separation of glucosamine and galactosamine, according to Gardell<sup>7</sup>. Fractions of 3 ml. were collected. One ml. of each was used to determine the amount of hexosamine while the remaining 2 ml. were dried, and the material—recovered three times with distilled water—was placed on aluminium disks. Activity was assessed with a windowless gas-flow counter, with anti-coincidence scintillation apparatus (Alberigi-Quaranta *et al.*<sup>8</sup>).

response of the connective tissue occurs rapidly. Moreover, of the two predominant MPS of the skin, one (hyaluronic acid) contains glucosamine and the other (chondroitin sulphuric acid, especially of B type) contains galactosamine, so that one can safely conclude that the highest increase is found in the synthesis of hyaluronic acid. The inflammatory stimulus, therefore, develops its action not only by promoting an accelerated synthesis of MPS in fibroblasts, but also by directing it chiefly towards the production of hyaluronic acid. This fact may be at least partly responsible for the reported finding<sup>1</sup> concerning the change in relationship between the two MPS, observed after one month's treatment with either irritants or carcinogenic polycyclic hydrocarbons.

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## HAEMATOLOGY

## Acid Deoxyribonuclease Activity in Peripheral Leucocytes of Swiss Mice

THE general morphological details of the blood cells of the common mammalian species have been thoroughly examined. Recently, increased interest in the distribution of the intracellular chemical components of these cells has suggested that cytochemical analysis of acid deoxyribonuclease activity in leucocytes of Swiss mice might prove profitable.

Recent findings on the occurrence of deoxyribonuclease in the different elements of the blood indicate that the enzyme is found in rather high concentration in the serum as well as in the cells, especially leucocytes<sup>1</sup>. Deoxyribonuclease is important in combating infectious viral nucleic acids which are not blocked by anti-serum against the intact virus. Herriot and Gupta reported that the major source of serum deoxyribonuclease is in the cellular elements of the blood. There are wide variations in reports on the concentrations of this enzyme in different cell types<sup>2,3</sup>. The platelets have been suggested as a possible source of serum DNase activity<sup>3</sup>. If this were correct, cytochemical analysis should reveal the presence of this enzyme in them, and in this investigation a cytochemical technique was applied to the investigation of this supposed source of serum deoxyribonuclease.

Blood smears were obtained from the tail of Swiss mice of the Webster strain and air dried for 10–30 min at room temperature. They were fixed for 10 min in formalin vapour. This was accomplished by adding enough 10 per cent formalin to moisten a thin layer of absorbent cotton at the bottom of a small Coplin jar which was covered with a large glass jar. Following fixation, the smears were incubated for up to 1½ h at 37° C. The

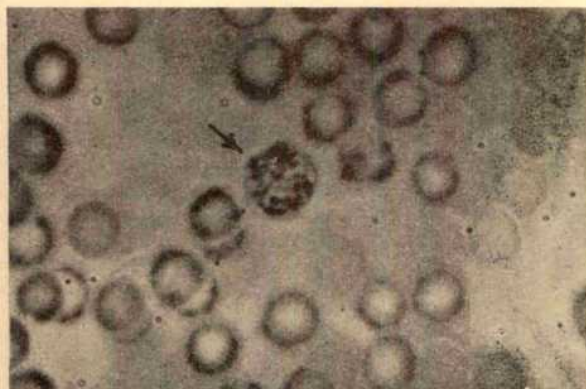


Fig. 1. Neutrophilic nucleus showing uniform distribution of enzyme activity throughout the chromatin

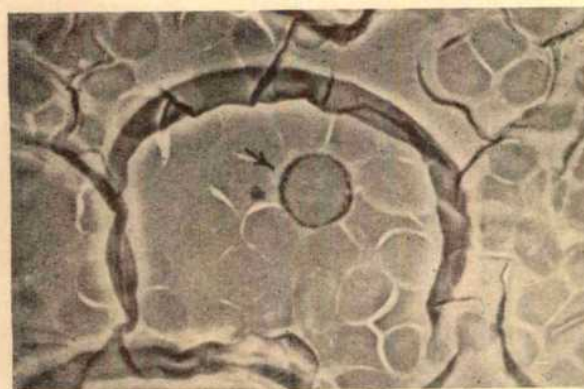


Fig. 2. Lymphocyte showing activity along the thin rim of the cytoplasm

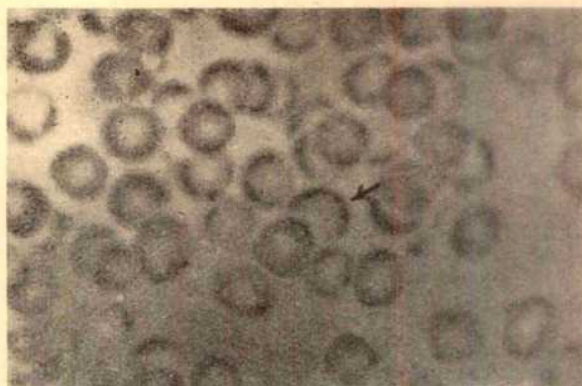


Fig. 3. Reticulocyte or late normoblast with detectable granules

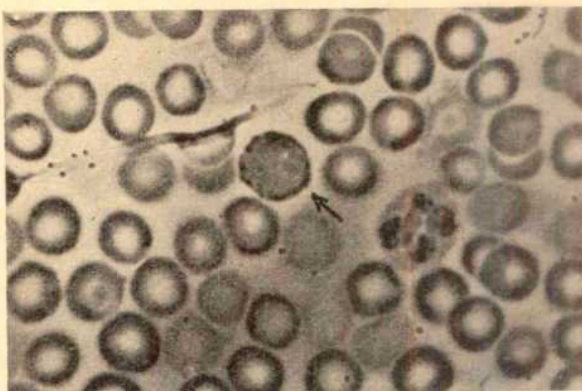


Fig. 4. A peripheral blood smear stained for acid deoxyribonuclease. The enzyme is demonstrated in the nuclei, especially at the nuclear membrane

incubation solution was prepared immediately before use from the stock solutions. The composition of the incubation medium was according to that described by Vorbrodt<sup>5</sup>. The staining procedure was that described by us in an earlier report. After incubation the smears were washed briefly in distilled water and placed in dilute ammonium sulphide solution for 10 min. After washing in distilled water, the smears were counterstained in 1 per cent safranin solution for 30 sec, rinsed in distilled water, dried and mounted in glycerine jelly. Photomicrographs were taken with phase contrast (oil immersion  $\times$  phase 3).

Acid deoxyribonuclease is indicated by black dye precipitated within the cells. It is consistently demonstrable in blood lymphocytes. It is also found occasionally in the nuclei of neutrophils, indicating a diffusely distributed activity throughout the chromatin structure (Fig. 1). In addition, granules, 5–10 in number, were detected in reticulocytes or late normoblasts (Fig. 3). The mature erythrocytes did not contain any detectable amount of deoxyribonuclease. Minute black granules were encountered throughout the entire periphery of the cytoplasm of lymphocytes (Fig. 2). These granules represent evidence of activity. The morphological characterization of the enzymatic activity in the form of dark granules was revealed best when examined with phase contrast microscopy.

When DNA was omitted from the incubation medium no staining occurred except for the presence of a few faintly stained black granules of some neutrophils. On the other hand, the addition of sodium fluoride (0.01 M) to the incubation medium completely inhibited the reaction.

Vorbrodt<sup>5</sup> demonstrated strong reaction in the cytoplasmic granules arranged along the bile canaliculi, and



positive reaction for DNase in the nuclei (or the nuclear membrane) and in the chromosomes of liver parenchymal cells. He also found enzyme activity in Kupffer cells, macrophages of the spleen, Novikoff hepatoma and Novikoff ascites hepatoma in the intestinal epithelium, and in the proximal convoluted tubules and nerve cells of the rat kidneys and brains, respectively. He noted that phagocytic cells generally showed a high enzymatic activity.

Acid DNase has mostly been associated with lysosomes. Considering this, the specificity of the reaction for DNase cannot be based solely on microscopic patterns, since investigation during these examinations revealed the positive reaction in the nuclei of the neutrophils. After the use of the inhibitor (sodium fluoride solution) the activity in both the neutrophilic nuclei as well as the cytoplasmic granules of the lymphocytes was lost. This was contrary to Vorbrodt's finding, the persistence of nuclear activity in spite of the addition of inhibitor  $\text{Na}_2\text{SO}_4$  in the incubation solution. He assumed that the cells of rat liver and other organs had two DNA-hydrolysing systems: one inhibited by the sulphate ions, located in the lysosomes; the other insensitive to sulphate ions in the cell nucleus. He contended that enzyme localized in the nucleus could be regarded as nucleotide phosphodiesterase or acid nuclease since positive reactions appeared when either DNA or RNA is the substrate. But this was not true of our experiments (ref. 4), since there was no hydrolysis of RNA by any kind of nuclear enzymes in any of the cell types in the peripheral blood. These two experiments suggest to us that the reaction in the peripheral leucocytes is specific for both DNase and RNase.

No enzyme activity was found in the blood platelets, which Herriot, Connolly and Gupta considered to be a major source of serum DNase in human blood. Only the lymphocytes showed the cytochemical activity and nuclei of some of the neutrophils.

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### Hæmoglobin N of Sheep

In a previous publication<sup>1</sup> the finding of a new type of sheep hæmoglobin was reported. This has a slower rate of migration on starch gels than the A and B hæmoglobins. It was first found in highly anæmic lambs where its relative amount of total hæmoglobin showed great variation. For these reasons it was called abnormal and tentatively given the symbol N. Blunt and Evans<sup>2</sup> reported a new hæmoglobin in sheep with hæmoglobin A type and made anæmic by experimental bleedings. This new type was electrophoretically similar to hæmoglobin B.

This communication deals with results from further investigation of the N hæmoglobin. It has been investigated by use of starch-gel electrophoresis, and its position in relation to the normal sheep hæmoglobins A and B is shown in Fig. 1, which is a photograph of a gel stained with benzidine.

Three of the six samples have the N hæmoglobin. Two of these have the A hæmoglobin in addition to the N hæmoglobin, and the third has the three hæmoglobins A, B and N. It can also be seen that of the two samples with A and N the N band is much stronger in one of them.

We have in the winter and spring of 1964 investigated 762 sheep living in Norway of which 130 were lambs. They represent five different breeds. In Old Norwegian sheep ('Spæl'), which is a rather primitive short-tail type, the N hæmoglobin was found in 99 of 105 investigated ewes. These were all of type hæmoglobin AA. It is, however, very weak in most of the samples. In two other native Norwegian breeds (Dala and Rygja) the N band even though not so frequent was commonly occurring, and also in the Cheviot breed which is based on original imports from Great Britain. Samples from 56 Oxford-down sheep of which 15 were AB and 41 BB did not reveal any N hæmoglobin. From Spain we received 59 samples of which 6 were AA, 25 AB and 28 BB. The N hæmoglobin was, however, found in one AB animal only.

The majority of our samples came from ewes, but the N hæmoglobin has been observed in rams and lambs also. The youngest lamb in which hæmoglobin N was observed was 10 days old. Accordingly it has been found together with fetal hæmoglobin.

One of the most typical features of the N hæmoglobin is its variation. Its migration rate relative to the A and B hæmoglobins on starch gels is, however, always the same. It is most often observed in AA animals and has never been found in animals of phenotype BB. Usually it is very faint, the percentage of total hæmoglobin being less than one. Animals having a higher percentage of N hæmoglobin are usually anæmic. The highest percentage of N hæmoglobin which has been observed was in an animal having a hæmoglobin content of 2.4 g/100 ml. In this sheep the total hæmoglobin was composed of approximately 80 per cent N hæmoglobin and 20 per cent A hæmoglobin. Three- to four-week-old lambs of phenotypes AA and AB usually had a rather high proportion of N hæmoglobin (10-30 per cent).

Even though the N hæmoglobin is not regularly detectable we are now inclined to consider it to be more normal than abnormal. In this connexion the similarity to the A<sub>2</sub> hæmoglobin in man may be mentioned, where this component may increase in the anæmic condition called thalassæmia minor, although to a much lesser extent<sup>3</sup>. Because of this resemblance and its occurrence in a high percentage of Old Norwegian sheep, we favour a similar genetic theory as for A<sub>2</sub> in man, where Ceppellini<sup>4</sup> suggested that the gene for the  $\delta$  chain of A<sub>2</sub> hæmoglobin was linked to the gene for the  $\beta$  chain. Accordingly our hypothesis is that one of the structural genes controlling N hæmoglobin is closely linked to one of the structural genes controlling the A hæmoglobin. Why the gene for the N hæmoglobin apparently usually is 'turned off' is for us a question difficult to answer at present, although several theoretical explanations might be given.

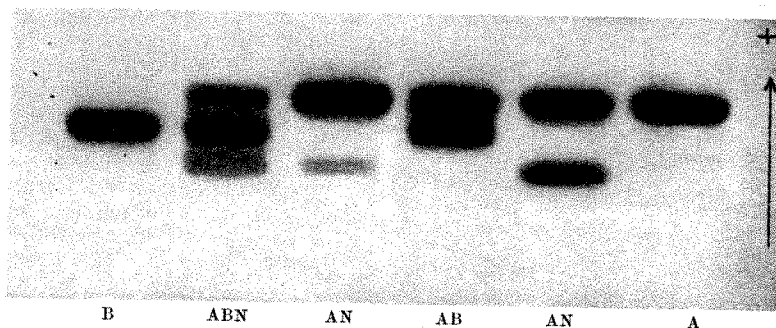


Fig. 1. Photograph showing relative position of sheep hæmoglobins tris-EDTA-boric acid buffer pH 9.0

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### Hæmoglobin O<sub>Arab</sub> in Bulgaria

SEVERAL cases of moderately severe hypochromic hæmolytic anaemia have recently been observed in Burgas, Bulgaria<sup>1</sup>. The affected individuals were unrelated white adult men and women. They showed splenomegaly, slight icterus, decreased osmotic fragility of the red cells and 40–90 per cent target cells. Paper and starch-gel electrophoresis<sup>2</sup> at pH 8.6 of their hæmoglobin showed complete absence of normal adult hæmoglobin (Hb A) and presence of a slow-moving abnormal hæmoglobin. The electrophoretic mobility of this abnormal hæmoglobin was similar in paper electrophoresis to that of hæmoglobin C (Hb C). In starch-gel electrophoresis the abnormal hæmoglobin moves slightly ahead of Hb C. In agar-gel electrophoresis at pH 6.0<sup>3</sup> it moved between Hb A and Hb C, in the position of hæmoglobin S (Fig. 1).

The abnormal hæmoglobin of the anæmic individuals was examined by 'fingerprinting', following the method of Ingram<sup>4</sup> as modified by Baglioni<sup>5</sup>. The hæmoglobin of the individuals A II-1, B II-3, B II-4, C II-2 and D I-1 of Fig. 2 gave an identical fingerprinting pattern. Peptide

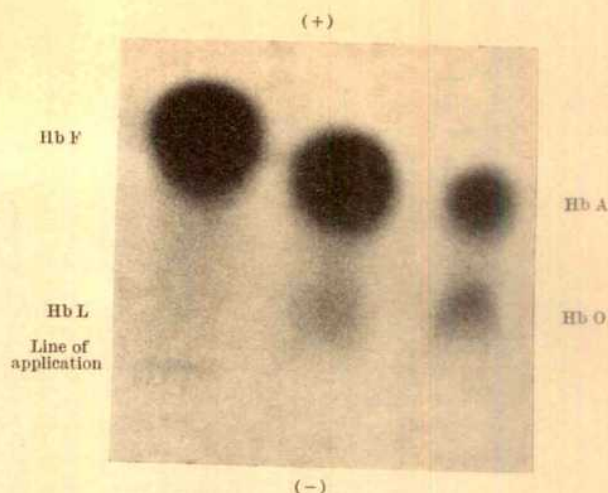


Fig. 1. Agar-gel electrophoresis at pH 6.0 of the hæmoglobin of an individual (A I-2) heterozygous for the Hb O<sub>Arab</sub> gene (left). Hæmoglobins A and L (centre) and F (right) are shown for comparison.

$\beta TpXIII$  (ref. 5) was absent from the position that it occupies in the fingerprint of Hb A and was moved to a more cathodic position. The fingerprinting pattern obtained (Fig. 3) was identical to that described by Baglioni and Lehmann<sup>6</sup> for hæmoglobin O<sub>Arab</sub> (Hb O<sub>Arab</sub>). The abnormal hæmoglobin observed in the affected individuals was thus identified with Hb O<sub>Arab</sub>: this hæmoglobin has shown a glutamic acid to lysine substitution in residue 121 of the  $\beta$ -peptide chain<sup>6</sup>.

The families of four affected individuals have been thoroughly investigated; less-complete information has been collected on the family of one more individual affected by the same type of anaemia. The family trees of the four propositi investigated are reported in Fig. 2. In the families A, B and C one of the parents of the propositus was shown to be a carrier of Hb O<sub>Arab</sub>, while the other parent was found to be a thalassaemia-trait

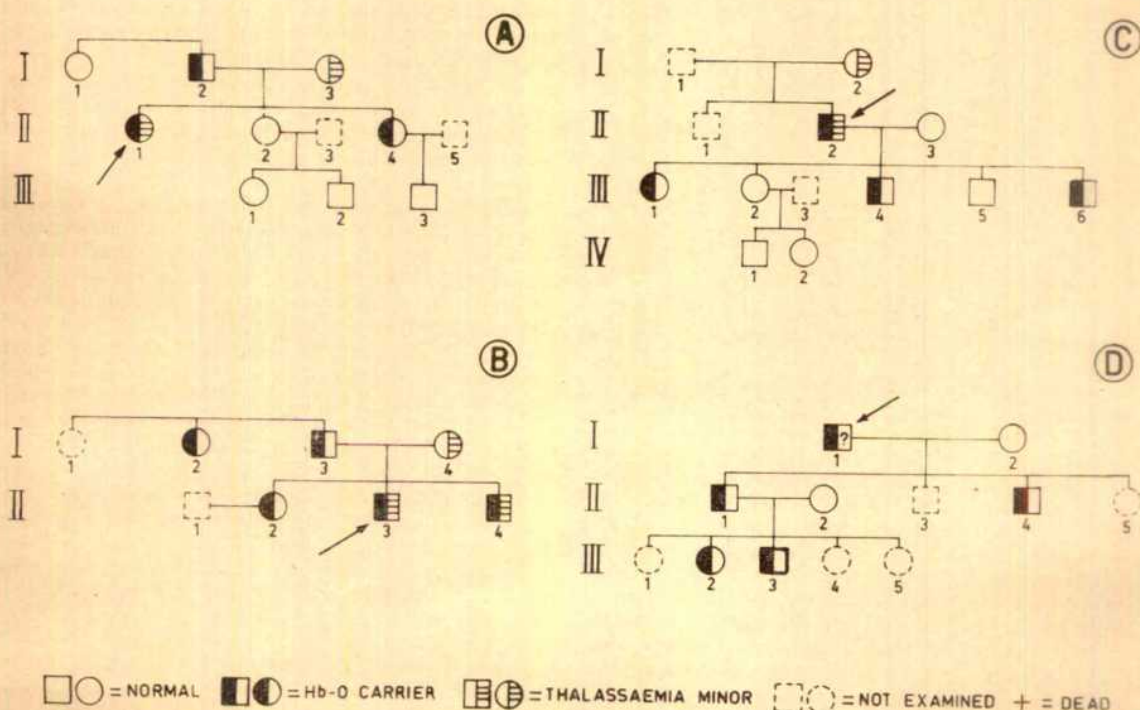


Fig. 2. Family trees of individuals affected by severe hypochromic hæmolytic anaemia (indicated by arrows). These individuals are  $\beta^{OAr}/\beta^{Thal}$  heterozygotes.



carrier. Thalassaemia is relatively frequent in some regions of Bulgaria, which have been infested by malaria in the past<sup>1</sup>. The combination of the thalassaemic gene with the Hb O<sub>Ar</sub> gene in the propositi causes the absence of normal haemoglobin and the moderately severe anaemia. The thalassaemic gene of the families investigated behaves as a gene allelic to the  $\beta^{0Ar}$  gene and it is thus a  $\beta$ -thalassaemic gene<sup>7</sup>. The propositi are very likely heterozygotes for the  $\beta$ -thalassaemic gene and for the  $\beta^{0Ar}$  gene ( $\beta^{Thal}/\beta^{0Ar}$ ). Not enough information has been obtained on family D (Fig. 2) to ascertain whether the propositus I-1 is also heterozygote for thalassaemia or whether he is homozygote for the  $\beta^{0Ar}$  gene. The haematological picture of this individual is extremely similar to that of the propositi of the other families, and it thus seems likely that his genotype is identical to that of the other propositi.

The occurrence of Hb O<sub>Ar</sub> in Bulgaria is of genetic and ethnological interest. Hb O<sub>Ar</sub> has so far been described in one Arab family only, observed in Israel<sup>8</sup>. It may be supposed that the Hb O<sub>Ar</sub> gene has been imported into Bulgaria during the five centuries of Turkish rule or more recently when a part of the Bulgarian population of the district of Burgas emigrated from the European part of Turkey (eastern Thrace) during 1912. Two of the families examined have indeed come from Eastern Thrace. However, Hb O<sub>Ar</sub> has not yet been reported to occur in Turkey. It seems more likely that Hb O<sub>Ar</sub> has appeared in Bulgaria as the result of an independent mutation. This mutation might have been preserved under the influence of severe malaria selection; other hereditary haematological disorders, like thalassaemia and favism, are quite common in the region of Burgas<sup>1</sup>.

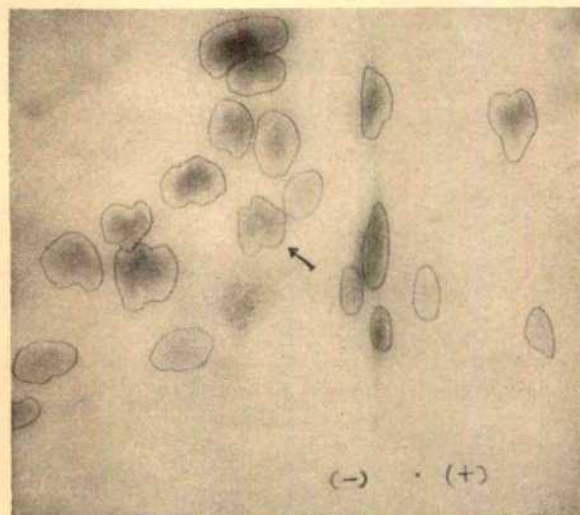


Fig. 3. Fingerprint of the Hb O<sub>Ar</sub> present in the individuals affected by severe hypochromic hemolytic anemia. The arrow indicates the abnormal peptide  $\beta^{0}T\text{pXIIIb}$ , where a lysine substitutes a glutamic acid residue.

Further investigations into the distribution of Hb O<sub>Ar</sub> in other regions of Bulgaria are needed. If Hb O<sub>Ar</sub> is found there also, the theory of an independent mutation will acquire further support. If Hb O<sub>Ar</sub> is found in those regions where malaria was widely spread, the selection of the  $\beta^{0Ar}$  gene by malaria may be considered likely. Malaria may possibly select all those mutations affecting red cell metabolism and/or haemoglobin, which by local circumstances are available. If further investigations show that the diffusion of the  $\beta^{0Ar}$  mutation is limited to the district of Burgas, very near to Turkey, the 'import' theory may gain some likelihood.

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## PATHOLOGY

### Recovery of Mycoplasmas in the Study of Human Leukemia and Other Malignancies

NUMEROUS reports have been published concerning the necessity of testing for the presence of mycoplasmas before the interpretation of experiments performed in cell cultures<sup>1-3,6</sup>. Such tests are especially important when the presence of viruses is suspected, since recent evidence indicates that some mycoplasmas are capable of eliciting a transmissible cytopathic effect<sup>4-7</sup>.

In addition, some mycoplasmas and viruses share properties, such as size, filterability, morphology in electron microscopy, sensitivity to ether, ability to haemagglutinate and cause haemadsorption, interference with virus replication *in vitro*, lack of inhibition by certain commonly used antibiotics and inhibition of growth by homologous antiserum. Thus, the absence of ordinary bacteria and moulds in preparations eliciting a cytopathic effect in cell cultures is an unreliable criterion for viral identification. Great caution should be exercised in classifying new agents as viruses, especially as myxoviruses, without adequate testing to exclude their identity as mycoplasmas.

The problem posed by the similarity of mycoplasmas to viruses is exemplified by a consideration of the properties of several agents isolated during work on human malignancies<sup>8,11-13</sup>. One of the more thoroughly investigated isolates is that reported by Negroni<sup>8</sup>, who obtained an agent while examining human leukemic bone marrows. These preparations were inoculated into tissue cultures of whole human embryos or kidneys. A 'virus' was isolated from each of ten cases and became cytopathic for the cells in the course of serial passage. Comparable cultures inoculated with bone marrow from control cases showed no such effect. Negroni concluded that the results did not justify any statement regarding the relationship of these viruses to leukemia itself, but that "the frequency of the association is, however, remarkable".

The Negroni agent was obtained from Dr. Negroni independently by our two laboratories (Wistar Institute and Laboratory of Infectious Diseases, National Institutes of Health) as a 0.2-ml. volume of infectious fluid from human embryo cells and the same results were obtained. Direct introduction of the material on mycoplasma agar of Hayflick's formula<sup>9</sup> revealed mycoplasma at a concentration of 10<sup>8</sup> colony-forming units/ml. The organism was gently scraped from agar into Eagle's basal medium containing penicillin as the only antibiotic. This infectious fluid and control fluid prepared from an uninoculated agar plate were introduced into tube cultures of primary human embryonic kidney and primary African grivet monkey kidney containing either chlortetracycline ('Aureo-



mycin', Lederle) (25 µg/ml.) or penicillin (100 units/ml.). In addition, the isolate was sub-cultivated for twelve passages on agar medium and then inoculated into similar cultures. The results presented in Table 1 indicate that the mycoplasma isolates caused a cytopathic effect in human embryonic kidney and grivet monkey kidney which could be inhibited by chlortetracycline but not by penicillin. Although human embryonic kidney was more sensitive as demonstrated by the earlier appearance of the transmissible cytopathic effect, grivet monkey kidney was also susceptible. Since the 12th agar passage, representing a dilution of approximately  $10^{-15}$  of the original isolate, caused a similar cytopathic effect, it is doubtful that a virus was present in the original fluid which was responsible for the cytopathic effect. This notion was further supported by evidence that fluid from the original Negroni agent sample was cytopathic for human embryonic kidney only in the absence of chlortetracycline.

Table 1. CYTOPATHIC EFFECTS IN TISSUE CULTURES PRODUCED BY MYCOPLASMAS ISOLATED FROM PREPARATIONS OF THE NEGRONI AGENT

Cell culture	Passages on agar	Cytopathic effect with mycoplasma isolate		Washing from sterile agar (control)
		Chlortetracycline present	Chlortetracycline absent	
Human embryonic kidney	0*	—	++	—
	1	—	+	—
Grivet monkey kidney	1	—	+	—
	12	—	+	—

\* Sample as obtained from Dr. G. Negroni

It was of interest to attempt to identify this mycoplasma with those of known human origin, and serological investigations were undertaken. The procedure used for the complement-fixation test was described previously<sup>10</sup> and utilized a broth-grown antigen concentrated 90-fold by centrifugation. This preparation was treated with guinea-pig complement for 60 min at 37° C followed by 30 min at 56° C in order to remove anti-complementary activity. This antigen was found to be non-reactive when tested against 4 units of antisera prepared against the known species of human mycoplasma: *Mycoplasma hominis*, type 1, *M. hominis*, type 2, *M. pneumoniae*, *M. fermentans*, *M. salivarium* and *M. orale*. The absence of complement-fixing reactivity in this test does not conclusively distinguish this agent from the known human mycoplasma species. A definite determination must await the outcome of investigations performed with homologous antisera prepared against the new isolate. Also, the antigen was non-reactive with antisera prepared against many of the mycoplasma species of animals, including *M. mycoides* var. *capri*, *M. gallisepticum* and *M. gallinarum*. Finally, antisera to the agent, kindly supplied by Dr. Negroni, give a positive reaction in the growth inhibition test with the mycoplasma isolated by us.

In addition to the agent described by Negroni<sup>6</sup>, four other agents recovered in the course of investigations of human malignancy have been identified as mycoplasmas<sup>11-13</sup>. Employing techniques described here, two of these<sup>11</sup>, studied more extensively at the Wistar Institute, were recovered repeatedly during investigations with two human tumours (haemangioma and retropharyngeal fibroma). These two strains of mycoplasmas as well as the others already mentioned here<sup>12,13</sup> share with the Negroni agent the characteristic of producing cytopathic effects in tissue culture. Some lack antigenic identity in complement-fixation tests with known human mycoplasma species. Similarly, some of these agents and the Negroni agent were capable of fermenting glucose, a property of human mycoplasmas shared only with *M. pneumoniae* and *M. fermentans*.

Thus, a group of previously unclassified mycoplasmas have been isolated from human malignancies as well as from normal tissue culture systems<sup>5,14</sup>. The significance of these findings relevant to human ecology is difficult to assess at present.

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## A Rapid Technique for detecting Multiple Antigen-Antibody Precipitin Reactions

USING standard immunoelectrophoretic techniques in gelatin<sup>1</sup> or on cellulose polyacetate<sup>2</sup>, the minimum time for an indication of multiple antigen-antibody precipitin reactions is from a few hours to two days. Even various gelatin diffusion techniques<sup>3-5</sup> may take from a few hours up to 22 days<sup>6</sup>. The need for a rapid means of detecting multiple antigen-antibody reactions as evidence of antigen homogeneity or heterogeneity is therefore apparent.

The gelatin electrophoretic technique of Bussard<sup>7</sup>, in which antisera are placed closer to the anode, antigen closer to the cathode, relies on endosmotic flow to carry the  $\gamma$ -globulin toward the advancing protein antigens. This technique is rapid, but, as with most gelatin techniques, is time-consuming in preparation and preservation of results. The 'crossing paper' electrophoretic technique of Nakamura and Ueta<sup>8</sup>, in which antisera are placed closer to the anode and antigen closer to the cathode at oblique angles to each other on filter paper, takes at best 8 h to detect precipitin arcs.

Realizing that  $\gamma$ -globulin migrates relatively slowly under electric current, the 'crossing paper' technique was modified and adapted for use with cellulose polyacetate electrophoretic support strips (Gelman's 'Sepharose III' strips, 1 in.  $\times$  6  $\frac{3}{4}$  in.; Ann Arbor, Mich.).

A veronal-barbital buffer, pH 8.6, ionic strength 0.05, is used routinely in a home-made electrophoretic chamber. One drop (about 5  $\mu$ l) of antisera (anti-fetal bovine sera prepared in rabbits) is applied to the strip 1 in. from the cathode, 4 in. from the anode, and electric current of 250 V, 5-7 m.amp current is passed for 30 min, permitting the antisera components to align and begin to separate. Then the antigens (fetal bovine serum diluted 1:20 in buffer) are applied as a straight line with a



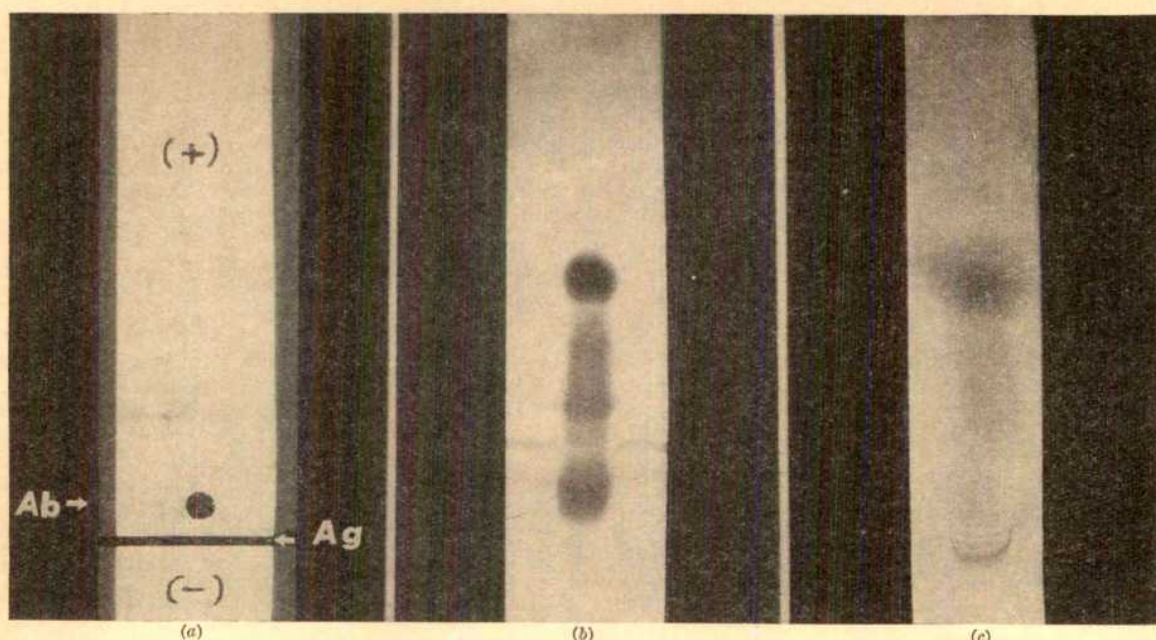


Fig. 1. (a) One drop of antisera (Ab) applied at the start. Antigens (Ag) applied as a straight line 30 min later. (b) The antigens passing through the  $\gamma$ -globulin. Strip stained without rinsing. (c) Strip stained after 10-min rinse in saline. Three precipitin arcs are visible

capillary tube immediately behind (closer to the cathode) the point of antisera application (Fig. 1a). Current is restored for 20 min. The antigens migrate through the  $\gamma$ -globulin and precipitate arcs form where equivalent proportions are reached (Fig. 1b). At the end of that time, the strip is removed to 0.85 per cent saline and allowed to rinse for 10 min. The strip is then blotted, dried at 37° C for 10 min, stained for 5 min in Ponceau S stain and rinsed in 5 per cent acetic acid following Gelman's instructions. From our test system we can identify at least three precipitin arcs (Fig. 1c). The entire procedure, to a permanent mount between two glass slides (cleared with mineral oil, if so desired), takes less than 2 h.

For illustrative purposes and to verify that multiple antigen-antibody reactions are indeed occurring in the test system, the same system was analysed immunoelectrophoretically on cellulose polyacetate (Oxoid, Colab, Chicago, Ill.) as described by Kohn<sup>2</sup>. The antigens were separated electrophoretically and antisera allowed to diffuse toward them. Three distinct antigen-antibody reactions were identified, which verifies the results presented (Fig. 2).

The procedure described here is effective so long as all the antibodies are contained in the slow-moving  $\gamma$  portion of the antiserum, such as with rabbit serum<sup>3</sup>, and provided the slowest-moving antigen will reach the  $\gamma$ -globulin in the time allotted. Although this technique lacks the specificity of standard immunoelectrophoretic techniques,

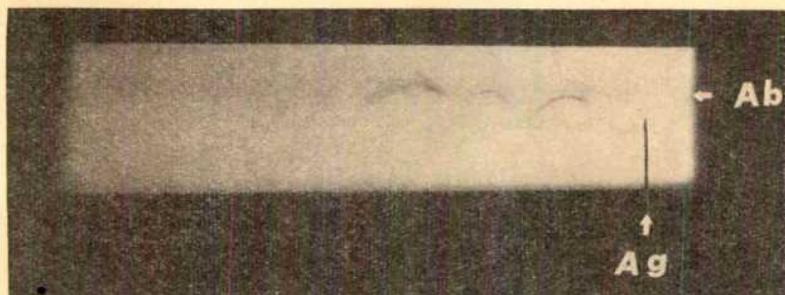


Fig. 2. Antigens (Ag) separated electrophoretically. Antisera (Ab) diffused toward them. Three precipitin arcs are visible

due to crowding of multiple bands, it is a rapid means of determining the existence of antigen-antibody precipitin reactions occurring in any given system. Where few antigen-antibody reactions occur, it does reflect specificity plus the advantage of speed.

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### Esterase Studies on Dog Mast-cell Tumours

RECENTLY our laboratory reported the isolation of proteolytic enzymes from a dog mast-cell tumour using DEAE-cellulose and CM-cellulose columns. An esterase which split *N*-acetyltyrosine ethyl ester (ATEe) and another which split *p*-toluene sulphonyl-L-arginine methyl ester (TAME) were described<sup>1</sup>. Using column chromatography and eluting with a concentration gradient of phosphate buffer with a pH of 8.0, several peaks of ATEe esterase and two TAME esterase peaks were found. All the peaks of ATEe esterase have since been found to have a similar Michaelis constant (*K<sub>m</sub>*) of 1.0–1.5 mM, except one which had a *K<sub>m</sub>* value of about 4 mM. From these observations the different peaks of ATEe esterase with similar *K<sub>m</sub>* values were postulated to be one enzyme.

In the present study we have shown the existence in dog mast-cell tumours of one



strong peak of ATEe esterase and one TAME esterase peak when eluted with a sodium chloride concentration gradient from a DEAE-cellulose column (Fig. 1). From these data it is apparent that the many peaks obtained by means of a concentration gradient of phosphate buffer (pH 8) indicate only minor differences in the enzyme molecule.

The mast cells used in this study were obtained from the mast-cell tumours of four dogs. The data presented are from one representative sample. Diagnosis on all tumours was established by histological examination.

The tissue was homogenized in a Potter Elvehjem homogenizer in cold 0.01 M phosphate buffer, pH 7.5. The supernatant fluid was suspended in a 40 per cent saturation of ammonium sulphate and then centrifuged. The supernatant was then dialysed against 0.005 M potassium phosphate buffer with a pH of 7.5 at 4° C for 48 h. The DEAE-cellulose column (1.2 × 20 cm) was equilibrated with 0.005 M potassium phosphate buffer (pH 7.5). The sample containing 86 mg of protein was applied to the column and then 20 ml. of 0.01 M phosphate buffer (pH 7.5) was added. The elution was performed with a linear concentration gradient of NaCl. The elution was initiated with 0.01 M potassium phosphate buffer (pH 8) and no NaCl, and increased linearly until the concentration of NaCl was 1 M in the same buffer. A total elution volume of 400 c.c. was used. The effluent fluid was collected in 10-c.c. samples and the protein content measured in a spectrophotometer at the optical density of 280 mμ. The proteolytic enzyme content of each tube was assayed by casein digestion at a pH of 7.6 (ref. 2). One unit of casein digestion was defined as that quantity of enzyme which produced an increase in trichloroacetic acid-soluble products of 10<sup>-3</sup> optical density units in digestion for 30 min by 1 ml. of the sample. ATEe- and TAME-esterase activities were assayed on a pH-stat (Radiometer, Copenhagen). The hydrolysis of each substrate was determined on 0.2 ml. of the test sample at room temperature. The pH was held constant at 7.9 by the continuous addition of 0.1 N sodium hydroxide for at least 10 min. A unit of enzyme was defined as that amount of enzyme which hydrolysed 1 μmole of substrate in 1 min (ref. 1).

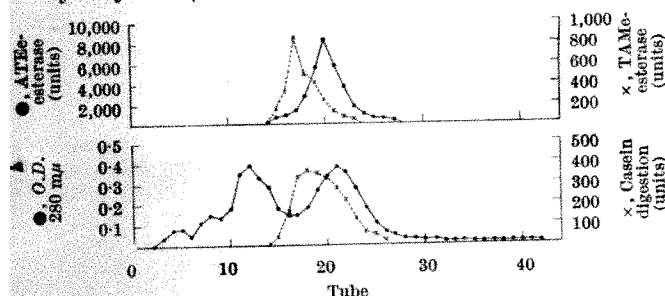


Fig. 1. DEAE-cellulose column chromatography of dog mast-cell tumour. Each tube contains 10 ml.

It was found that the ATEe-esterase was eluted around 0.4 M NaCl and the TAME-esterase at a slightly higher molarity. Each esterase showed only one peak. The tubes showing significant activity (Nos. 15–18 and 19–22, Fig. 1) were dialysed with 0.005 M potassium phosphate buffer (pH 7.5) at 4° C for 48 h and then re-applied to the DEAE-cellulose column and eluted by the same method as that described above. Although similar patterns for ATEe-esterase and TAME-esterase were again obtained, some of the TAME-esterase activity was lost. Varying the pH at which the sample was initially applied to the column over a range of 6.5–7.8 revealed a good recovery of ATEe-esterase throughout this range, but the best recovery of TAME-esterase occurred when the procedure was started at pH 7.4–7.5. At lower pH, 6.5–7.2, it was possible to obtain ATEe-esterase fractions without TAME-esterase activity. We have been unable, however, to obtain TAME-esterase activity without ATEe-esterase activity by changing the pH of application and/or elution.

Three additional dog mast-cell tumours were treated similarly by a linear concentration gradient of NaCl. These neoplasms showed similar ATEe-esterase and TAME-esterase activity to the one described in detail above. In an occasional tumour, however, a weak ATEe-esterase was eluted when the NaCl concentration was around 0.05 M. The separation of ATEe- and TAME-esterases in these three tumours was again found to be incomplete. Each dog, and different neoplasms obtained from the same animal, had a wide variation of both the enzyme content and specific activity, but all the mast-cell tumours showed a similar elution pattern which indicates similar enzymes.

From these results we conclude that in dog mast-cell tumours one strong ATEe-esterase and one TAME-esterase exist and are clearly different enzyme molecules. In addition, one weak ATEe-esterase may exist in dog mast-cell tumours.

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## IMMUNOLOGY

### A Radioimmunoassay for Human Chorionic Gonadotrophin

A NUMBER of immunological assay methods for human chorionic gonadotrophin (HCG) have been described recently. These include haemagglutination<sup>1</sup>, latex particle agglutination<sup>2</sup>, and complement fixation<sup>3</sup>. These methods have been devised principally to detect or confirm pregnancy, and qualitative tests are usually adequate for this purpose. Patients with untreated choriocarcinoma excrete HCG in the urine. During the treatment of these patients with anti-metabolite drugs the concentration of HCG in the body fluids, which provides an index of the tumour's behaviour, falls rapidly to low levels. In these circumstances an assay method which is both more precise and more sensitive is needed. With this objective a radioimmunoassay technique, similar to that described by Hales and Randle for insulin<sup>4</sup>, has been applied to HCG.

HCG-antibody complexes normally give a heavy precipitate; but at the low concentrations of reagents required to give a sensitive assay the complexes are soluble. These soluble complexes can be precipitated by the addition of anti-γ-globulin serum and they can then be filtered off on Oxoid filter membranes. When HCG labelled with iodine-131 is included in the system the radioactivity recovered on the membrane is related to the concentration of unlabelled HCG present.

Antiserum to HCG was prepared in rabbits using a crude preparation of the hormone (2,000 i.u./mg) with Freund's complete adjuvant. Horse anti-(rabbit γ-globulin)—a gift from Dr. A. J. Fulthorpe, Wellcome Research Laboratories—was used as the precipitating antiserum.

Highly purified HCG (10,000–12,000 i.u./mg) was prepared from the urine of patients with choriocarcinoma by methods which will be described elsewhere. This preparation was labelled with iodine-131 by the method which Greenwood, Hunter and Glover<sup>5</sup> used for human growth hormone to give an activity of 140–170 μc./μg.

Assays were performed by the addition of HCG  $^{131}\text{I}$  to HCG standards and to serial dilutions of the unknown specimen. These were incubated with anti-HCG serum at  $2^\circ\text{C}$  in a total volume of 0.3 ml. and all dilutions were made in 0.04 M phosphate buffer pH 7.0 + 0.4 M sodium chloride. After 4 h, 0.1 ml. anti- (rabbit  $\gamma$ -globulin) serum was added and the incubation was continued for periods of 18 h–5 days depending on the concentrations of antisera used.

The concentrations of antisera for use in the assay were determined by previous titration to find the region of slight antigen excess. In a titration of HCG antiserum in the presence of 250  $\mu\text{g}$  of labelled HCG using the precipitating antiserum at a dilution of 1:20 and an 18-h second incubation, 60 per cent of the added label was precipitated in the antibody excess zone. Using an HCG antiserum dilution of 1:6,400 (slight antigen excess) 45–50 per cent was precipitated. These recoveries could be increased by longer incubations, but this was not necessary for practical purposes. In the assay it was found that the percentage of HCG  $^{131}\text{I}$  which was precipitated was progressively reduced by the addition of unlabelled HCG and showed a linear relationship to the log of the (unlabelled) HCG concentration (Fig. 1a).

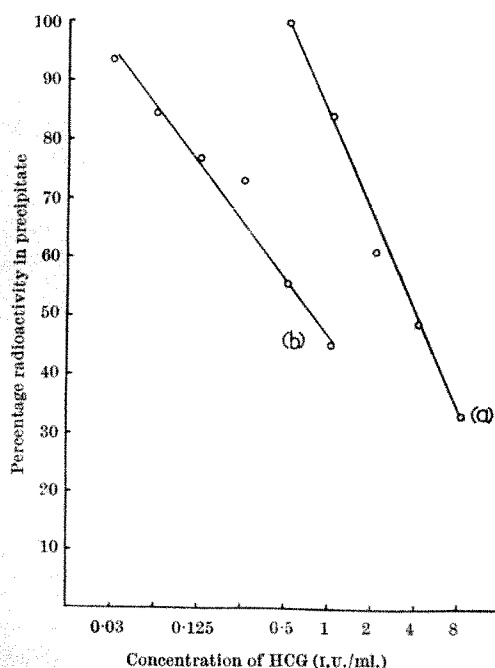


Fig. 1. Standard assay curves for HCG. Effect of unlabelled HCG on recovery of  $^{131}\text{I}$ -labelled HCG in precipitate (100 per cent = recovery with no added unlabelled HCG). (a) Anti-HCG serum added at a concentration of 1:6,400, precipitating antiserum at 1:20; second incubation of 1 day. (b) Anti-HCG serum 1:25,000, precipitating antiserum 1:80; second incubation of 4 days. 250  $\mu\text{g}$  labelled HCG added in each case.

Attempts to increase the sensitivity of the assay by lowering the concentrations of all the components were successful, but at low concentrations of labelled HCG the precision was inadequate because of low count rates in the precipitate. It was found that with fixed concentrations of antisera an increase in the amount of labelled material did not result in any loss of sensitivity. This showed that the system did not follow the principles of isotope dilution. This appeared to be because there was a considerable range where further addition of HCG resulted in a greater total uptake of the hormone in the precipitate. In practice the sensitivity could be increased without loss of precision by reducing the concentration of antisera relative to the amount of labelled HCG if the incubation times were increased.

In this way two different ranges of sensitivity have been established (Fig. 1) and concentrations down to 0.06 I.U./

ml. HCG can be detected. The method is therefore considerably more sensitive than previously published methods and it is possible that this sensitivity could be increased still further. Preliminary assays on extracts from the urine of choriocarcinoma patients excreting HCG have been in good agreement with the results from other assay methods.

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### Effect of Purinoyl Human Serum Albumin on Transplanted Ehrlich Ascites Tumour in Mice

PURINOYL human serum albumin elicits in rabbits purine-specific antibodies which also cross-react with single-stranded deoxyribonucleic acid<sup>1</sup>. It seemed of interest to investigate the effect of this antigen on tumour-bearing animals.

Purinoyl human serum albumin (Pu-HSA) was prepared by a modification of a procedure described earlier<sup>2</sup>: 6-Trichloromethyl purine (10 mg) and human serum albumin (HSA) (fraction V, Magen David Adom, Israel) (120 mg) were stirred together in water (15 ml.) at pH 9–9.5 for 2 h at  $25^\circ\text{C}$ . The resulting solution was first dialysed against tap water (24 h), then against distilled water (24 h) and then used immediately without further processing. The ultra-violet absorption spectrum of the product indicated the presence of 14 purinoyl groups per mole protein (mol. wt. 70,000).

Diploid Ehrlich ascites tumour cells ( $1 \times 10^7$ ) grown for eight days in the peritoneal cavity of female white Swiss mice (7–8 weeks old, 20–25 g) were incubated with gentle shaking for 1 h at  $37^\circ\text{C}$  with Pu-HSA (1.00 mg) in 2 ml. phosphate buffer-saline solution (PBS, M/60; pH 7.4). Cell viability was then determined by the trypan blue adsorption method. Cell mortality (5–10 per cent) was the same for cells incubated with HSA or with PBS alone.

An inoculum consisting of  $10^6$  cells + 100  $\mu\text{g}$  Pu-HSA was then administered intraperitoneally to each of a group of female mice. Animals in the control group were inoculated with  $10^6$  cells + 100  $\mu\text{g}$  HSA. In both groups tumour was established almost at the same rate in nearly all the animals. However, tumour regression occurred in some of the animals that had received Pu-HSA. The results of these experiments are given in Table 1 (Exps. 1 and 2).

In a modified experiment, mice were injected intraperitoneally with  $3 \times 10^5$  tumour cells and, 2 h later, with 500  $\mu\text{g}$  Pu-HSA. Two successive booster doses of Pu-HSA, 500  $\mu\text{g}$  each, were then given, on the second and fourth day following the initial injection. This treatment, too, had no effect on the establishment and growth of the tumour, but regression occurred in a number of mice (Table 1, Exp. 3).

The growth rate of tumour cells in animals inoculated with  $10^6$  cells and treated with 100  $\mu\text{g}$  Pu-HSA was compared with controls treated with 100  $\mu\text{g}$  HSA. This was done by counting the tumour cells on various days following inoculation. The results show that Pu-HSA

Table 1. EFFECT OF PURINOYL-HSA ON THE COURSE OF TUMOUR DEVELOPMENT BY TRANSPLANTED EHRLICH ASCITES TUMOUR CELLS

	Expt. 1	Expt. 2	Control	Expt. 3	Control
No. animals used	45	20	28	38	16
Tumour 'takes'	45	20	28	38	16
No. animals showing regression	12	9	0	13	0
Av. survival time (days) of animals not showing regression	30	(a)	26	34	34
Onset of regression (days)	26	14	—	37	—
No. animals showing relapse of tumour	6	9	—	9(b)	—
Av. survival time (days) of animals showing relapse	61	(a)	—	(a)	—

Exp. 1 and Exp. 2: Animals inoculated with  $10^6$  tumour cells + 100  $\mu$ g Pu-HSA. Exp. 3: Animals inoculated with  $3 \times 10^6$  tumour cells, followed by three successive injections, 500  $\mu$ g each, of Pu-HSA. (a) Not determined. (b) This figure includes 3 solid tumours.

had no apparent inhibitory effect during the logarithmic phase of growth (first nine days).

The total uptake of Pu-HSA (adsorbed + ingested) by the tumour cells was also studied. Tritiated 6-trichloromethylpurine (10 mg = 1.38 mc.) was coupled to HSA and the labelled protein (1.00 mg = 40,200 c.p.m.) was incubated with  $1 \times 10^7$  cells in 2 ml. PBS solution. After 1 h at 37° C, the cells were centrifuged, washed twice with PBS solution and resuspended in 1 ml. PBS solution. This suspension gave a reading of 1,960 c.p.m. in a Tri-Carb liquid scintillation counter, model 500 B, roughly corresponding to  $4 \times 10^7$  protein molecules per cell, most probably adsorbed on the cell membrane<sup>2</sup>. According to Ryser, a sphere of the size of an ascitic tumour cell would be completely covered by an albumin monolayer consisting of  $3.7 \times 10^7$  molecules. Since the value obtained is about two orders of magnitude larger than that reported for  $^{125}$ I-HSA under comparable conditions<sup>2</sup>, it seemed of interest to investigate the uptake of Pu-HSA *in vivo*. After an incubation period of 1 h at 37° C, a mixture of tumour cells ( $10^6$ ) and tritiated Pu-HSA (100  $\mu$ g = 4.25 c.p.m.) in 0.2 ml. PBS solution was injected intraperitoneally to mice. For the next five days, two animals were killed daily, the tumour cells counted and the radioactivity contents of these cells determined.

The results show that at no time was the uptake of Pu-HSA by the cells higher than 6 per cent of the quantity injected, irrespective of cell number which reached the value of  $2.8 \times 10^7$  at the end of five days. Although the radioactivity level of the preparation was somewhat too low for autoradiography, this was nevertheless attempted in order to reveal the possible concentration of the label in particular cells. The results, however, were negative.

In view of these results, it is concluded that tumour regression is the outcome of a delayed action of Pu-HSA either on the tumour cells or on the host: (a) Pu-HSA is not toxic to the cells *in vitro*; (b) it does not inhibit cell growth *in vivo*; (c) cells from a regressing tumour are still capable of establishing a new tumour by transplantation.

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## Reticulo-endothelial Function in Thymectomized Rats

ANIMALS thymectomized at, or shortly after, birth show a decreased ability to develop a normal immune response. This is manifested by an impaired ability to reject homografts<sup>1-3</sup> and heterografts<sup>1,3</sup> and a lowering of the levels of circulating antibody to various antigens, both soluble and particulate<sup>1,3-5</sup>. This failure to develop immunological maturity possibly results from the loss of a major source of lymphoid cells, to be seeded in peripheral lymphoid organs, or the loss of the source of a humoral factor which would permit existing lymphoid tissue to participate in the immune responses<sup>5</sup>.

The role of the reticulo-endothelial system (RES) in the immune mechanism has been reviewed by Thorbecke and Benacerraf<sup>6</sup>. Work in this laboratory has shown that alterations in RE function are associated with corresponding changes in antibody formation to a particulate antigen<sup>7</sup>. These studies also indicated that the induction of RE depression is associated with a reduction in the immune response to a particulate antigen essentially equivalent to splenectomy, thymectomy, and partial lymphadenectomy<sup>8</sup>. In view of these observations, and recent studies which have indicated that macrophages interact with potential antibody cells<sup>9</sup>, the immune defect in thymectomized animals could well reflect an alteration in phagocytic function. Indeed, recent studies by Osoba and Miller<sup>10</sup>, and Levey *et al.*<sup>11</sup>, demonstrate that the humoral factor elaborated by the thymus is derived not from lymphoid cells but from reticular cells.

One- to four-day-old Holtzman rats were thymectomized according to the method of Janković *et al.*<sup>4</sup>. Sham-operated litter mates were treated identically except that the thymus was left in place.

At 8-12 weeks of age, the phagocytic activity of the RES of the thymectomized and sham-operated rats was evaluated by measuring the intravascular removal rate of colloidal carbon<sup>12</sup> (Günther Wagner, Hanover, Germany; preparation C11/1431a). Each rat received an intravenous injection of 8 mg of colloidal carbon/100 g body-weight. A series of tail blood samples of 0.01 ml. were obtained from the tail veins and hemolysed in 0.1 per cent Na<sub>2</sub>CO<sub>3</sub>. The colloidal carbon concentration was determined spectrophotometrically and the half-time (*t*/2) was calculated. The absence of thymic tissue in thymectomized rats was verified.

As observed by Janković *et al.*<sup>4</sup>, the rats which were thymectomized at birth or shortly thereafter gained weight normally (Table 1). In similar confirmation, the spleen weight was not changed in thymectomized rats when compared to control animals.

Table 1. PHAGOCYTIC FUNCTION IN SHAM-OPERATED AND THYMECTOMIZED RATS

Group	Final body-weight (g)	Organ weight (g)			Colloidal carbon removal ( <i>t</i> /2 min)
		Liver	Lung	Spleen	
Thymectomized	234 ± 20	9.17 ± 0.78	1.52 ± 0.23	0.56 ± 0.05	11.6 ± 1.0
Sham-operated	220 ± 10	8.31 ± 0.31	1.47 ± 0.23	0.48 ± 0.03	10.0 ± 1.3

Values are expressed as means ± standard errors and are derived from 10 rats/group.

The phagocytic activity of the RES, as evaluated by the removal rate of colloidal carbon, was not altered in the thymectomized group. The maintenance of normal phagocytic activity following thymectomy is in basic agreement with the observation of Waksman *et al.*<sup>13</sup> that no histological abnormality in RE cells followed thymectomy. The increased susceptibility to intercurrent infection previously observed in thymectomized rats<sup>4</sup> cannot be related to an impaired phagocytic activity.

Recently the role of the macrophage in antibody formation has been predicated. This involves the essential



phagocytosis of the antigen<sup>6,14</sup> followed by transfer of cytoplasmic content as RNA or an RNA-antigen complex<sup>14</sup>. The demonstration of a direct cytoplasmic connexion between macrophages and antibody producing cells further established this concept<sup>9</sup>. Since no change in the RE function was apparent on the removal of the thymus, it is apparent that alterations in phagocytic activity of the RES are not involved in the immunological defects which follow neonatal thymectomy. The possible influence of thymectomy on other metabolic activities of the macrophage system remains to be established.

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### Immune Response induced by RNA-Immuno-carrier extracted from Heterologous Immune Sera

THE importance of nucleoproteins in antibody-globulin production is well known<sup>1,2</sup>. In previous experiments<sup>3,4</sup> an evident increase of the content of RNA in the  $\gamma$ -globulin fraction in immune sera has been observed. The RNA extracted from the serum of immunized rabbits is capable of eliciting in normal rabbits the production of antibodies against the same antigens used for immunizing the animals from which this RNA-immuno-carrier (RNA-I-C) was taken. The following investigations were undertaken to ascertain whether an RNA-I-C extracted from the serum of immunized animals of one species was able to induce antibody production in animals of a different species.

Young male rabbits were immunized by 6-8 intravenous injections of red blood cells (RBC) of rat or guinea-pig. The RNA-I-C extracted<sup>4</sup> from sera of rabbits immunized with guinea-pig RBC was introduced into normal rats; normal guinea-pigs were treated with RNA-I-C obtained from sera of rabbits immunized with rat RBC. The amount of RNA introduced in each animal of both groups, by a single intracardiac injection, was 0.28 mg/100 g

Table 1. IMMUNE RESPONSE OF RATS TREATED WITH RNA FROM ANTI-GUINEA-PIG RBC HYPERIMMUNE RABBIT SERA AND OF GUINEA-PIGS TREATED WITH RNA FROM ANTI-RAT RBC HYPERIMMUNE RABBIT SERA

	Guinea-pig RBC		Rat RBC	
	Rats treated with anti-guinea-pig RBC RNA	hemagglutination hemolysis	Guinea-pigs treated with anti-rat RBC RNA	hemagglutination hemolysis
Controls	0	0	0	0
24 h	1:80	1:80	1:80	1:40
48 h	1:320	1:160	1:320	1:80
72 h	1:80	1:80	1:40	1:20
96 h	neg.*	neg.	neg.	neg.

\* Negative is less than 10.

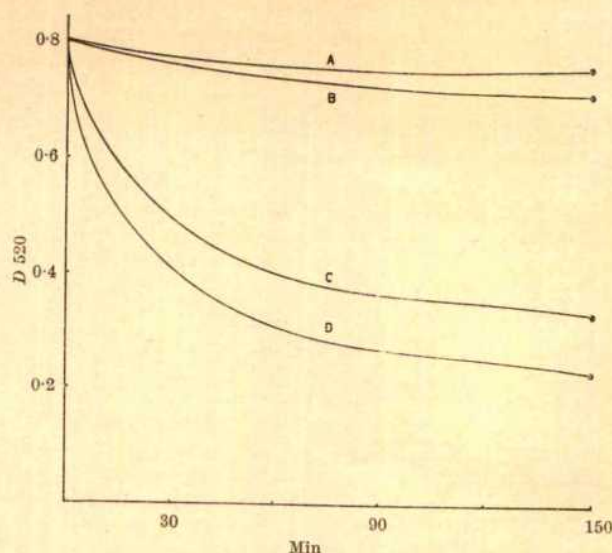


Fig. 1. Spectrophotometric behaviour of rat RBC or guinea-pig RBC hemolysis by: A, normal rat serum; B, normal guinea-pig serum; C, serum from rat 48 h after injection of anti-guinea-pig RBC RNA; D, serum from guinea-pig 48 h after injection of anti-rat RBC RNA

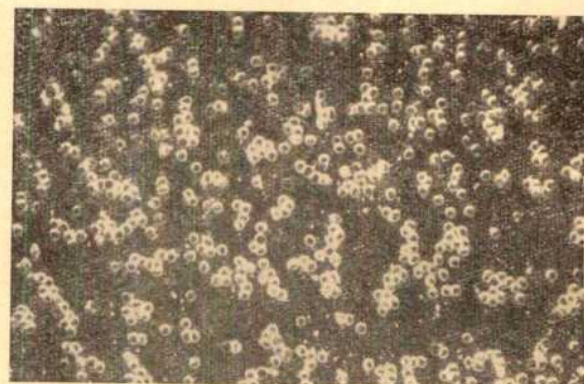


Fig. 2. Immunofluorescence test: guinea-pig RBC incubated at 37°C with serum (1:10) from rat 48 h after injection of RNA-I-C from rabbit anti-guinea-pig RBC sera, and then incubated with fluorescent anti-rat globulins

body-weight. At intervals of 24, 48, 72 and 96 h after RNA injection, blood samples were collected and the sera were subjected to haemagglutination tests and macroscopic and spectrophotometric<sup>4</sup> hemolysis tests.

The results of haemagglutinations and hemolysis are summarized in Table 1. The kinetic behaviour of hemolysis observed spectrophotometrically is shown in Fig. 1. The antigen-antibody reaction was also shown by the indirect immunofluorescent technique (Fig. 2). Check tests of RNA preparations were made by spectrophotometry in ultra-violet light.

The data confirm the possibility of eliciting an immune response by RNA-I-C extracted from the serum of immunized animals of a different species.

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## HISTOLOGY

Cyclic and Experimentally-induced Changes in the Histology of the Adrenal Gland in *Rana temporaria*

THE anuran adrenal gland consists of tissue strands in which the interrenal and chromaffin cells are intermingled. In some species a third cell type (summer- or Stilling-cell) has been described which has an eccentrically placed nucleus and cytoplasmic granules which are eosinophilic and stain metachromatically with basic dyes like toluidine blue. In *Rana temporaria* chromotropic cells are also present but differ from the foregoing in histological appearance; moreover, the cytoplasmic granules are not eosinophilic.

With the annual variation in environmental factors and the considerable seasonal changes in metabolic state, it might be expected that, in *R. temporaria*, the histological appearance of the adrenal gland might also vary throughout the year. The work recorded here is based on animals caught in the open during February 1962–January 1963. Five adult frogs were collected and killed every two weeks throughout this period. Adrenals were rapidly extirpated and fixed in Helly's fluid. Paraffin sections of 7  $\mu$  were stained with hæmatoxylin–eosin and alcian blue.

Cytologically two quite different cortical cells could be distinguished: (1) Cells with a compact cytoplasm containing few small vacuoles. The large nucleus was round or oval in outline with 1 or 2 clearly defined nucleoli ('compact cells'). (2) Cells with a highly vacuolated cytoplasm and an irregular crenated nucleus in which the nucleolus was difficult to distinguish ('clear cells'). Both cell types, and in addition several intermediate forms, were present at all periods of the year. The clear cells were always situated in the strands of the central part of the adrenal whereas the compact cells were present in the peripheral strands.

The cytological variations during the year were found to be in accordance with those reported by Fowler<sup>1</sup>, who stated that in spring and summer compact cells were more numerous than during the winter period. This author did not, however, mention changes in the general histological appearance of the gland. In the present material, in addition to strands in which each cell fits closely with its neighbours we frequently found strands in which cavities were present (Fig. 1). These cavities varied in size and were surrounded by cortical cells. The cavities were found at all times of the year, but were most numerous in April and August–September. In those parts of the gland where cavities were present, most of the chromotropic cells were situated within the cavities and not scattered among the cortical tissue. Sometimes the cavities were follicle-like, that is, surrounded by a single layer of cortical cells and mostly contained a colloid-like substance. Bulliard *et al.*<sup>2</sup> and Maillet<sup>3</sup> observed cortical cavities in the adrenal of *Rana esculenta* after treatment with adrenocorticotrophic hormone. The same were also described by Olivereau<sup>4</sup> in the eel after treatment with metopirone and adrenocorticotrophic hormone. It seems therefore not unlikely that the above cavities in the adrenal of *R. temporaria* reflect an active phase of the gland.

Since aldosterone has been reported in high concentration in Amphibia<sup>5,6</sup> a second investigation was undertaken to determine the effects of exogenously administered aldosterone, a known aldosterone antagonist. Moreover, the effect of metopirone, an inhibitor of the 11 $\beta$ -hydroxylation in the synthesis of corticoids, was also investigated.

After oral administration of 5 mg aldosterone/day during 18 days, the histological alterations pointed to an increased activity of the cortical tissue as a whole. As compared with the controls, all cortical cells of the aldosterone-treated animals showed a compact cytoplasm containing large



Fig. 1. Normal adrenal gland of *Rana temporaria* in April ( $\times$  c. 170)

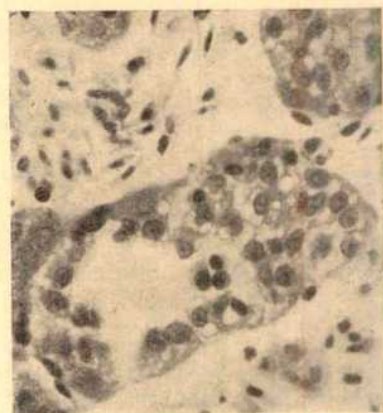


Fig. 2. Adrenal gland of *Rana temporaria* after treatment with aldosterone in September ( $\times$  c. 170)

vacuoles which were quite different from those found in the compact cells already described here (Fig. 2). The nuclei were large and round in outline with 1 or 2 clearly defined nucleoli. Moreover, the cortical tissue showed an increased mitotic activity and the number of cavities was much higher than in control animals. The fact that these alterations occurred in the peripheral as well as the central part of the cortical tissue suggests that the aforementioned cytological differences between the cells of these two parts of the gland do not represent a functional zonation as can be seen in the mammalian cortex, but rather reflect a difference in activity of both kinds of cells.

Administration of 5 mg metopirone/day during 18 days resulted only in a small increase in mitotic activity of the cortical tissue. According to Olivereau<sup>4</sup> the effect of metopirone in the eel is maximal at the fourth day and thereafter diminishes rapidly in spite of prolonged treatment, even with high doses. It is therefore not impossible that the absence of marked changes after administration of metopirone can be explained by the long duration of the experiment.

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## HISTOCHEMISTRY

## Effect of Denervation on the Lactate Dehydrogenase Isozymes of Skeletal Muscle

THE lactate dehydrogenase (LDH) isozyme pattern of many mammalian tissues has been shown to change with development<sup>1,2</sup>. Persistence of the foetal LDH pattern in skeletal muscle has been noted in chickens with hereditary muscular dystrophy<sup>3</sup> and in children with myopathy<sup>4</sup>. In the work recorded here an adult skeletal muscle was denervated in order to ascertain the effect of an acquired lesion on the LDH isozyme pattern.

The soleus muscle of an adult guinea-pig was unilaterally denervated by severing the sciatic nerve at the trochanteric level under general anaesthesia. A portion of nerve 1.5 cm long was removed. The animal was then permitted free movement in its cage and killed after 11 weeks. The soleus muscles from both hind-legs were excised, homogenized, submitted to starch-gel electrophoresis, and incubated to demonstrate LDH activity, according to the methods described previously<sup>5</sup>. LDH isozyme patterns of soleus muscles from new-born and normal adult guinea-pigs were prepared in the same manner. The LDH activities of all extracts were measured in a spectrophotometer and equalized at 30,000 units/ml. by dilution before application to the gel.

Predominance of the faster-moving LDH isozymes (LDH 1 and LDH 2) was found to characterize normal adult guinea-pig soleus<sup>6</sup> (Fig. 1C). The pattern of new-born soleus differed from that of the normal adult (Fig. 1B). It showed less activity of the fast-moving isozymes and greater activity of the slow-moving isozymes. The pattern of the denervated soleus also differed from that of the normal adult and resembled the pattern of the new-born (Fig. 1A). The soleus from the unoperated side of the denervated animal had an isozyme pattern identical to that of normal adult soleus.

Microscopic examination of the denervated soleus revealed only slight infiltration of connective tissue in addition to neurogenic atrophy of muscle fibres.

These results were confirmed with three adult guinea-pigs and with new-born guinea-pigs from three different litters. The observations are in accord with a recent chemical analysis of the forms of LDH in rabbit soleus by Dawson *et al.*<sup>7</sup>, who found that 'H-LDH' (which predominates in the fast-moving isozymes) increases more rapidly in the first several weeks of life than 'M-LDH' (which predominates in the slow-moving isozymes). Denervation

was found by these workers to produce greater loss of the H type than of the M.

The work recorded here indicates that experimental denervation of muscle may cause reversion to the immature LDH isozyme pattern. Not only does the intensity of the fast-moving isozymes decrease in the reversion of the guinea-pig soleus pattern but also the intensity of the slow-moving isozymes increases.

Resemblance to the immature LDH isozyme pattern is, therefore, a non-specific effect of either myopathic or neurogenic change in skeletal muscle and occurs in hereditary and acquired conditions.

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## RADIOBIOLOGY

Retention of <sup>90</sup>Sr in Lactating Rats

IN previous investigations we were able to demonstrate that elimination of <sup>90</sup>Sr is enhanced in lactating rats<sup>1-4</sup>. Similar findings were reported by other investigators under various experimental arrangements and with various experimental animals<sup>5-10</sup>. To gain more quantitative information on Sr-metabolism in the course of lactation the following experiment has been performed.

Experimental animals were 32 albino rats of the 'Heiligenberg' strain, 15 weeks of age. 29 were dams carrying litters, the mean number of young being 9-10. Three virgin rats served as controls. Each rat received an intravenous injection of 2  $\mu$ c. of <sup>90</sup>Sr-<sup>90</sup>Y chloride in physiological saline and was killed 48 h thereafter. Time of injection varied with experimental groups so that 48-h retention could be evaluated for the end of gestation, the beginning of lactation and the end of lactation. Femora of dams and total litters were ashed dry. Radioactivities of samples of the ash from each femur were measured, and means were calculated for the litter. The experimental arrangement and results are recorded in Table 1.

As can be seen, ash-weights were elevated with respect to controls at the end of gestation and at the beginning of lactation. At the end of lactation they were lowered. Radioactivities of the femora were lower with all dams, the difference between groups 2 and 3 not being significant ( $P > 0.05$ ). In an earlier experiment<sup>2</sup>, different retentions of injected <sup>90</sup>Sr, compared with virgin controls, could not be established. With the present arrangement retention at the end of gestation was significantly lower. Diminution of radioactivity in the femora at days 15-17 post partum was highly significant. Radioactivities of the juveniles were roughly doubled for days 3-5 post partum compared to days 17-19 of gestation, and then once more for days 15-17 post partum. Therefore, participation of the litter on a dose of <sup>90</sup>Sr injected during lactation was growing continuously.

An approximation to the amount retained by the whole animal can be calculated from the figure derived from the femora by multiplication by a factor of 20. Retentions calculated for groups 1-4 are 51, 36, 42 and 17 per cent of the injected dose, respectively. Radioactivities retained in the biological system comprising mother and litter 48 h

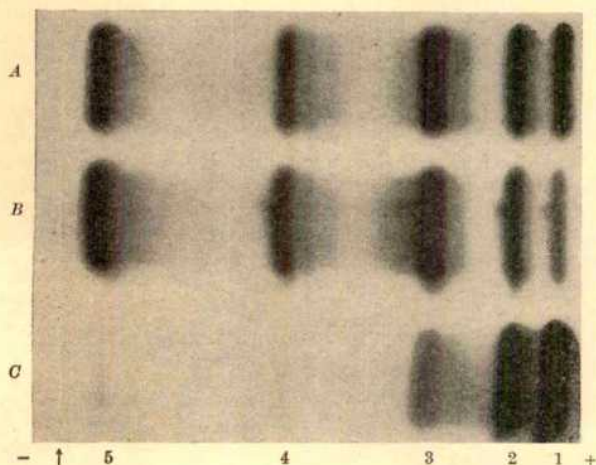


Fig. 1. Photograph of a starch gel showing electrophoretic patterns of guinea-pig muscles. The LDH activity of each extract was adjusted to 30,000 units/ml. before application to gel. Arrow indicates origin: LDH isozymes are numbered from anode. A, adult soleus denervated 11 weeks; B, new-born soleus; C, normal adult soleus. Note predominance of fast-moving isozymes in normal adult soleus. In denervated soleus, the fast-moving isozymes are decreased in intensity, the slow-moving isozymes are increased, and the pattern resembles that of the new-born



Table 1. 48-h  $^{86}\text{Sr}$  RETENTION AND ASH-WEIGHT OF FEMORA, AT VARIOUS STAGES OF GESTATION AND LACTATION

Group	No. rats	Mean No. * young/litter	Time of retention of the isotope †	Mean ash-weight ‡ per femur (mg)	Mean radioactivity ‡ per femur (% of injected dose)	Mean radioactivity per liter (% of injected dose)
1 Virgin controls	3	—	—	213 ± 24	2.55 ± 0.08	—
2 Dams	9	10	17–19 of gestation	242 ± 12	1.84 ± 0.12	15.07
3 Dams	12	10	3–5 post partum	244 ± 11	2.10 ± 0.14	30.78
4 Dams	8	9	15–17 post partum	190 ± 8	0.84 ± 0.43	66.13

\* Or embryos in group 2. † From injection of the isotope to time of killing. ‡ Means with single standard deviations of the means ( $s_x$ )

after injection are therefore 51, 72 and 83 per cent of the injected dose for experimental groups 2–4. This indicates that at the end of gestation the retention of this system is roughly the same as that of virgin controls and is steadily elevated during lactation in contrast to the retention in the femora of dams. It is not clear, from the present experiment, whether this is accompanied by lowering of the faecal and urinary excretion of the mineral by the dams or the young or both. It may be mentioned, however, that Lenkeit<sup>6</sup> reported, in lactating sows, a considerable decrease of faecal and urinary excretion of Ca and a negative balance of Ca due to elevated secretion with milk.

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### Absorption and Retention of Ingested Strontium and Calcium in Beagles as a Function of Age

INVESTIGATIONS of the metabolism of strontium and calcium in various species have shown that the two elements, though qualitatively similar, have distinct quantitative differences. The differential movement of strontium and calcium across physiological barriers is expressed by the term 'Strontium-Calcium Observed Ratio' (*OR*) and is given by:

$$OR \text{ sample-precursor} = \frac{\text{Sr/Ca sample}}{\text{Sr/Ca precursor}}$$

The  $OR_{\text{bone-diet}}$  values for laboratory and domestic animals (cow, goat, sheep and rat) under usual dietary conditions range from 0.2 to 0.5 with most of the values at about 0.25 (refs. 1 and 2). Limited data for man suggest a range of 0.25–0.5 under different dietary and environmental conditions. Unfortunately, few data have been reported for the dog.

In the present work, the absorption and retention of strontium and calcium were determined in beagles of different ages after ingestion of a single meal containing known amounts of  $^{86}\text{Sr}$  and  $^{45}\text{Ca}$ . The value of the  $OR_{\text{bone-diet}}$  in a given species can be used to estimate the deposition and retention of strontium. Thus, at a continuous or chronic intake of  $^{86}\text{Sr}$  per g of dietary calcium the skeleton would be uniformly labelled, and the body burden estimated as follows:  $\mu\text{c.}^{86}\text{Sr/g Ca}_{\text{diet}} \times OR_{\text{bone-diet}} \times \text{total Ca in species} = \mu\text{c.}^{86}\text{Sr in skeleton}$ . The data obtained on the differential uptake of  $^{86}\text{Sr}$  and  $^{45}\text{Ca}$  (*OR*) in the dog were used as a guide in the preparation of diets containing  $^{86}\text{Sr}$  to yield predictable body burdens

under a continuous dietary regimen. Such an experiment is in progress at the University of California at Davis, to investigate the long-term effects of chronic ingestion of  $^{86}\text{Sr}$  at given  $^{86}\text{Sr}/\text{Ca}$  ratios<sup>3</sup>.

Sixteen pure-bred beagles were used, ranging from 48 to 2,060 days of age. Eleven dogs were maintained on a compounded 1 per cent calcium diet (Ca/P weight ratio of 1.5) for several months before the experiment and in most cases from birth. Five dogs were maintained on a 2 per cent calcium diet (Ca/P weight ratio 1.5) for a 30-day equilibration period before the experiment. In both cases, the diets were composed of 60 per cent dry kibbled ration plus 20 per cent meat and 20 per cent (by weight) water, and required vitamin supplements. The dietary calcium was essentially all in the dry ration and was controlled within reasonable limits by selection of natural materials only. The calcium content of the two dry rations was 1.7 and 3.3 per cent by weight, respectively.

High specific activity  $^{45}\text{Ca}$  was obtained from Oak Ridge National Laboratory, Tennessee. It decays with a 4.7-day half-life and emits  $\gamma$ -rays at 1.29, 0.81 and 0.50 MeV. Carrier-free  $^{86}\text{Sr}$  (65 days half-life) emits a 0.513-MeV  $\gamma$ -ray. It was obtained from Nuclear Science and Engineering Corp., Pittsburgh, Pa. The energies of the  $\gamma$ -rays differ sufficiently to permit good pulse-height differentiation. The  $^{45}\text{Ca}$  contribution to the 0.513-MeV  $^{86}\text{Sr}$  photo peak can be corrected by comparison of the  $\gamma$ -ray spectra of the two radionuclides in the calibration standards. The  $^{86}\text{Sr}$  estimate for each dog was reduced by that fraction of the standard  $^{45}\text{Ca}$  spectrum detected at the  $^{86}\text{Sr}$  window. A single channel analyser was adjusted to record the  $\gamma$ -ray pulses within a 0.120-MeV energy band centred over either the 0.513-MeV  $^{86}\text{Sr}$  or the 1.29-MeV  $^{45}\text{Ca}$  photo peaks.

Standards of each radionuclide were prepared at the time of feeding by placing 200  $\mu\text{c.}^{86}\text{Sr}$  and/or 500  $\mu\text{c.}^{45}\text{Ca}$  in a 2-l. plastic bottle the approximate size of the smallest test animal. Two annular paraffin cylinders into which these standards could be placed were also prepared to approximate to the geometry of medium and larger-sized dogs.

The test meals were prepared by using aliquots of the radionuclides, as used in the standards, which were added to the mixture of meat and water, followed by thorough blending with the dry ration. To ensure complete consumption, the food was offered after a short fast and, in the case of the very young animals, in two divided portions a few hours apart.

The first  $\gamma$ -ray counts were made within 1 h after ingestion. The dog measurements were compared to the standards at each counting time and the data expressed as percentage of the initial dose. This eliminated any necessity for corrections for the physical decay of the radionuclides.

The single-channel analyser used for these investigations was fed by a 5 × 5 cm Na I (Tl) scintillation crystal mounted in a shield constructed of 5 × 10 × 20 cm lead bricks. The detector, surrounded by 10 cm of lead, faced out into an open lead-walled 'horn' the wide end of which was the same size as the lateral face of a dog restraint box made of wood and 'Lucite'. A constant distance (63 cm) was maintained between the midline of the dog (unanaesthetized), and/or standards, and the crystal face.



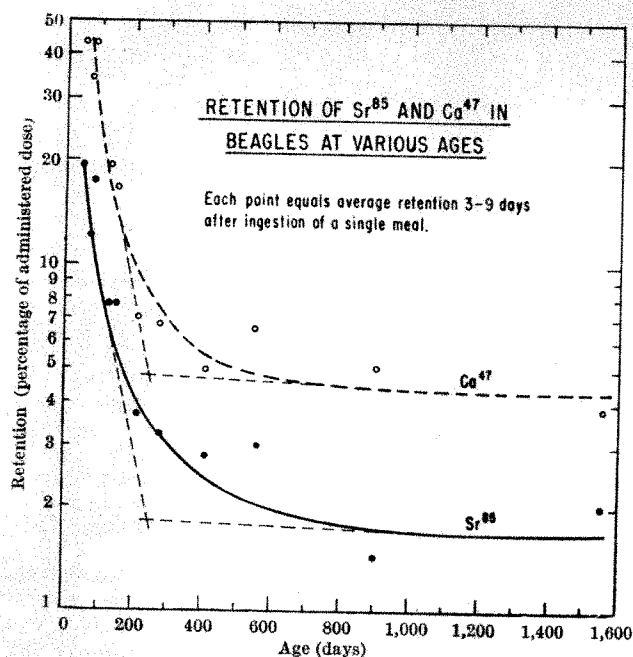


Fig. 1. Biological retention curves of  $^{85}\text{Sr}$  and  $^{47}\text{Ca}$ , percentage of administered dose versus age averaged, 3-9 days after ingestion, 1 per cent calcium diet

Fig. 1 shows the biological retention curves of  $^{85}\text{Sr}$  and  $^{47}\text{Ca}$  expressed as the percentage of administered dose versus age averaged at 3-9 days after ingestion of the 1 per cent stable calcium diet. The retention curves indicate that strontium and calcium are absorbed in a qualitatively similar manner. The absorption and retention of administered  $^{85}\text{Sr}$  and  $^{47}\text{Ca}$  decrease rapidly with increasing age of the animal. After 250 days there is little effect of age on the percentage retention of the two radionuclides, which average about 5 per cent for calcium and 2 per cent for strontium. The highest absorption was observed in the youngest dog (48 days old at feeding) at about 44 per cent for calcium and 19 per cent for strontium.

Fig. 2 shows the values of the observed ratios ( $OR$ ) expressed as the ratio of  $^{85}\text{Sr}/^{47}\text{Ca}$  in the 1 per cent stable calcium diet. A line shows the average  $OR$  value of 0.45 for all ages. Under the ages and conditions of this experiment, no effect of age on the  $OR$  was evident, despite the age-dependency of the uptake of these elements.

The effect of increased dietary calcium (2 per cent) on the absorption of  $^{85}\text{Sr}$  and  $^{47}\text{Ca}$  in five beagles is shown in Table 1, and the data are compared with those obtained

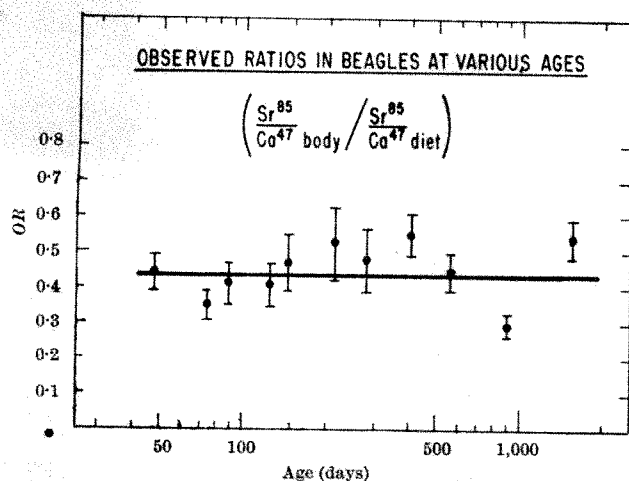


Fig. 2. Observed ratios, body to diet, in beagles at various ages, 1 per cent calcium diet

Table 1. RETENTION OF  $^{85}\text{Sr}$  AND  $^{47}\text{Ca}$  IN BEAGLES  
Per cent of dose: average 3-9 days after ingestion of single meal

Age (days)	$^{85}\text{Sr}$	1% Ca diet $^{47}\text{Ca}$	$OR^*$	$^{85}\text{Sr}$	2% Ca diet $^{47}\text{Ca}$	$OR$
48	19.2	43.7	0.44			
74	12.0	34.1	0.35			
79	17.3	43.2	0.41			
83				8.7	16.9	0.52
83				10.0	17.1	0.59
94				12.9	23.6	0.55
125	7.8	19.2	0.41			
145	7.8	16.7	0.47			
215	3.7	7.0	0.53			
279	3.2	6.8	0.48			
405	2.8	5.1	0.55			
560	3.0	6.6	0.45			
589				1.2	2.4	0.49
900	1.4	5.0	0.29			
1,550	2.0	3.8	0.54			
2,057				1.1	2.1	0.52
Average			0.45			0.53

\*  $OR = \frac{\text{Sr/Ca body}}{\text{Sr/Ca diet}}$

from the 1 per cent calcium diet. The average percentage retention (3-9 days after ingestion) for both  $^{85}\text{Sr}$  and  $^{47}\text{Ca}$  is significantly reduced in beagles on the 2 per cent calcium diet. A two-fold increase in the calcium ingestion resulted in a nearly two-fold decrease in the absorption of  $^{85}\text{Sr}$  and  $^{47}\text{Ca}$ . The  $OR$  body-diet values appear to be somewhat higher at the 2 per cent dietary calcium level; that is, the average  $OR$  is 0.53 compared with 0.45. The decreased absorption and retention of  $^{85}\text{Sr}$  and  $^{47}\text{Ca}$  observed here confirm the observations made by Wasserman *et al.*<sup>4</sup> in growing rats over a four-fold variation in dietary calcium with the Ca/P maintained at a constant ratio. No significant change in the value of the  $OR$  was observed by these investigators; the difference seen in the present investigation could be attributed to the small number of dogs, to age distribution, or to possible species differences.

The differential metabolism of strontium and calcium in beagle dogs has been shown to be comparable in magnitude to that in other mammalian species. The value of the observed ratio ( $OR$ ) in beagles in equilibrium with the dietary calcium and phosphorus environment varies between 0.3 and 0.5 (average 0.45) at widely different ages. A preliminary report by Stover, Goldman and Andersen<sup>5</sup>, based on a single dog, indicated that the value of the  $OR$  might approximate to unity under conditions of continuous ingestion. It now appears that the reported  $^{90}\text{Sr}$  to calcium ratio in the diet was in error by a factor of about 2. Only the diet data appear erroneous, for the skeletal data in the original report have been verified. Further work by Della Rosa<sup>6</sup> under conditions of continuous feeding of  $^{90}\text{Sr}$  at constant  $^{90}\text{Sr}/\text{Ca}$  ratio confirms the findings of the single double double tracer feeding reported here.

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<sup>2</sup> Comar, C. L., Wasserman, R. H., and Nold, M. M., *Proc. Soc. Exp. Biol. and Med.*, **92**, 859 (1956).

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## BIOLOGY

Locomotion in the Australian Marsupial  
*Antechinomys*

THE so-called 'jerboa marsupial' of Australia (*Antechinomys*) is widely held to be bipedal and convergent on bipedal saltatory murids or jumping mice (*Notomys*); various species of these occur in identical localities with *Antechinomys* in rather arid parts of Australia from Western Australia to Queensland. This interpretation of locomotion in *Antechinomys* is to be found in most general works on Australian marsupials and, as an example of evolutionary convergence, in zoological text-books<sup>1-4</sup>.

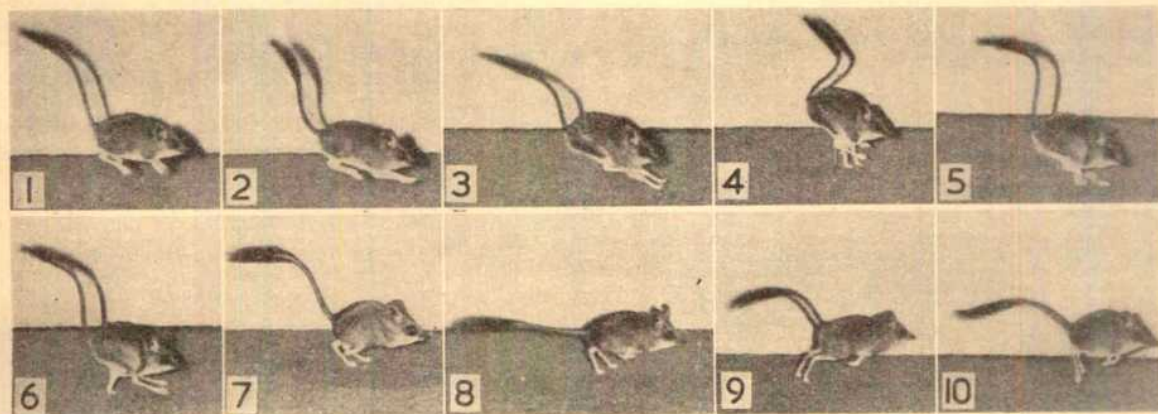


Fig. 1

During the past year I have kept four *Antechinomys spenceri* and one *Notomys richardsoni* in captivity; they are often allowed free to run in my home. Electronic-flash photographs and ciné-film have been taken of them, and these reveal that *Antechinomys* is consistently quadrupedal. Fig. 1 illustrates typical stages in the gallop. The sequence illustrated is made up from selected electronic-flash photographs arranged in the order shown to be natural in cinematographic film taken under similar conditions. This sequence takes from 0.2 to 0.25 sec to complete.

The jumping-mouse, *Notomys richardsoni*, photographed under these conditions is bipedal and saltatory.

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<sup>1</sup> Jones, F. Wood, *The Mammals of South Australia*, Part 1 (Govt. Printer, Adelaide, 1923).

<sup>2</sup> Troughton, E. Le G., *Furred Animals of Australia*, seventh ed. (Angus and Robertson, 1962).

<sup>3</sup> Parker and Haswell, *A Textbook of Zoology*, 2, seventh ed. (Macmillan, 1962).

<sup>4</sup> Troughton, E. Le G., in Keast, A., Crocker, R. L., and Christian, C. S., *Biogeography and Ecology in Australia* (Junk, 1959).

## Mechanism of Anaphylactic Death in the Mouse

GUINEA-PIG anaphylaxis has for many years been considered the classical example of anaphylaxis. In this animal, challenged intravenously, anaphylactic shock is believed to be due to liberation of histamine with subsequent contraction of smooth muscle. Contraction of the bronchial musculature completely shuts off the alveoli and prevents exhalation of air, and the animals die of asphyxia. If the shocking dose of antigen is given by the intraperitoneal or subcutaneous route, death in many cases is not due to asphyxia, but occurs slowly (protracted anaphylaxis), and cannot be attributed to respiratory failure<sup>1,2</sup>. In other animals, anaphylactic death has also been associated with a 'selectively' shocked organ. Thus,

in the rabbit, the constriction of pulmonary arterioles and extreme dilation of the right heart lead to acute fatal heart failure. In the dog, marked liver congestion seems to be responsible for death<sup>3</sup>.

In the mouse, anaphylactic shock is always accompanied by respiratory distress, cyanosis and some degree of oedema. Symptoms associated with smooth muscle contraction are evident, but not striking. Other characteristic symptoms are marked lack of activity and uncoordinated motor response. A mouse which has just died of anaphylaxis has collapsed and unobstructed lungs, a heart that is still beating and an uncongested liver. All the rest of the abdominal organs appear normal, except for a generalized injected and oedematous appearance of

the intestines and stomach. Consequently, death of the mouse is not due to obstruction of the lungs or liver or to cardiac failure. The mouse undergoing anaphylaxis does show signs of respiratory distress and anoxia. Within 1 min after antigen injection an intense spasm of the arterioles and venules occurs followed within 5-20 min by a marked relaxation of these vessels and a fall in blood pressure. All the vessels become dilated with slow-moving blood<sup>4</sup>. In the experiments to be reported, mice undergoing anaphylaxis appeared to experience an actual loss in blood volume, and replacement of the blood volume protected them from anaphylactic death.

Experiments were conducted to explain the obvious respiratory distress always observed in mouse anaphylaxis. It is known that the capillaries become strikingly dilated and that circulation is retarded<sup>4</sup>. During anaphylaxis mice are extremely difficult to bleed; little blood can be obtained by cutting the tail veins and arteries or by puncturing the infra-orbital sinus. If the heart of a mouse which has just died of anaphylactic shock is cut open, little bleeding occurs. These observations suggested that a marked loss of effective blood volume has occurred or that dilation of vessels has produced circulatory collapse.

The following experiments were performed to determine the cause of this circulatory failure. Mice were immunized with 0.5 mg of egg albumen in incomplete Freund's adjuvant, and 21 days later the hematocrit values were

Table 1. HEMATOCRIT VALUE CHANGES DURING ACTIVELY INDUCED ANAPHYLAXIS

Mouse No.	Sensitized mice*		Normal mice*	
	Pre-challenge	Post-challenge	Pre-challenge	Post-challenge
1	49	72	51	50
2	45	60	44	45
3	52	70	49	47
4	45	65	46	46
5	52	74	49	46
6	50	73	49	49
7	47	64	48	46
8	48	65	46	46
9	51	67	51	50
10	47	69	51	49
Average	48.6	67.9	48.4	47.4

\* All sensitized mice eventually died of shock, while all the normal controls survived without symptoms of anaphylaxis.



determined for them and for normal controls before and after an intravenous challenge with 0.5 mg egg albumen. The post-challenge sample of blood in sensitized mice was taken when strong symptoms of anaphylaxis had developed. Control mice were bled 5 min after challenge. The results given in Table 1 indicate that, in all cases where anaphylaxis occurred, the haematocrit value was increased markedly. From these values, it could be calculated that 20–30 per cent of the blood volume had disappeared from the circulation within 5 min after challenge. No such effect was observed in the normal control animals. These observations indicated that fatal anaphylaxis might be the result of insufficient oxygenation of the tissues due to severe loss of blood volume and to dilatation of the capillary bed. The respiratory distress, oedema and cyanosis observed are thus explained. If this is indeed the main cause of death, it should be possible to save these mice by merely replacing the blood volume.

Table 2. PROTECTIVE EFFECT OF BLOOD VOLUME REPLACEMENT IN MOUSE ANAPHYLAXIS\*

Fluid used	No. of injections	Vol. per injection (ml.)	Results survivors/total
6 per cent dextran	2 to 3	0.3 to 0.5	9/9
Physiological saline	2 to 3	0.3 to 0.5	9/10
Untreated controls	—	—	2/10

\* Sensitized mice were challenged intravenously and as soon as strong symptoms of anaphylaxis appeared treatment was initiated. An intravenous injection of 0.5 ml. plasma volume extender was first given. The animals were observed and, if severe shock was again obvious, another intravenous injection of 0.3–0.5 ml. was given. In a few cases, a third injection was needed. Treatments were stopped 1 h after the shocking dose.

Mice sensitized as in the previous experiment were similarly challenged, and, as soon as anaphylactic symptoms appeared, intravenous treatment with either 6 per cent dextran solution or with plain physiological saline was begun. Complete and dramatic protection of mice resulted with as few as 1–3 injections of 0.3–0.5 ml. of either dextran solution or physiological saline (Table 2). In many animals anaphylactic shock had progressed so far as to seem irreversible. At this point, injection of 0.5 ml. of 6 per cent dextran solution or physiological saline restored life to many of these mice. The only animals which were not protected were those in which the veins of the tail were extremely difficult to inject and replacement therapy was impossible. Mice received no further treatment 1 h after challenge. Deaths seldom occurred after this time, and the following day these mice seemed normal. Similar experiments in which the mice were passively sensitized gave comparable results.

These findings indicate that the mouse undergoing anaphylactic shock dies of circulatory failure due mainly to loss of effective blood volume. This effective loss of volume must be due not only to dilation of blood vessels but also to an increased permeability of the capillaries which allows fluids to escape. This would explain the oedema and haemoconcentration observed. The anoxic condition may be due to lack of circulation of blood and insufficient oxygen transport to the tissues, which in turn would explain the cyanosis observed. The oedema noticed in the peritoneal cavity is probably due to increased permeability of vessel walls. Other symptoms associated with smooth muscle contraction (ruffled hair, defaecation and urination) are also observed and are most likely due to release of substances, such as serotonin or histamine, from the anaphylactically shocked tissues.

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<sup>1</sup> Kabat, E. A., *Experimental Immunochimistry*, second ed. (Charles C. Thomas, Springfield, Illinois, 1961).

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## A Cytoplasmic Organelle associated with the Cell Walls of *Chara* and *Nitella*

MATURE internodal cells of the algae *Chara* and *Nitella* are several centimetres long, and develop from meristematic cells about 20  $\mu$  across. In an investigation of the fine structure of developing internodal cells in *Chara* and *Nitella*, an unusual organelle has been seen in the cytoplasm. In young internodal cells the organelle had a superficial resemblance to a mitochondrion, but a mitochondrion is always surrounded by a double membrane, whereas the organelle had a single outer membrane about 75  $\text{\AA}$  thick which resembled the 'unit' membrane of Robertson<sup>1</sup>. In immature cells the organelle membrane appeared to be discontinuous on its cell-wall side, leaving small gaps through which there was contact between the inner structure of the organelle and the cell wall (Fig. 1). In developing cells the organelles were elongated in the direction of cell growth (Figs. 2 and 3) and the membrane surrounding the organelle appeared to be continuous with the cell membrane, of which it may be an extension.

The organelle contained a system of interconnected tubules with diameters varying from 200  $\text{\AA}$  at their narrowest to 500  $\text{\AA}$  at their widest parts. The walls of the tubules were single membranes like those of the cristae in the mitochondria, but a granular material within the tubules made them appear dense, whereas the mito-

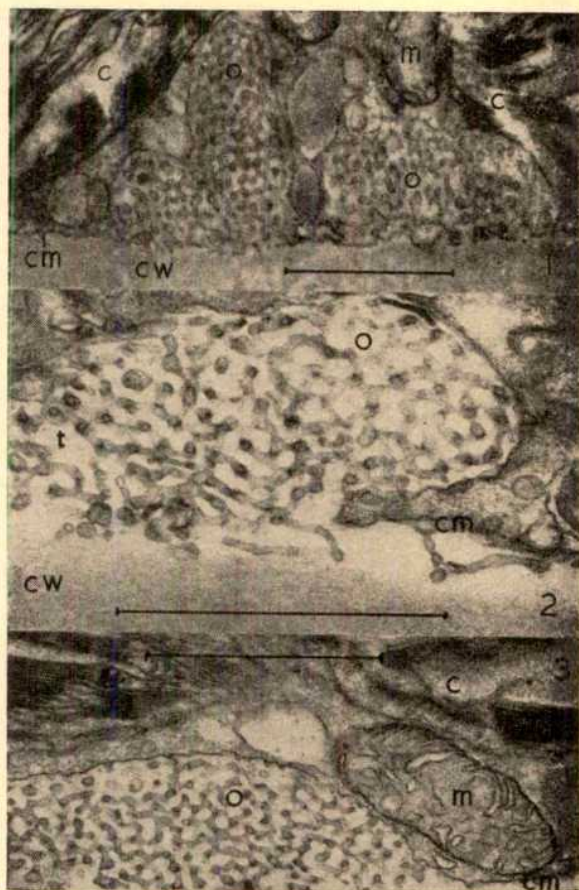


Fig. 1. Part of a young internodal cell in *Chara*. There is close contact between the organelles and the cell wall through gaps in the cell membrane. The single membrane of the organelle passes near the double membranes of chloroplasts. ( $\times 21,000$ )

Fig. 2. This organelle is elongated in the direction of the long axis of a developing internodal cell. The membrane of the organelle is continuous with the cell membrane, and the tubules within the organelle are in very close contact with the cell wall. ( $\times 42,000$ )

Fig. 3. At a later stage of cell development the membrane of the organelle appears to be part of the cell membrane. The structure of the tubules can be compared with that of the cristae within the mitochondrion. ( $\times 30,000$ ). o, organelle; t, tubule; m, mitochondrion; c, chloroplast; cm, cell membrane; cw, cell wall. Scale represents one micron



chondrial cristae appeared to be empty (Fig. 3). When differentiating cells were plasmolysed during preparation for the electron microscope, some of the tubules remained attached to the cell wall. The organelles were not seen in the segment cell or in mature internodal cells, and Green<sup>2</sup> has shown that the cell walls are isotropic at both these stages of development. The organelles were seen in developing internodal cells, which have birefringent walls<sup>2</sup>.

The close association between the cell wall and this organelle suggests that it may be involved in the organization of cell wall structure or the synthesis of cell wall material. Frei and Preston<sup>3</sup> have shown that microfibrils in the cell walls of a number of species of algae are arranged in a regular pattern, and that the angles between them are similar. Preston<sup>4</sup> proposed a structure capable of synthesizing cell wall material, which consisted of three layers of spheres, each about 500 Å in diameter and arranged in cubic close packing: such a structure could synthesize microfibrils at the angles which he observed. The organelle described here does not have the structure proposed by Preston, but Green<sup>2</sup> has shown that the microfibrils in the walls of internodal cells in *Nitella* are arranged transversely.

Ledbetter and Porter<sup>5</sup> fixed root tips of higher plants in glutaraldehyde and found tubules in the cytoplasm which, they suggested, were associated with cell wall structure. The organelles observed in *Chara* and *Nitella* were not so well preserved by fixation in osmium tetroxide or potassium permanganate as they were by fixation in glutaraldehyde. Glauert and Hopwood<sup>6</sup> saw membranous structures near the growing cross walls of bacteria, which they also interpreted as organelles responsible for cell wall synthesis.

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<sup>1</sup> Robertson, J. D., *Biochem. Soc. Symp.*, **16**, 3 (1959).

<sup>2</sup> Green, P. B., *J. Biophys. Biochem. Cytol.*, **4**, 505 (1958).

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<sup>5</sup> Ledbetter, M. C., and Porter, K. R., *J. Cell Biol.*, **19**, 239 (1963).

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### An Unusual Organelle in the Peripheral Cytoplasm of *Chara* Cells

DURING the course of an investigation of the fine structure of the development of laterals of limited growth of *Chara*, unusual structures were observed at the surface of some of the cells (Fig. 1). They consisted of an invagination of the plasmalemma, approximately ovoid in shape, containing a knot of anastomosing tubules. Each tubule, when cut transversely, showed a circular outline, 200–500 Å in diameter, bounded by a single unit membrane the same thickness as the plasmalemma. The organelles were present in the cylindrical cells of well-developed laterals, being confined to the cytoplasm adjacent to the outer walls, where they lay next to the layer of chloroplasts found in this region. Mitochondria also occurred in this position but, while superficially resembling the organelles, they could be distinguished because of their double outer membrane. The organelles were not observed in meristematic cells of young lateral branches.

In some cases the tubules were observed to be extensions of the plasmalemma. The organelles described here could therefore be interpreted as localized modifications of the surface membrane, the increased surface area probably being associated with some specific enzymatic activity.

Since the organelles are situated in contact with the developing cell wall it seems probable that they are sites of synthesis and/or organization of wall material. Frei

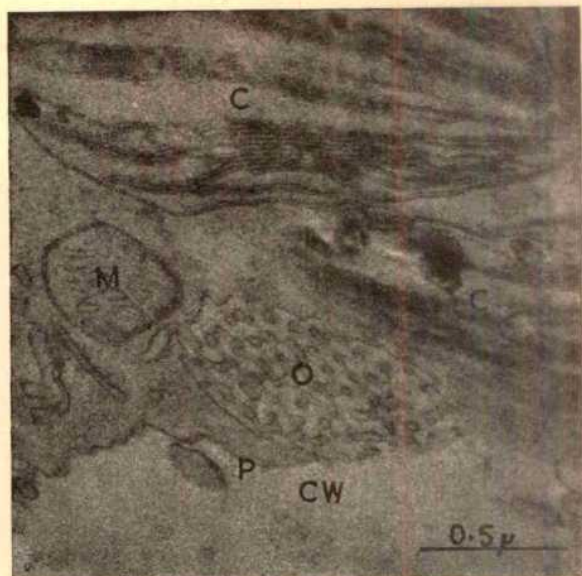


Fig. 1. Part of a section through the edge of a cell from a developing lateral branch of *Chara*. Fixed in glutaraldehyde and potassium permanganate. O, organelle containing anastomosing tubules; C, chloroplast; CW, cell wall; M, mitochondrion; P, plasmalemma.

and Preston<sup>1</sup> put forward the hypothesis that synthesis and organization of cellulose fibrils are associated with granular regions observed on the interior surfaces of the walls of *Cladophora* and *Chaetomorpha*, and Preston<sup>2</sup> postulated a three-dimensional model at or near the cell surface to account for this synthesis. On the other hand, these organelles might be involved in the formation of other extra-cellular materials such as non-cellulosic wall substances.

Although it is unlikely that the invagination of the plasmalemma reported here is some stage in pinocytotic activity, the increase in surface area of the outer membrane in these organelles might be related to the uptake of substances into the cells.

I thank Prof. P. F. Wareing for his advice and P. Henley for assistance.

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<sup>1</sup> Frei, E., and Preston, R. D., *Proc. Roy. Soc. B*, **154**, 70 (1961).

<sup>2</sup> Preston, R. D., in *The Formation of Wood in Forest Trees*, edit. by Zimmermann, M. H., 169 (Academic Press, London, 1964).

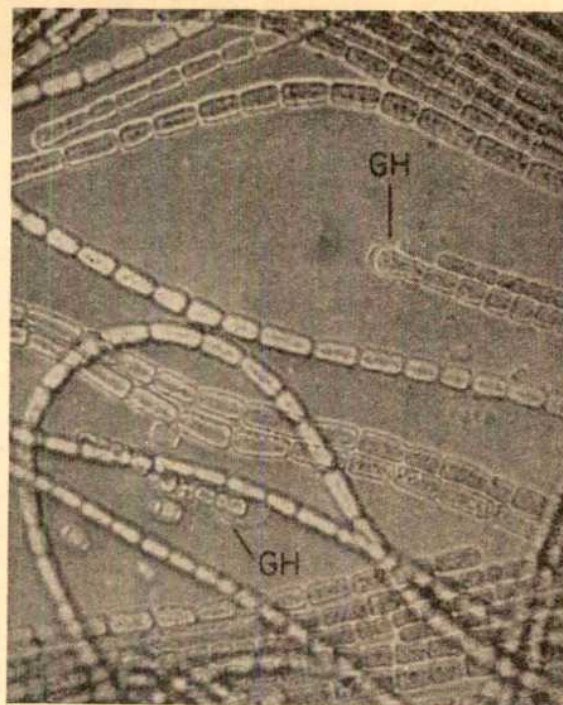
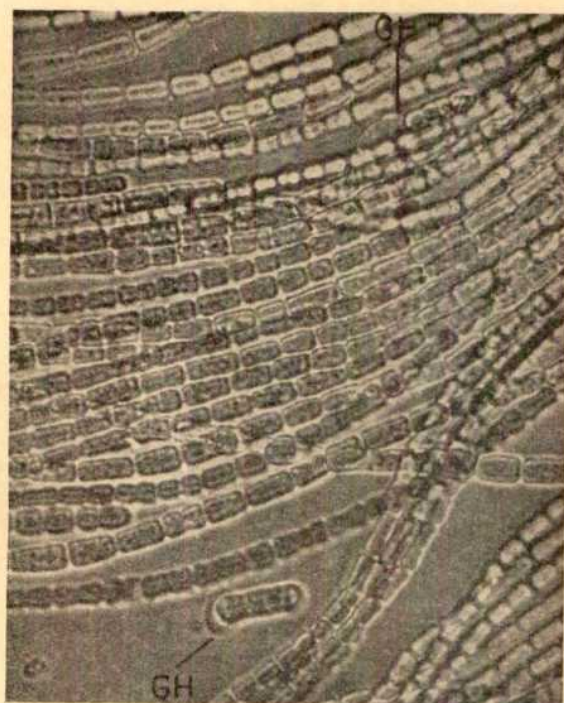
### Heterocyst Germination under Defined Conditions

ONLY on rare occasions have heterocysts of blue-green algae been seen to germinate. The observations of a number of investigators have supported various suggestions as to non-reproductive functions of these cells. However, no definitive elucidation of their function has yet been presented<sup>1</sup>. Indicative of the apparent infrequency of heterocyst germination under natural conditions is Fritsch's<sup>2</sup> comment that in a six-month survey of algal material from all over the world, he had observed no single case of a germinating heterocyst. If conditions could be established which would invariably result in germination of these cells, these conditions could then suggest natural environments in which heterocyst germination might be successfully sought.

A clonal, axenic culture of a blue-green alga identified as *Anabaena cylindrica* Lemm. was derived from culture B381 *Anabaena* sp. of the Indiana University algal culture collection<sup>3</sup>.

In accordance with the results of Fogg<sup>4</sup> and Pandey and Mitra<sup>5</sup>, heterocyst formation in this alga was found





Figs. 1a and b. Pairs of germinating heterocysts (GH) from a Petri dish culture

to be inhibited by ammonium ions. This finding led to a test of the ability of ammonium to induce heterocysts to germinate.

Medium C of Kratz and Myers<sup>6</sup> was modified by the substitution of  $\text{NH}_4\text{Cl}$ , at 4 mmoles/l., for  $\text{KNO}_3$ , and by the replacement of  $\text{Ca}(\text{NO}_3)_2$  by  $\text{CaCl}_2$ . Glucose was added at a concentration of 50 mmoles/l. To prevent germination products from floating apart, the medium was solidified with 0.92 per cent agar. Use of highly purified agar<sup>7</sup> was essential. Cooled but liquid double-concentration agar, autoclaved in 'Pyrex'-redistilled water, was combined with an equal volume of a filter-sterilized double-concentration solution of the remaining constituents of the medium. Two-ml. aliquots of medium were pipetted into disposable sterile Petri dishes (35 mm  $\times$  10 mm) (Falcon Plastics), and let set for at least several hours. Algal inocula were grown on a variant of medium C of Kratz and Myers<sup>6</sup> as described elsewhere<sup>8</sup>. A very small amount of inoculum-suspension was streaked on the agar. The cultures were exposed to continuous illumination from General Electric cool-white fluorescent lamps. Light intensity, as measured with a Photovolt Corporation model 200A photometer, was 175 ft.-candles. Temperature was maintained at 23.5° C. The cultures were examined 9–13 days later. Inhibition of heterocyst formation ceased soon after the thirteenth day. Assays of heterocyst germination were made at 400 times magnification using a 40 times magnification long working distance objective lens (Cooke—A.E.I.). Cover slips were never used, so that the observations themselves did not result in mechanical opening of heterocysts.

Consistently, from 3 to c. 10 per cent of the identifiable heterocysts observed were found to be germinating or to have germinated. That is, the thick heterocyst coats had broken open, and in the great majority of cases a multicellular germling was observed extending through the opening in the coat. Figs. 1a and b show pairs of germinating heterocysts encountered in a representative culture. In the absence of glucose and  $\text{NH}_4\text{Cl}$ , germination was rarely observed.

It cannot be stated that germination is a common fate of heterocysts until evidence to this effect is found

in natural environments. There are several possible ways of explaining the fact that although very infrequently observed in Nature, heterocyst germination may be obtained under rather simple conditions in the laboratory. The identification of a germinated heterocyst as such can be difficult, particularly if germination has occurred in a liquid medium, where germling and heterocyst coat can become easily separated. According to Brand<sup>9</sup>, a discarded heterocyst coat soon becomes unrecognizable. Desikachary<sup>10</sup> states that discarded coats eventually dissolve. Geitler<sup>11</sup> has pointed out the ease of overlooking germination stages. Sites rich in ammonia might well be the best in which to start a search for evidence of abundant heterocyst germination under natural conditions.

It has been found<sup>3</sup> that phosphate-starvation stimulates sporulation of *A. cylindrica*, and that spores which develop under such conditions germinate on a phosphate-containing agar medium. Low-nitrogen media, on the other hand, stimulate heterocyst-formation, and an ammonium-containing medium leads to heterocyst germination. These results suggest that the spores of *A. cylindrica* may have become specialized for surviving periods of phosphate deficiency, and the heterocysts for surviving periods of nitrogen deficiency. Different germination requirements for two resting-cell types of the same organism should be useful for investigations of the comparative physiology of germination.

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<sup>1</sup> Geitler, L. v., "Schizophyzeen", 2 Aufl. *Handbuch der Pflanzenanatomie*, 6, Teil 1, 79 (Berlin, Borntraeger, 1960).

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<sup>3</sup> Wolk, C. P., Ph.D. thesis, The Rockefeller Institute, New York (1964).

<sup>4</sup> Fogg, G. E., *New Phytol.*, **43**, 164 (1944).

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<sup>6</sup> Kratz, W. A., and Myers, J., *Amer. J. Bot.*, **42**, 282 (1955).

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<sup>8</sup> Brand, F., *Ber. deutsch. bot. Ges.*, **19**, 152 (1901).

<sup>9</sup> Desikachary, T. V., *J. Indian Bot. Soc.*, **25**, 11 (1946).

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### Isolation of Salivation Factor from *Rhizoctonia leguminicola* on Red Clover Hay

IN 1959 O'Dell, Regan and Beach<sup>1</sup> in Missouri reported a new widespread toxicity problem associated with second-cutting red clover hay. Cattle and sheep which ate such hay slobbered excessively, went off feed, developed diarrhoea, bloat, stiff joints, and sometimes died. The toxic principle was organic in nature, slowly lost activity in solution and was soluble in water, ethyl alcohol and chloroform. Byers and Broquist<sup>2,3</sup>, investigating similar cases in Illinois, suggested that an alkaloid was involved, but did not identify the causal agent. Watson<sup>4</sup> and Guss<sup>5</sup> observed similar problems in dairy cattle consuming red clover hay.

Smalley *et al.*<sup>6</sup> and Crump *et al.*<sup>7</sup> investigated a similar disorder in ruminants in Wisconsin. They observed that samples of red clover forage associated with the excessive salivation in dairy cattle were infested with a dark-brown coloured fungus identified as *Rhizoctonia leguminicola* Gough and Elliott, the cause of the black patch disease of red clover. These authors concluded that the 'salivation factor' was contained in the mycelium of *R. leguminicola* and was not a product of red clover *per se*.

The objectives of the work recorded here were to purify the salivation factor, elucidate its chemical structure, and determine the effects of the purified toxin on animals.

*R. leguminicola* isolated from second-cutting red clover hay from Galesville, Wisconsin, was grown as still cultures on a medium containing 20 g soybean meal, 20 g dextrose, 5 g CaCO<sub>3</sub>, and 5 g corn steep liquor per litre distilled water. This medium<sup>8</sup> yielded consistent and reproducible production of the active principle. Optimum age for growth of the cultures was 30 days.

For bioassay of the salivation factor, guinea-pigs (200–300 g) were anaesthetized with medicinal grade ether, then stomach-fed 10 ml. of the test solution and placed in cages for observation. Every 15 or 20 min for the next 5–6 h each animal was rated for the degree of response to the feeding trial. Numerical ratings were assigned as follows: 1 = no response; 2 = slight salivation; 3 = extensive salivation, lacrimation and defaecation; 4 = severe depression, salivation, lacrimation, defaecation and dyspnoea; 5 = death. This rating provided a semiquantitative measure of the activity of the preparation tested.

For a typical purification run, about 100 cultures from 500-ml. Erlenmeyer flasks each containing 150 ml. of medium were used. The mycelial mats were removed, air dried (about 1 g dry wt./mat), ground to a fine powder in a Wiley mill, stirred with water (2 l./100 g dry wt.) at pH 6 for 2 h at 70° C and filtered. The filtrate was adjusted to pH 1 with hydrochloric acid and extracted three times with an equal volume of ether each time. The aqueous layer was then adjusted to pH about 10.5 with solid sodium carbonate and again extracted three times with an equal volume of chloroform each time. The active chloroform extract was evaporated to dryness, the residue redissolved in about 15–20 ml. of dilute hydrochloric acid and re-extracted three times with 15–20 ml. of ether. The aqueous fraction (about 20 ml.) was mixed with an equal volume of aqueous ammonium reineckate solution (saturated at 0° C), the mixture kept for 1 h at 0°, and the precipitate collected on a sintered glass filter and redissolved by washing with acetone through the filter. An equal volume of water was added to the acetone solution (15–20 ml.), and the acetone removed under vacuum. The aqueous solution was then made basic (pH 10) with sodium carbonate, extracted three times with an equal volume of chloroform each time, and the chloroform evaporated. The crude bases so obtained weighed about 80 mg.

This material was dissolved in 15 ml. of chloroform and chromatographed on 50 g of silicic acid (325 mesh) contained in a column (2 cm internal diam.). Successive passage through the column of 200 ml. chloroform, 200 ml. methanol and 200 ml. of 50 per cent aqueous methanol failed to elute the active material (as tested by spotting on filter paper and spraying with iodoplatinate reagent). The activity was then eluted with 0.01 N hydrochloric acid, the eluate (100 ml.) made basic with sodium carbonate (pH 10) and extracted with chloroform as before. Evaporation of the chloroform extract left a 20 mg residue which gave a rating of 3 when tested at a level of 1.4 mg/kg body wt.

The foregoing preparation was dissolved in 5 ml. of chloroform and the solution introduced into a chromatographic column which had been prepared by slurring 20 g of 'Florisil' in chloroform and pouring into a 1.5 cm internal diameter tube. Elution with 50 ml. of chloroform followed by 50 ml. of 25 per cent methanol in chloroform removed no active material. The iodoplatinate-positive material did not appear in the eluate until the column was developed with pure methanol. Evaporation of the active fractions left a residue weighing 4 mg. This was dissolved in 2 ml. water containing a trace of hydrochloric acid, and a saturated aqueous solution of picric acid was added drop by drop until no more precipitate formed. The product was collected and crystallized from 20 per cent ethanol. One recrystallization from the same solvent gave 2.8 mg of yellow needles, m.p. 178°–181°. The picrate was decomposed with excess hydrochloric acid, picric acid removed by extraction with ether, and the aqueous solution tested on guinea-pigs after neutralization. Ratings of 2 and 3 were obtained with doses corresponding to 0.17 and 0.29 mg of the picrate/kg body-wt., respectively.

The most highly purified material gave positive alkaloid tests (precipitates) with phosphotungstic acid, potassium bismuth iodide, and potassium mercuric iodide. At various stages of the purification the concentrates were examined by chromatography on Whatman No. 1 paper with the solvent system butanol:acetic acid:water (120:30:50 by vol.). The ascending technique was used. Under these conditions the most highly purified material gave only one spot at  $R_F$  0.35. The material gave an orange colour with Dragendorff's reagent, a grey colour with iodoplatinate and was negative to Ehrlich's reagent. With some of the less-highly purified fractions, faint ninhydrin positive spots were noted at  $R_F$  0.35 and 0.1.

The purified alkaloid obtained from the recrystallized picrate exhibited a weak band at 264 m $\mu$  and end absorption below 240 m $\mu$  when examined in aqueous solution containing excess hydrochloric acid.

The general chemical behaviour of the *R. leguminicola* toxin leaves little doubt that the substance is an alkaloid. Its physiological properties as an irritant are somewhat similar to those of physostigmine. However, physostigmine is distinctly different from the salivation factor chemically and at similar toxic doses on guinea-pigs caused severe griping and defaecation with only slight salivation. No signs of pulmonary involvement, lacrimation and prolonged salivation were apparent as seen with the salivation factor.

Cattle, swine, sheep, chickens, guinea-pigs, rats and mice have been investigated with respect to their response to the salivation factor. All were sensitive to the material, but ruminants seemed to be somewhat less reactive than other animals. Efforts to characterize the toxin are in progress.

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### Isolation of a Parasympathomimetic Alkaloid of Fungal Origin

SINCE 1947, reports have reached us of excessive salivation in ruminants after consuming certain forages. Frequently these forages were red clover hay, but other types of forages were involved. The cattle would consume from 1 to 3 feedings of such forage, salivate excessively, and then refuse further feed. The refusal of the cattle to consume 'slobber forage' represents an economic loss to the farmer, including a loss of milk production and the necessity of purchasing replacement forage.

Byers and Broquist<sup>1</sup> reported that slobbering in cattle and guinea-pigs could be also induced following the administration of a hot water extract of 'slobber forage'; certain properties of such extracts suggested that the salivation factor might be alkaloid in nature<sup>1,2</sup>. Smalley *et al.*<sup>3</sup> observed that all samples of 'slobber forage' were infested with a black fungus. They isolated the fungus in pure culture and identified it as *Rhizoctonia leguminicola*; guinea-pigs force-fed the mycelia salivated profusely. This report concerns the isolation of an alkaloid from this fungus having salivation factor activity, which thus appears to account for the early observations from this laboratory on 'slobber forages'.

*Rhizoctonia leguminicola* was obtained from Dr. J. W. Gerdemann of this University, who had isolated a pure culture of this fungus from red clover. The culture was maintained on potato-dextrose-agar slants and produced the salivation factor maximally when grown aerobically on a cold-water extract of second-cutting red clover hay. Medium was prepared by infusing 4 kg chopped hay with 20 l. cold water for 4 h and straining through cheesecloth. The medium was dispensed in 300-ml. quantities in 1-l. Roux bottles and autoclaved for 30 min at 120° C. The flasks were then inoculated from agar slants by the aseptic addition of *R. leguminicola* mycelium to the side of the glass bottles at the surface of the medium. The mould was grown in stationary culture at room temperature until the mycelial pad had covered the surface of the medium (generally about three weeks). Salivation factor activity was assayed qualitatively by injecting intraperitoneally guinea-pigs weighing 200–300 g with appropriate test materials and observing the degree of salivation at 15-min intervals. The degree of salivation was graded from 0 to 3; only sub-lethal doses were graded.

Approximately 2 kg of mycelia (wet wt.) was homogenized in a Waring blender with 95 per cent ethanol. The homogenate was filtered through Soxhlet extraction thimbles. The thimbles containing the homogenized mycelia were then placed in Soxhlet extractors and

extracted with the ethanolic filtrate for 48 h. The extract was then concentrated *in vacuo* to remove the ethanol and water added to a volume of 1 l. This solution, made slightly acidic (hydrochloric acid), was repeatedly extracted with CHCl<sub>3</sub> until fresh CHCl<sub>3</sub> extracts were colourless. The organic layer was discarded and the aqueous layer was then brought to pH 10 (powdered Na<sub>2</sub>CO<sub>3</sub>) and extracted with three volumes of CHCl<sub>3</sub>. The CHCl<sub>3</sub> fraction was retained, concentrated *in vacuo* to dryness, and the residue taken up in 100 ml. 0.01 N hydrochloric acid. The process of extracting the aqueous layer with CHCl<sub>3</sub> under acidic and then alkaline conditions was repeated. Following removal of the CHCl<sub>3</sub> from the latter step, the salivation factor concentrate was taken up in water and lyophilized, finally to yield 143 mg crude salivation factor which served as convenient source material for subsequent purification procedures.

When this material (50y) was chromatographed on paper in a butanol:acetic acid:water (4:1:1) system, one major and two minor components were found which stained pink following spraying with Dragendorff's reagent, a reagent diagnostic for alkaloids<sup>4</sup>. The major component ( $R_F = 0.25$ ) was accumulated by large-scale preparative paper chromatography and when eluted from the chromatogram elicited salivation factor activity.

Crude salivation factor was dissolved in dry CHCl<sub>3</sub> and hydrochloric acid gas passed through the solution. On standing overnight a reddish, semi-solid oil formed. Attempts were made to crystallize this material, thought to be salivation factor hydrochloride, from propanol-ethylacetate mixtures, but only amorphous precipitates were obtained. Such material was further purified by dissolving in water and then adding stepwise one volume, and then a second volume of Mayer's reagent<sup>5</sup>. The buff-coloured precipitates that resulted were combined and contained the bulk of salivation factor activity. Crystalline material was obtained, however, when the residual mother liquor was allowed to stand for several days at room temperature. The crystals melted with decomposition at 230°–235° C.

5.5 mg of such crystals was suspended in water and hydrogen sulphide passed through the suspension until no further precipitation occurred. HgS was removed by centrifugation and excess hydrogen sulphide removed by aeration. An aliquot of this solution when chromatographed on paper in the solvent system previously described gave a single Dragendorff positive spot,  $R_F = 0.25$ . A second aliquot of this solution, equivalent to 0.5 mg original crystalline material, gave a rating of 3 in the salivation factor assay in guinea-pigs.

The parasympathomimetic action of salivation factor suggests that it may be an anticholinesterase or an acetylcholine-like substance. Its action in this respect is very much like those of physostigmine or pilocarpine, and is reversed by atropine. Salivation factor can be distinguished from these latter alkaloids, however, by paper chromatography and other chemical criteria. *In vitro* experiments have failed to show anticholinesterase activity. Experiments are being carried out to determine the chemical nature of the salivation factor together with its mode of action.

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## Factors affecting the Stimulation of Fungal Development in the Root Region

OVER recent years a justifiably large amount of attention has been directed to the role of root exudates in the stimulation of microbial development in the root region. There is, however, the possibility that the importance of exudates in this stimulation has been exaggerated and that some micro-organisms could be stimulated by the mere presence in the soil of a solid structure such as a root. With this possibility in mind, a comparison in colonization was made using live and dead roots and inert solid material (nylon thread) placed in the same soil.

(a) *Live roots.* Barley grains were planted in soil from the University of Liverpool Botanic Garden and grown for 10 days. Roots were taken from the young plants at 2, 5 and 10 days after germination of the grain, and were subjected to serial washing with sterile water (20 washings). Segments of washed root, 2 mm long, were then plated out on to Czapek-Dox + yeast extract agar (pH 5.0). The plates were kept at room temperature, and fungi developing from the plated root segments were isolated and identified.

(b) *Dead roots.* Fresh barley roots were killed with propylene oxide, and pieces 4 cm in length were buried in soil obtained from the Botanic Garden. Samples of the buried dead roots were taken 2, 5 and 10 days after burial, and fungi were isolated from washed root segments, as in the case of the live roots.

(c) *Nylon thread.* 4 cm pieces of thoroughly washed nylon thread (4 cm long and of approximately similar diameter to barley roots) were buried in the Botanic Garden soil, and sampled 2, 5 and 17 days after burial. Fungi were isolated from the pieces of thread as was done with the roots.

Table 1. PERCENTAGE FREQUENCY OF OCCURRENCE OF FUNGI ON LIVE AND DEAD ROOTS AND ON NYLON THREAD AFTER VARIOUS PERIODS IN SOIL

Time in soil (days)	Live roots			Dead roots			Nylon thread		
	2	5	10	2	5	10	2	5	17
<i>Fusarium oxysporum</i>	—	27	18	17	11	18	—	—	2
<i>Fusarium sambucinum</i>	—	5	7	7	11	14	—	—	—
<i>Fusarium culmorum</i>	—	—	—	1	—	—	—	—	—
<i>Fusarium solani</i>	—	1	—	1	—	—	—	—	—
<i>Cylindrocarpum radiculicola</i>	—	12	8	1	—	—	—	—	—
<i>Mortierella vinacea</i>	13	4	4	3	15	—	—	—	2
<i>Mortierella</i> spp.	7	—	7	4	10	14	—	—	—
<i>Gliocladium</i> spp.	—	5	5	1	4	—	—	—	—
<i>Trichoderma viride</i>	—	6	3	29	74	65	—	—	2
<i>Penicillium lilacinum</i>	—	1	12	—	—	—	—	—	—
<i>Penicillium</i> spp.	7	3	9	7	20	5	—	1	8
<i>Coniothyrium</i> sp.	6	3	5	3	—	2	—	—	—
<i>Phoma</i> spp.	—	2	2	—	—	—	—	—	—
<i>Trichocladium opacum</i>	—	5	1	1	1	—	—	—	—
Sclerotial forms	—	1	1	3	17.5	—	—	—	—
Sterile dark forms	20	13	8	3	—	—	—	—	—
Sterile hyaline forms	7	—	1	—	—	—	—	—	—
<i>Cladosporium herbarum</i>	—	—	—	—	—	—	—	—	1
<i>Botrytis cinerea</i>	—	—	—	—	—	—	—	—	1
<i>Stysanus medius</i>	—	—	—	—	—	—	—	—	1
<i>Verticillium lateritium</i>	—	—	—	—	—	—	—	—	1

The results of the isolation of fungi from the three types of buried substrate are presented in Table 1; they show that for live roots the typical pattern of colonization occurs with *Fusarium oxysporum*, *Cylindrocarpum radiculicola*, *Penicillium lilacinum* and sterile dark forms being prominent colonizers. For dead roots, colonization appears to occur much more quickly (80 per cent of the plated root material yielding fungi after 2 days as compared with 60 per cent of the live root material) and by a wider range of species (14 species, as compared with 6 from live roots after 2 days), of which typical 'root surface fungi' were well represented; however, as colonization proceeded *Trichoderma viride* assumed dominance over *Fusarium* spp. and *Mortierella* spp. Sterile dark fungi, important initial colonizers of live barley roots, never

Table 2. FUNGI FROM THE WASHING WATER USED IN THE SERIAL WASHING OF NYLON THREAD SAMPLED AFTER 17 DAYS BURIAL IN SOIL

	1	Washing		14
		2	10	
<i>Mortierella vinacea</i>	6	—	—	—
<i>Penicillium</i> spp.	4	1	1	1
<i>Penicillium lilacinum</i>	2	—	—	—
<i>Mortierella</i> spp.	3	—	—	—
<i>Fusarium culmorum</i>	1	—	—	—
<i>Fusarium oxysporum</i>	—	1	—	—
<i>Coniothyrium</i> sp.	1	1	—	—
<i>Humicola grisea</i>	1	—	—	—
<i>Stysanus medius</i>	2	—	—	—
<i>Cladosporium herbarum</i>	1	—	—	—
<i>Trichoderma viride</i>	—	1	—	—
<i>Phoma</i> sp.	—	2	—	—
<i>Acrostalagmus</i> sp.	—	1	—	—

played an important part in the colonization of dead roots and quickly died out. The results obtained by plating segments of washed nylon thread showed a very marked contrast to those obtained using live and dead roots (Table 2). Here the substrate was inert and any stimulation of the soil mycoflora was caused merely by the physical presence of the thread and not by the presence of any organic materials (that is, root exudates, sloughed-off root cells, materials diffusing from the dead root material). Throughout the period of burial, colonization was extremely sparse, and because of the paucity of fungal development the final sampling time was delayed until 17 days after burial. Even after 17 days the fungi isolated were species typical of the soil rather than of the root surface (*F. oxysporum* being the only root surface form isolated—at 2 per cent frequency).

As the nylon thread was very smooth it could have been that the paucity of fungal colonization recorded was due to the fact that most of the hyphae had been removed from the thread during the washing process. However, isolations from the water used in each of the serial washings of nylon thread, which had been buried in soil for 17 days, yielded only small amounts of the 'root surface' species (that is, *Fusarium* spp.); sterile mycelia, *Cylindrocarpum* spp., etc., were not isolated. These data do not support the suggestion that typical root surface fungi develop on the nylon thread but are removed in the washing procedure.

The results obtained from plated nylon thread indicate how marked is the stimulation of soil micro-organisms by live and dead roots, and that this stimulation (or removal of fungistatic factors) is mainly due to nutrient materials supplied to the soil—the effect of their physical presence being negligible (even after 17 days burial there was little evidence of stimulated fungal development on the nylon thread).

It is interesting to compare these results with those obtained by numerous workers applying the Rossi-Cholodny buried-slide technique. These slides often show the development on their surface of complex branching hyphal systems, the development of which is thought to take place because of the physical presence of the glass slide in the soil offsetting the effects of fungistasis. It may be that the small size of the nylon thread used here allowed rapid equilibration with the soil environment (for example, the temperature might quickly have adjusted to that of the soil so that less water condensed on the thread and hence the fungistatic activity of the soil was little affected). However, the nylon threads were of much the same dimensions as the barley roots used, and therefore the physical effects of the substrates examined may be compared.

The living and dead roots are examples of organic substrates which, when added to the soil, stimulate the micro-organisms in their immediate vicinity; living roots, however, support a different mycoflora from that of dead roots. Obviously factors, such as exudates, CO<sub>2</sub> concentrations, etc., intimately associated with living roots are



of prime importance in the development of fungi on root surfaces.

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## ENTOMOLOGY

### Persistence of *Erwinia amylovora* in the Apple Aphid (*Aphis pomi* DeGeer), a Probable Vector

INSECTS of the family Aphidae and particularly the apple aphid, *Aphis pomi* DeGeer, have long been suspect as significant vectors of the phyto-bacterial pathogen *Erwinia amylovora* (Burrill) Winslow *et al.*, 1920. That these insects could artificially transmit the fireblight bacterium and establish progressive infections in apple and pear tissue was demonstrated by Stewart<sup>1</sup>, Stewart and Leonard<sup>2</sup>, and Merrill<sup>3</sup>.

It was, however, not known how soon after feeding, or for how long, the pathogen could be detected within the insect. These questions remained unanswered because feeding the apple aphid *in vitro* was, until recently, an all but impossible task.

Recently in our laboratory, and independently by Mittler and Dadd<sup>4</sup> and Strong<sup>5</sup>, a system has been developed by which aphids can be successfully fed a synthetic substrate through an artificial membrane. Our simple glass feeding chamber is shown in Fig. 1 and is essentially two 3-in. pieces of glass tubing 4 mm in diameter, separated by a thinly stretched membrane of 'Parafilm-M' (Marathon Co., Menasha, Wisconsin). The Parafilm was secured with a rubber band and one of the glass tubes was drawn slightly smaller in diameter than the other so that the smaller one could be inserted into the larger.

The aphids were placed on one side of the membrane and a droplet of sap expressed from young shoots, containing a suspension of the fireblight bacterium, was placed on the other side. Active feeding by the aphid through the membrane is shown in Fig. 2. During the feeding period, the tubes were inverted so that the insect adopted approximately its normal feeding attitude on the under side of the apple or pear leaf. Furthermore, feeding in this manner prevented the dropping of ingested bacteria to the membrane surface in aphid excrement, and reduced the likelihood of contaminating the external surface of the insect.

To obviate the necessity for aseptic procedures, a virulent strain of *E. amylovora* was developed that was resistant to 1,000 µg/ml. streptomycin. The use of this genetically marked mutant permitted ready separation of *E. amylovora* from the endogenous bacterial flora of the aphid as well as atmospheric contaminants.

In replicate experiments, ten single aphids were fed the apple juice-bacteria mixture for intervals of active feeding ranging from 5 min to 2 h. In another experiment, three groups of insects fed for 2 h were placed on vigorously-growing apple shoots. The insects were subsequently removed from their natural host at intervals, and macerated in streptomycin-containing broth which was plated on streptomycin-containing agar.

The results from these experiments appear in Tables 1 and 2. They reveal that the pathogen could be found in

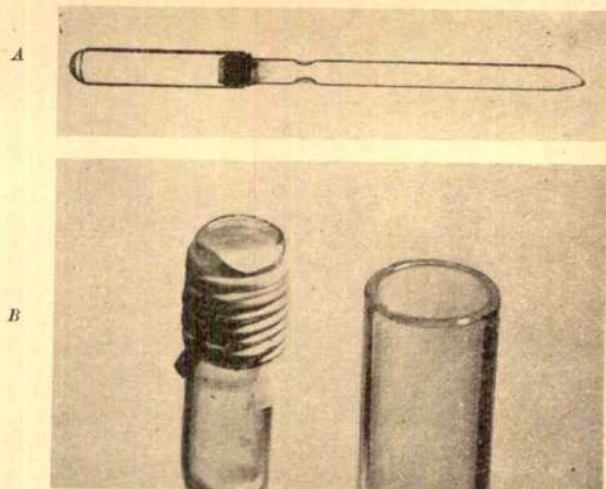


Fig. 1. Insect feeding chamber. A, assembled; B, unassembled

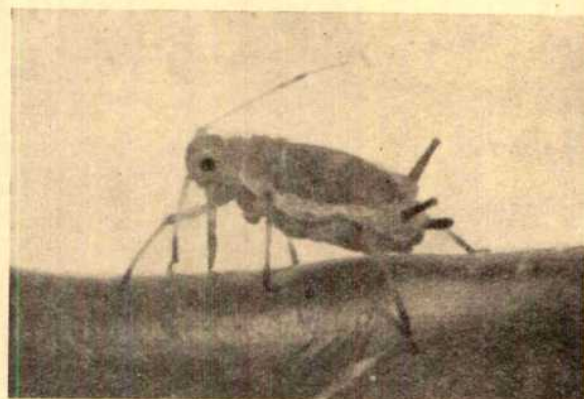


Fig. 2. Aphid actively feeding through a 'Parafilm-M' membrane

the bodies of the insects after as short a feeding period as 5 min. Moreover, the bacteria persisted in the aphid for at least 72 h, the duration of the longest experiment.

To determine whether or not contamination of the external surface of the membrane had occurred as a result of feeding, this surface, on which the aphids had rested, was swabbed with cotton and promptly streaked over the streptomycin-containing agar. In no instance was the pathogen recovered from the surface of the membrane. Hence, it would appear that our isolation of *E. amylovora* from *Aphis pomi* was, in fact, bacteria that had been ingested by the insect.

Experiments are now in progress to determine the distribution of the pathogen in the internal organs of the aphids and the approximate number of bacterial cells required to establish a progressive infection by *Aphis pomi* in host tissue.

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Table 1. TIME INTERVAL AFTER FEEDING WHEN BACTERIA COULD BE RECOVERED FROM THE INSECTS

Time (min)	5	15	30	60	120
No. of positives (out of 10)	2	5	3	4	6

Table 2. PERSISTENCE OF BACTERIA IN THE INSECT

Time (h)	24	48	72
No. of positives (out of 10)	4	4	2

<sup>1</sup> Stewart, V. B., *New York (Cornell) Agr. Exp. Sta. Bull.*, 329 (1913).

<sup>2</sup> Stewart, V. B., and Leonard, M. D., *Phytopath.*, 5, 273 (1915).

<sup>3</sup> Merrill, J. H., *J. Eco. Ent.*, 8, 402 (1915).

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### Pore Canals in the Cuticle of *Hypoderma bovis* (Diptera)

THE pore canals of the larval cuticle of *Hypoderma bovis* are extremely conspicuous. When the cuticle is treated with saturated potassium hydroxide solution followed by iodine and sulphuric acid<sup>1</sup>, they are seen to be occupied by helical strands of chitosan 2–3 $\mu$  thick, which give a much stronger reaction than the remainder of the cuticle. The helical nature of the canals is readily seen in a horizontal optical section. On changing the plane of focus the optical section of the canal clearly described a circular path. The diameter of the helix is about 5 $\mu$  and in vertical sections its pitch is seen to be about 6 $\mu$ . There is no relation between the pitch of the helix and the spacing of the laminae of the cuticle. Between 5 and 6 laminae are transversed by one complete turn of the helix (Fig. 1).

In electron microscope investigations on the pore canals of *Tenebrio*, *Calpodes*, *Galleria* and the honey bee, Locke<sup>2</sup> found a correspondence between the pitch of an apparent helix and the spacing of the laminae, and since the distribution of the canals in tangential section did not conform to the pattern expected for a random distribution of helical pore canals he concluded that the canals are not in fact helical, but pursue an undulating course from lamina to lamina. It is interesting, however, that the pattern seen by Locke in tangential sections resembles that seen by Drach<sup>3</sup> in similar sections of the cuticle of the crab *Carcinides* in which, as in *Hypoderma*, the canals appeared to be helical, rotating as the plane of horizontal section was changed.

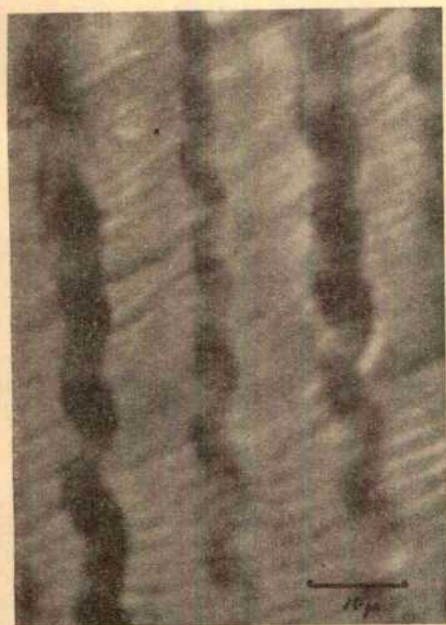


Fig. 1. The pore canals of *Hypoderma bovis* as seen after the chitosan reaction

These observations on the very large and readily observed canals of *Hypoderma* are clearly in agreement with the opinion that the arthropod pore canal is helical<sup>4</sup>, and contrast with the condition reported by Locke in the insects he examined.

I thank Prof. E. A. Vincent for the photograph of the pore canals and Mr. J. C. Wood for living *Hypoderma* larvae.

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### CYTOLOGY

#### Glutaric Acid Dialdehyde; a Routine Fixative for Central Nervous System Electron Microscopy

THE central nervous system of mammals presents special problems to the electron microscopist, because of its relative inaccessibility, and the fact that the brain is by no means a uniform organ but is composed of sub-units, about the structure or function of which we know little, and because of the extremely soft nature of fresh cerebral tissue. Although the most accessible area, the cerebral cortex, has been investigated extensively, it still remains to investigate the less superficial sub-units in detail. Attempts to dissect out such deeper structures from slices of soft, fresh brain, and then to cut these into smaller pieces for adequate fixation in osmium tetroxide, are often attended by disruption of structural organization resulting largely from mechanical manipulation—dark neurones are familiar to both light and electron microscopists.

The slow penetration of buffered osmium tetroxide during the first 2 h results in only superficial fixation when thin coronal slices of fresh brain are completely immersed in solutions of the fixative. The reduction of osmium tetroxide during fixation produces a diffuse blackening of these slices, so that all detail of deeper structures—the contrast between grey and white matter so important in gross anatomical localization—is obscured. Likewise, attempts to fix the central nervous system *in situ* by perfusing osmium tetroxide through the vascular system produce excellent tissue preservation in skilled hands<sup>1</sup>, but again, due to diffuse blackening of the fixed organ, specific anatomical localization cannot be readily performed.

Osmium tetroxide, though an excellent fixative, destroys enzymatic activity very quickly, and for this reason less-destructive fixatives such as formalin, acrolein and glutaric acid dialdehyde<sup>2–4</sup> have been introduced in an attempt to preserve fine tissue structure as well as enzymatic activity. We have found that glutaric acid dialdehyde (glutaraldehyde) used as a routine primary fixing agent for the central nervous system, followed by post-fixation in osmium tetroxide, gives very adequate fine structural preservation of whole coronal slices of the brain of the mouse and the Syrian hamster.

We have used a 6.5 per cent solution of glutaraldehyde buffered at pH 7.4 with either 0.1 M phosphate or 0.1 M cacodylate buffer, though the phosphate buffer (Sorensen) has been found to be the most stable. Excellent tissue preservation was obtained both by perfusion via the left ventricle and ascending aorta, and by immersion of whole coronal slices, 2–4 mm in thickness, in buffered glutaraldehyde. We prefer the immersion method, because the majority of small vessels are greatly distended by perfusion, making their structure and their relationship to the neighbouring parenchyma difficult to interpret.

Fixation was allowed to proceed for 1.5 h at 4° C, then the coronal slices, after preliminary washing in 0.2 M sucrose in either 0.1 M phosphate or 0.1 M cacodylate buffer at pH 7.4, were examined under a dissecting microscope. Glutaraldehyde caused a slight yellowing of all brain areas, but did not interfere with gross anatomical localization. Small blocks could then be removed at leisure with a sharp razor blade from any selected area, and shaped for later orientation when embedding. The selected blocks were then washed further in the sucrose phosphate or sucrose cacodylate buffer for 24–48 h before post-fixation for 1.5 h at 4° C in 1 per cent osmium tetroxide buffered in veronal acetate. We have used Vestopal 'W' as an embedding medium, but other plastics—'Araldite' and 'Epon', etc.—may also be used. However, as indicated by Sabatini *et al.*<sup>4</sup>, mixtures of methyl and butyl methacrylate were not suitable, as extensive sublimation

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## SOIL SCIENCE

## Dispersion of Mineral Colloids in Soils using Cation Exchange Resins

THE techniques for particle size distribution analysis of soils which have gained general acceptance are those which include a pre-treatment with an oxidizing agent such as hydrogen peroxide<sup>1</sup> or sodium hypobromite<sup>2</sup> to destroy, as completely as possible, the organic materials present. It is customary also, where hydrogen peroxide has been used, to add a peptizing or dispersing agent, usually in the form of a soluble salt such as sodium metaphosphate<sup>3</sup>, before the oxidized soil material is shaken in water suspension to obtain separation of the mineral particles. However, for a realistic investigation of the processes of dispersion and aggregation in soils, a method is required which results in complete separation of the mineral particles without the destruction or irreversible alteration of any organic materials present and without degradation of the mineral colloids<sup>4</sup>. These requirements preclude the use of bases or soluble salts, since those which are efficient dispersing agents invariably dissolve soil constituents, and the materials solubilized can be lost from the system along with the added chemicals if desalting is attempted. These stringent requirements led to the development of the method of dispersion described here.

Dispersion with cation exchange resins was accomplished by shaking 10 g of < 2 mm soil in 60 ml. of de-ionized water with an amount of any one of the three major types of synthetic cation exchangers (iminodiacetic, carboxylic or sulphonate acid resins, arranged in order of decreasing speed of dispersion) sufficient to supply 15 m.equiv. of a monovalent cation such as Na<sup>+</sup>, K<sup>+</sup> or NH<sub>4</sub><sup>+</sup>. Shaking was carried out in 100-ml. cylindrical, wide-mouthed, screw-topped bottles of pliable polyethylene to minimize mechanical abrasion, and an equipoise shaker, with a 'sponge' rubber-lined box, was used at a regulated voltage. The time required to obtain maximum dispersion varied, not only with the type of resin used, but also with the cation saturation, bead size, surface area and hardness of the various resins and also with the soil material being dispersed. Soils high in both montmorillonite clay and organic matter were most difficult to disperse, and, with these, a clay-percentage plateau was reached, using 16-50 mesh sodium-saturated resins, in 2 h with 'Dowex A-1' (iminodiacetic acid structure), 10 h with 'Amberlite IRC-50' (carboxylic acid structure) and 20 h with 'Amberlite IR-120' (a sulphonate acid resin). However, the shaking time required using 'Amberlite 200', a sulphonate acid exchanger with a mechanically stable macroreticular structure and relatively large surface area compared with the gel matrix of the 'Amberlite IR-120', was comparable with that necessary for maximum dispersion using 'Amberlite IRC-50' (10 h).

The foregoing dispersion technique was designed initially to serve as a pretreatment for particle size distribution analysis by the pipette method. For this purpose the soil-water-resin mixtures were transferred, following shaking, directly to 1-l. glass cylinders. This practice was adopted after establishing, for a wide range of soils, that removing the resin and sand in the process of making the suspensions up to volume for sedimentation gave results, for percentages of silt and clay, identical to those achieved with the coarse materials present. Following sedimentation and the removal of aliquots of silt plus clay and clay, the resin (normally in the 16-50 mesh size range) was separated from the finer sand fractions by sieving and from the coarser sands and gravel by flotation in a stream of water. The dissolved organic materials present in the silt plus clay and clay aliquots, and the small amounts of insoluble plant residues remaining with the sand fractions, were destroyed before weighing by treatment with 3 per cent hydrogen peroxide (5 ml. of 30 per cent added to 50 ml. of water or suspension).

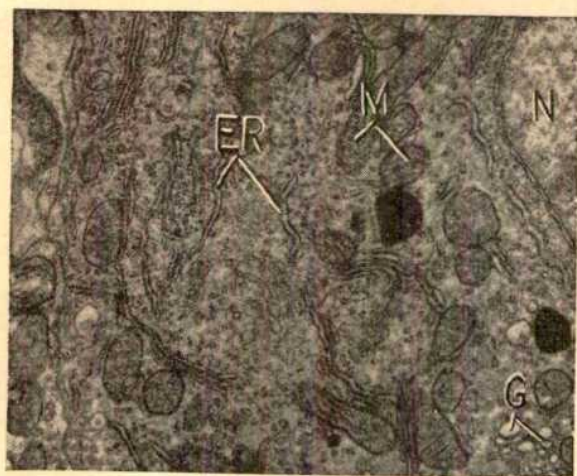


Fig. 1. Well-preserved Palade granules, granular (ER) and agranular (G) endoplasmic reticulum and mitochondria (M) occupy the cytoplasm of a Purkinje cell. ( $\times 15,000$ )

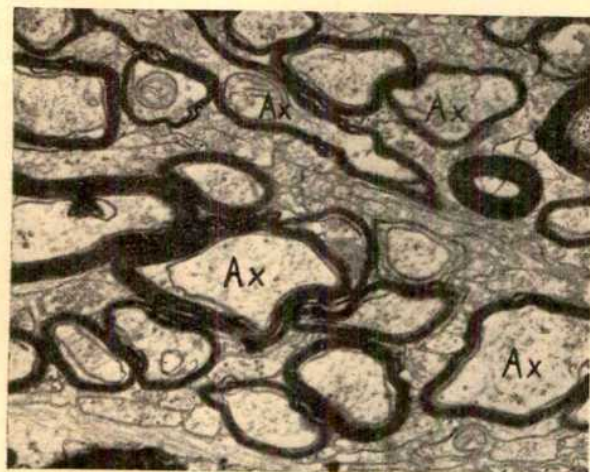


Fig. 2. All available space between myelinated axons (Ax) in the brain stem is occupied by closely apposed cell processes. ( $\times 15,000$ )

of the ultrathin sections occurred in the electron beam, grossly distorting the fine structure.

Neurons and glial cells from the cortex, hippocampus, thalamus, cerebellum (Fig. 1) and brain stem were uniformly preserved. The spaces between the apposed filaments of the granular endoplasmic reticulum were slightly wider than those observed with tissue fixed in osmium tetroxide alone, and they enclosed an amorphous material. There was neither undue mitochondrial swelling nor clumping of cell cytoplasm. The grey and white matter (Fig. 2) showed no large extracellular spaces either in the parenchyma or around capillaries, although appreciable spaces did appear between cell processes in the region of the area postrema.

These observations suggest that glutaraldehyde may be extremely useful, not only for the preservation of fine structure for enzymatic investigations, but as a routine fixative for the preservation of the central nervous system for both anatomical and pathological electron microscopic examination.

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Separation of the sand and resin was not required where 'Amberlite 200' was used, because of the mechanical stability of the resin and its resistance to oxidation in 3 per cent hydrogen peroxide.

In the absence of any absolute standard of dispersion, the cation exchange resin technique was compared, on a wide range of surface and sub-soil materials from the United States, Canada and Australia, with the results from the procedures found to yield the highest clay percentages in a recent investigation by Protz and St. Arnaud<sup>5</sup>. The most effective of these was a method based on the work of Troell<sup>2</sup> in which sodium hypobromite was used as an oxidant. The cation exchange resins (sodium saturated) gave clay percentages which compared favourably with those from the hypobromite procedure applied to the same soils. The reproducibility of size distribution analyses carried out on resin-dispersed suspensions has also been excellent. For example, the percentage of <2 $\mu$  material in a Nicollet soil from Iowa, based on mechanical analyses of 6 separate 10-g samples, was shown to be  $23.26 \pm 0.25$ .

The soils effectively dispersed using the cation exchange resin technique have been grassland and forest soils with high base status in which aggregation is due largely to the presence of a clay-organic matter complex. The maximum dispersion of mineral colloids in such soils has been achieved coincident with a high degree of dissolution of soluble organic materials. The dispersion procedure described here was therefore used as the initial step in a method for the extraction of humus, the next step being the removal of the sand and resin by sieving. The silt and part of the clay-sized mineral fraction were then removed, largely free of organic matter, by sedimentation in a 2-l. separatory funnel having a stopcock fitted with a large-bore 'Teflon' plug for bottom withdrawal of the sediment. The small volume of suspension removed with the sediment was collected in a plastic suction filter funnel fitted with a 'Millipore' or 'Polypore' filter paper with pores about 0.3 $\mu$  in diameter. To minimize re-formation of a clay-organic matter complex during filtration, a few grams of a finely divided (200-400 mesh) sodium-saturated cation resin such as 'Amberlite IRC-50' were added to the funnel prior to the application of suction. When sedimentation had essentially ceased, the filtrate was returned to the bulk of the extract. The resulting solution-suspension contained, in essentially unaltered form, most of the humified organic materials, along with finely divided and highly dispersed clay minerals. The latter could not be completely removed by centrifuging or filtering without loss of some of the organic materials of higher molecular weight, but the possibility of destroying the silicate minerals in the extract with a mild hydrofluoric acid treatment is being investigated.

The only modification of the dispersion procedure required to carry out investigations on the process of soil aggregation was a preliminary grinding of the soil samples to permit separation of the resin from the whole soil following dispersion. This separation was made by sieving, under suction, using small stainless steel screens in plastic mounts which were fitted into Buchner funnels with rubber rings. After washing several times with small volumes of de-ionized water, the suspensions were evaporated down at room temperature by using a stream of air from a hot-air drier. Stirring to obtain uniform distribution of the dissolved organic matter was necessary when the paste stage was reached. When dried to original moisture content, the soil samples were crushed to their original state (to pass a 2-mm sieve) and were then subjected to particle-size distribution analysis following partial dispersion of 10-g samples by shaking in 60 ml. of de-ionized water for 10 h. The results were compared with those obtained on samples of the original soils shaken in water in the same manner. The analyses showed that, even with the exchange complex partially saturated with monovalent ions, the soils dispersed and

then air-dried contained almost the same proportions of stable aggregates as were present originally. The effects of restoring different di- and poly-valent cations were also examined using suitably saturated cation exchange resins which were added and removed before the drying step.

The use of cation exchange resins as dispersing agents and extractants of soluble organic matter has shown clearly that the polyvalent metal ions known to complex organic materials in soils into insoluble forms<sup>6</sup> also are involved in formation of micro-aggregates which include clay minerals. Contact exchange of monovalent ions on the resins for some of these polyvalent ions in the clay-polyvalent metal ion-soil organic matter complex, achieved by shaking a mixture of soil and resin in water suspension, results in both dispersion of the clay fraction and dissolution of that portion of the humus which is potentially water-soluble.

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## GENETICS

### Serum Albumin and Transferrin Polymorphism in East African Cattle

A SURVEY of the serum proteins of several breeds of East African cattle by starch-gel electrophoresis has recently been carried out. During this survey, a new transferrin phenotype was seen and a previously undescribed variation in the serum albumin, presumably representing serum albumin polymorphism, was found.

The starch-gel procedure used was essentially that described by Ashton and Braden<sup>1</sup> for mice serum proteins and by Ashton and Ferguson<sup>2</sup> for sheep. In this system the electrolyte was composed of 2.2 g lithium hydroxide monohydrate (53-55 per cent LiOH) and 11.8 g boric acid per litre of solution. The gel was prepared from hydrolysed starch (Connaught Laboratories, Toronto, Canada) at the recommended strength, and the buffer consisted of 450 ml. of a solution containing 1.6 g *tris* (hydroxymethyl)-aminomethane, and 7.8 g citric acid per litre, to which was added 50 ml. of the electrolyte. The hot gel solution was poured into a tray containing six adjacent compartments, each 0.3 × 4 × 25 cm internally, and the electrolyte was connected to the gel by a thin plastic sponge (Wettex, Aust. Pty., Ltd., Sydney, Australia) which overlapped the gel by 4 cm at either end. The samples were inserted 8 cm from the cathode end of the gel, on very thin filter paper (Ekvip Industrial Equipment Pty., Ltd., Sydney, Australia). Either three or four samples per compartment were inserted, one a reference sample, and the gel was covered with polythene film to avoid evaporation during electrophoresis.

With this system, when an initial current of 100 mA per gel was passed, optimal resolution was obtained after about 2 h. The albumin zone, which should be narrow (2-3 mm), had then migrated about 9-10 cm, and the brown zone of discontinuity about 13-14 cm. This system gives excellent resolution of transferrin, post-albumin<sup>3</sup>, and albumin polymorphism<sup>4</sup>.

The serum samples examined were from Boran, Sahiwal, Nganda, Teso, Ankole and Tanganyika shorthorned Zebu cattle. Full details of the gene frequencies and other information will be reported elsewhere. From the total



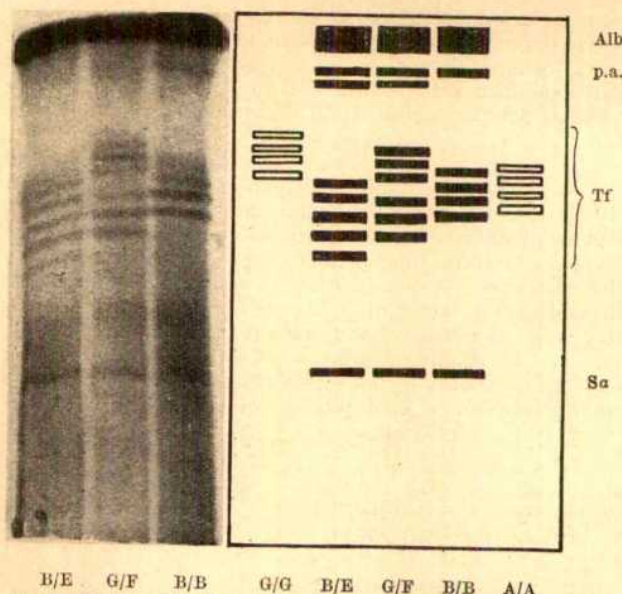


Fig. 1. Photograph and corresponding diagram of a gel carrying three serum samples, showing (left to right) transferrin types B/E, G/F and B/B. The diagram also shows the expected position of the postulated homozygote G/G, and the relative position of an A/A phenotype. Alb, albumin; p.a., post-albumins; Tf, transferrins; Sa, slow- $\alpha$  globulins.

of about 1,100 samples representing the different types of cattle, three Borans out of a group of approximately 300 from the East African Veterinary Research Organization. Genetics herd showed a previously undescribed transferrin phenotype (Fig. 1). The three animals showing this type were members of the same family, and it seems probable that they represent the heterozygote of  $Tf^{FF}$  (ref. 5) and a 'new' allele which, following the pattern previously adopted<sup>2,3</sup>, will be coded provisionally with the next available letter,  $Tf^G$ . The new phenotype is accordingly  $Tf^{G/F}$ . The heterozygote  $Tf^{G/G}$  will presumably show the usual four zones<sup>6</sup>, and the slowest of these will align with the second fastest zone of  $Tf^{A/A}$  (Fig. 1). The phenotypes of other heterozygotes of  $Tf^G$  may be forecast by the diagrammatic approach used for  $Tf^{B}$  and  $Tf^{F}$  in Zebu cattle<sup>5</sup>.

Serum albumin polymorphism is shown in Fig. 2. Although it can be detected in the described system in gels with undiluted serum samples inserted, it is revealed more readily when the serum is diluted to one part in six with water before electrophoresis. Five albumin phenotypes have been recognized, three of which appear to be

common and two rare. The three common types have been coded A, AB and B, and the two rarer types AC and BC (Fig. 2). The three common types occurred in all the breeds examined, but the two rarer types were found only in the Tanganyika shorthorned Zebu. However, only the latter and the Ankole samples were examined in detail for albumin polymorphism, and the presence of the AB and C phenotypes in the other breeds is not excluded.

The 74 Ankole sera which were examined in detail included 17 A, 29 AB and 28 B phenotypes, and among 83 Tanganyika shorthorned Zebu sera there were 16 A, 25 AB and 42 B types. In both breeds the distribution of phenotypes was compatible with Hardy-Weinberg distribution for a two-allele, three-genotype system. It seems probable that the albumin variants described reflect serum albumin polymorphism. It is therefore proposed that the locus symbol for this system is  $Alb$  and the two common alleles,  $Alb^A$  and  $Alb^B$ . The three common phenotypes found may then prove to be  $Alb^{A/A}$ ,  $Alb^{A/B}$ , and  $Alb^{B/B}$ . The two rarer phenotypes BC and AC may represent the products of  $Alb^A$  and  $Alb^B$ , with a third allele,  $Alb^C$ . So far, no mating data are available to test this hypothesis. European and other breeds of cattle are to be examined for this polymorphism.

McIndoe<sup>7</sup> has described serum albumin polymorphism in the domestic fowl. Two serum albumins have also been described in some of the members of a few human families. Serum albumin polymorphism may well be a widespread phenomenon.

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## VIROLOGY

### Behaviour of an Attenuated Strain of Foot-and-mouth Disease Virus in Culture

In previous articles<sup>1,2</sup> it was reported that differences were found between virulent and attenuated strains of foot-and-mouth disease virus in surviving cultures of calf tongue epithelium. The attenuated strains (a) gave rise to a lower number of infective centres after adsorption, (b) were less efficiently titrated in the epithelial cultures, (c) multiplied to a lower titre, but (d) produced more interferon, and (e) were more sensitive to interferon action than the virulent strains. From the findings it was suggested that at the most only 5 per cent of the attenuated strain was competent, that is, was able to initiate infection and the further production of virus. The other 95 per cent was thought to enter the cell, inducing cellular resistance and the production of interferon. As a result of successive multiplication cycles of competent virus there was a build-up of non-competent virus and of interferon, which, in turn, inhibited the growth of competent virus. Hence there was a failure of lesion development in animals but still sufficient antigen production to stimulate immunity.

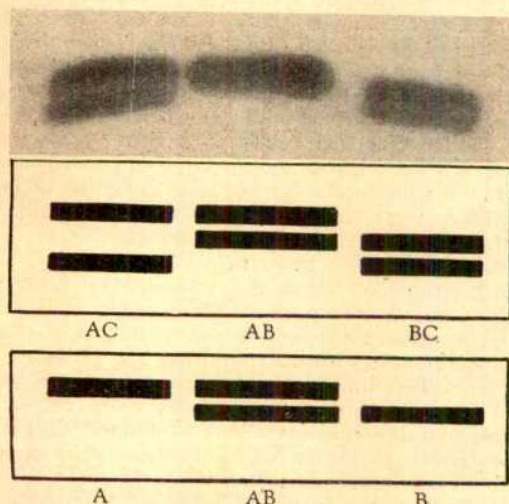


Fig. 2. Photograph and corresponding diagram (centre) of the albumin region of a gel carrying three diluted serum samples, showing (left to right) the albumin phenotypes AC, AB and BC. The lower diagram shows the three common phenotypes A, AB and B.



It was decided to investigate the behaviour of the attenuated strain after repeated passage in the calf tongue epithelial cells, for which the strain before attenuation had been fully virulent. In addition actinomycin D, which had been shown to block the production of interferon<sup>3-5</sup>, was investigated for its effect on the production of competent and non-competent virus and of interferon.

Virus of Venezuelan strain, type 'O', after 95 passages in 1-day-old chicks, six passages in 9-day-old chick embryos<sup>6</sup> and four passages in BHK 21 cells<sup>7</sup>, was used as the attenuated strain (ATT VI BHK 4). The virus of the first BHK 21 cell passage was passaged in surviving calf tongue epithelial cells (BTE cells) and used as the 10th passage (ATT VI BHK 1 BTE 10). BTE tube cultures were infected with about  $10^4$ – $10^{4.6}$  mouse ID<sub>50</sub> of virus. Actinomycin D (kindly supplied as 'Lyovac' 'Cosmogen' (Meractinomycin) by Dr. R. A. Pingeon of Merck, Sharp and Dohme Research Laboratories, New York) was added to one group of cultures at a concentration of 0.06 µg/ml. and was left in the culture throughout the experiment. The cultures were incubated at 37° C and three tubes were collected daily. The contents were pooled, and the supernatant fluid divided into two parts. One part was tested for virus content by inoculation of unweaned mice and, for comparison, of BTE cultures<sup>2</sup>. The other part was treated with acid and tested for interferon content in BTE cells<sup>1-2</sup>.

Table 1. TITRES OF VIRUS AND INTERFERON PRODUCED AFTER INOCULATION OF SURVIVING CALF TONGUE EPITHELIAL CULTURES

Virus strain	Treatment		Day	Day	Day	Day
			1	2	3	4
O ATT VI BHK 4	Nil	V*	6.3	5.6	5.0	4.2
		I†	0.0	1.8	3.0	3.0
O ATT VI BHK 4	Actinomycin D 0.06 µg/ml.	V	7.0	5.5	5.0	4.1
		I	0.0	0.0	0.2	0.0
O ATT VI BHK 1 BTE 10	Nil	V	5.1	6.3	5.6	6.25
		I	0.0	0.0	0.1	0.2

\* Virus titre expressed as log<sub>10</sub> mouse ID<sub>50</sub>/ml.

† Interferon titre expressed as log<sub>10</sub> VSV production in control minus log<sub>10</sub> VSV production in interferon-treated cultures.

The results of one experiment are shown in Table 1. It can be seen that in the ATT VI BHK 4 actinomycin D-treated cultures there was more virus on the first day than in the untreated ATT VI BHK 4 cultures, but this was not found in every experiment. With the passaged strain (ATT VI BHK 1 BTE 10) virus production was delayed, but titres reached were of the same order and in some experiments higher than those of the original attenuated strain. A constant feature of all experiments was that little or no measurable interferon was detected in cultures treated with actinomycin D or in cultures infected with the BTE-passaged virus. However, in these cultures the composition of the virus population which was produced remained similar to that of the original strain. In Table 2 the results of comparative titrations in unweaned mice and BTE cells are shown, and from this and other comparative titrations it was demonstrated that the log difference between the titres in mice and BTE cells was of the same order and the virus populations in all three instances consisted of about 1 per cent competent virus and 99 per cent non-competent virus.

Table 2. COMPARATIVE TITRES IN UNWEANED MICE AND IN TONGUE EPITHELIAL CULTURES

Virus strain	Previous treatment	Mouse log	BTE log	Log
		ID <sub>50</sub> /ml. (a)	TCD <sub>50</sub> /ml. (b)	difference (a-b)
O ATT VI BHK 4 BTE 1	Nil	5.4	3.1	2.3
O ATT VI BHK 4 BTE 1	Actinomycin D	4.75	2.5	2.25
O ATT VI BHK 1 BTE 11	Nil	5.9	3.5	2.4

The finding that interferon production was inhibited by actinomycin D confirms the results of other workers<sup>3-5</sup>. No change, however, was noted in the virus population produced, which with 1 per cent competent and 99 per cent non-competent was the same as that produced by the attenuated strain without treatment. This confirms that actinomycin D probably has a direct effect on a cellular

mechanism involved in interferon production<sup>3,4</sup>. The finding that the virus passaged 10 times in BTE cells gave results similar to those obtained in actinomycin D-treated cultures can be interpreted by assuming a change in virus nucleic acid composition during passage. The virus after passage probably contained calf-cell-synthesized components in its nucleic acid. It no longer appeared to the cell as foreign nucleic acid<sup>8</sup>, and thus did not set off the cellular mechanism for interferon production.

Lack of virulence on the part of the attenuated strain appears to be due to at least two factors. First, the virus population consists of 1–5 per cent infective or competent virus and 95–99 per cent non-infective or non-competent virus in contrast to the virulent strain, which has been shown to consist of 50 per cent or more competent virus<sup>2</sup>. Secondly, the cell reacts to the introduction of foreign nucleic acid by the attenuated strain with the production of interferon, which inhibits in turn further infection by competent virus. Actinomycin D and passage in BTE cultures abolished or reduced the second factor, but the first factor was unaltered. To what extent the virulence for cattle as compared with virulence in BTE cultures was regained by these treatments must await tests in susceptible cattle.

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## Electron Microscopy of a Tick-borne Encephalitis Virus

CLARKE<sup>1</sup> showed that a strain of tick-borne encephalitis virus, 'A52', isolated in Finland<sup>2</sup>, is antigenically identical with the Central European tick-borne encephalitis viruses. Sokol *et al.*<sup>3</sup> reported that the particles of the 'Hypr' strain of Central European tick-borne encephalitis virus purified from mouse brain suspension were predominantly spherical, and about 30 mµ in size. The same strain in HeLa cells has been demonstrated to be round-shaped with a dense inner body and a transparent outer zone surrounded by a surface membrane<sup>4</sup>. In the present work the negative staining technique<sup>5</sup> was used to determine the size and shape of particles of the strain 'A52', grown in tissue culture.

Monolayer cultures of a continuous line of human amnion cells<sup>6</sup> were inoculated with a virus grown in mouse brain at a multiplicity of approximately one mouse infectious dose per cell. After two weeks incubation at +37° C, virus was collected, clarified from cell debris by centrifugation at low speed and concentrated ten times in 'Cellophane' tubes by evaporation at room temperature for 30 h. The preparation with its high salt content was dialysed against phosphate-buffered saline, pH 7.4, overnight at +4° C. After 5 days storage at +4° C, 10 ml. of the preparation was diluted 1 to 5 with 1 per cent ammonium acetate containing 0.1 per cent bovine albumin (Armour, Fraction V). The pH of the diluent was adjusted to 7.3 with 1 N potassium hydroxide. The diluted preparation was centrifuged in a Spinco Model L centrifuge at 105,000g for 2 h. Four-fifths of the supernatant from the top was discarded, and the pellet was re-suspended in the remaining part and clarified by low-speed centrifugation. The supernatant in an equal volume of diluent was re-spun at 105,000g for 100 min.



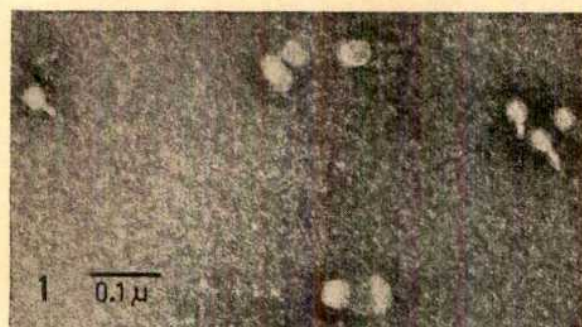


Fig. 1. Particles of a tick-borne encephalitis virus stained with PTA

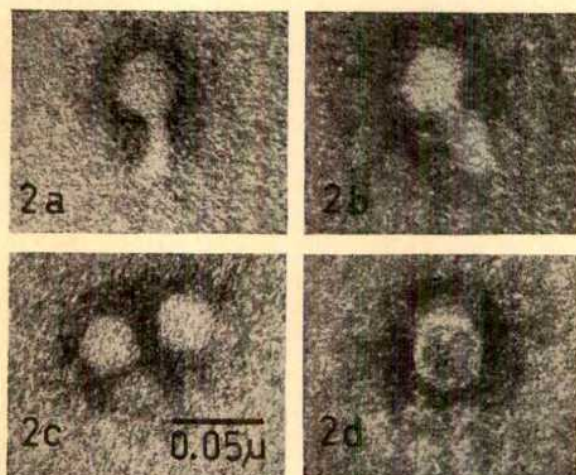


Fig. 2a. Particle with a curved tail. 2b. Particle with a bud-like bulb. 2c. Two spherical particles. 2d. Particle filled with PTA

Nine-tenths of the supernatant was discarded, and the pellet was re-suspended in the remaining part. After these centrifugations the evaporated preparation was concentrated five-fold in volume but haemagglutinins were increased only four-fold. The virus, in an equal volume of 4 per cent phosphotungstic acid (PTA) containing 0.4 per cent sucrose, was sprayed on carbon-coated grids and, after air-drying, was examined with a Siemens Elmiskop I electron microscope at an original magnification of 29,000.

The particle size varied from 30  $\mu$  to 40  $\mu$ , the average being 36  $\mu$ . About one-third of the particles had tail-like projections, some of which were straight, others slightly curved (Figs. 1 and 2a). The length of the tails varied from 16  $\mu$  to 32  $\mu$ ; the width was mostly about 12  $\mu$ . In some cases the heads and tail-like projections had the same electron density, but bud-like bulbs were also seen (Fig. 2b) and were mostly less dense than the heads. The particles were mainly spherical, but angular ones, hexagonal or pentagonal, were also seen (Fig. 2c). Some of them seemed to be filled with PTA (Fig. 2d). Structures similar to those described above were not observed in uninfected tissue culture fluids prepared in the same manner as the virus preparations.

Smith and Holt<sup>7</sup>, using negative staining, demonstrated that the particles of another tick-borne encephalitis virus, strain 'TP 21' of the Langat virus, obtained from a chromatographic fraction of mouse brain material, were roughly spherical, 32–37  $\mu$  in size, and several particles appeared to have a triangular facet. Tail-like particles of an A-group arbovirus, 'WEE' virus, have previously been described by Sharp *et al.*<sup>8</sup> in unfixed preparations of the purified virus stored 16 days at +4°C.

Some recent work indicates that certain animal viruses may have a tail<sup>9–11</sup>. On the other hand, distortion in

shape may be due to the conditions of preparing the virus for electron microscopy<sup>12,13</sup>. Further investigations are required to determine whether the tail-like projections described in the present report do exist under natural conditions or if they are produced in the preparation of the virus.

We thank Prof. N. Oker-Blom, Prof. A. Telkka, and Prof. P. Halonen for advice, and Mr. M. Lehtimäki for photographic assistance.

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## ARCHAEOLOGY

### Radiocarbon Dating of the Nok Culture, Northern Nigeria

AN early occupation site was located at Taruga, south-east of Abuja, in Niger Province of Northern Nigeria in November 1960, and fourteen exploratory trenches were dug by me early in January, 1961, for the Federal Department of Antiquities. Figurines characteristic of the Nok style had been found by tin miners in sample pits close by, and many other figurine fragments were afterwards excavated *in situ* together with decorated pottery, querns, quartz hammerstones, iron slag and quantities of charcoal. Four, and in some places five, distinct layers were observed in a depth of about 4 ft.

Charcoal excavated from layer three was submitted for determination to Isotopes Incorporated of Westwood, New Jersey, with the following result:

Isotopes Inc. Determination No.	Sample	Age (years B.P.)
I-1458	Sample B Taruga	2,230 ± 120

A date of 280 B.C. for an undisturbed Nok site containing abundant evidence of iron-working correlates satisfactorily with the original pre-radiocarbon date for the Nok Culture (based on geomorphological evidence) of the last four centuries B.C. The only other indisputably undisturbed wood specimen (excavated in a completely fresh condition with the bark still unscratched in the heart of the grey clay beds at Nok) gave a carbon date of 206 A.D. ± 50 years (Y 474). These two dates provide evidence of the survival of a single early iron age culture for nearly five centuries and there is at present no reason at all to believe that this did not begin earlier and survive later.

BERNARD FAGG

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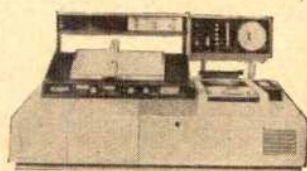
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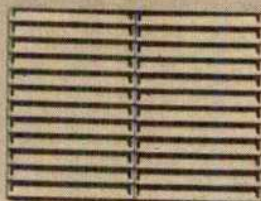


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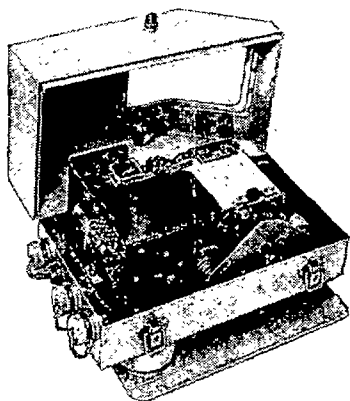


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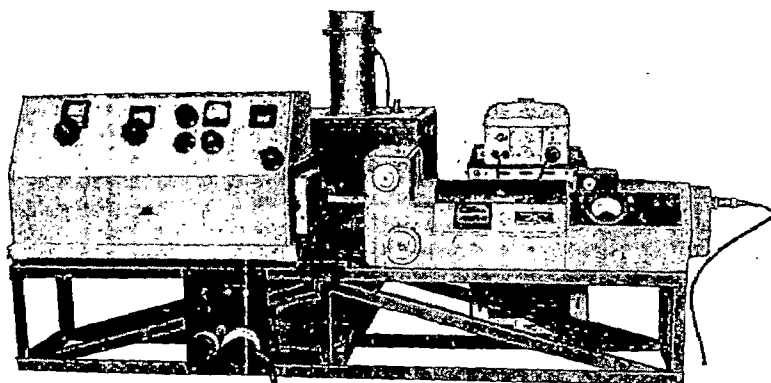
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## REPLANNING BRITAIN'S SCIENCE AND TECHNOLOGY

THE anniversary address which Sir Howard Florey delivered to the Royal Society on November 30 (*Nature*, 204, 1129; 1964) is of outstanding interest on two counts, both of which have a direct bearing on the present proposals of the Government in respect of science and technology. First, Sir Howard, in announcing the Society's decision to increase the number of annual elections to the Fellowship from 25 to 32, intimated that it was proposed to work out procedure for ensuring that a large proportion of future elections should comprise Fellows whose distinction lay in the applied sciences, both on the biological and physical sciences, and with some consideration too of disciplines at present inadequately represented or not at all. Sir Howard made it clear that this step was being taken deliberately to demonstrate the interest of the Society in technology and all that it meant to the Nation.

As a means of enhancing the prestige of technology and the technologist this step should be far more effective than the establishment of a separate Royal Society of Technologists, as was advocated, for example, by Sir Gordon Sutherland. Moreover, the Royal Society is taking other steps which should assist to that end. Lectures on applied science are to be given in 1965 and 1966 particularly for the information of the Fellows, and a conference for headmasters and others interested in education is being arranged in conjunction with the Engineering Institutions Joint Council with the view of letting it be known, as widely as possible, that the applied sciences offer just as splendid careers as the so-called 'basic sciences'. Taken with such forthright addresses as those given recently by Prof. I. D. Rattee (*Nature*, 204, 1017; 1964) and Vice-Admiral Sir Frank Mason (*Nature*, 205, 2; 1965), such measures should make an invaluable contribution towards the removal of the false idea that applied science is in any way inferior as an intellectual effort to pure science, though it may be a slower task to remove the attached social disadvantage.

That, however, should be assisted by the increasing relations between Government and science, to which Sir Howard Florey next referred, at least if those relations are wisely elaborated with clear ideas, on one hand, as to the nature of politics itself and, on the other, as to what is involved in science and technology. Pointing out that to-day the ability to pursue research increasingly depends on complicated Government organization, Sir Howard noted that one effect of the proliferation of the bodies to which the Government turned for advice on scientific and technical matters had been to diminish the direct relation of the Royal Society to the Government, although many Fellows of the Society served on these advisory bodies. He thought the time was now ripe for reconsidering the specific part which the Society itself could play and that the proposed increase in the technological element in the Society's fellowship could increase its capacity to serve in this way.

Obviously this has a very close bearing on the new arrangements being made for the organization of civil science, and it should not be overlooked that the decision to place the National Physical Laboratory under the Ministry of Technology, announced in the House of Commons on November 26, and to combine the Laboratory

with the National Chemical Laboratory under one director was only taken after discussion with the President of the Royal Society, and arrangements were included to continue the long-standing association between the National Physical Laboratory and the Royal Society. Sir Howard thought that the Society could increase its value to science if it reviewed at this time the methods by which it could furnish information and advice to the Government when required, but he also claimed that in broad outline the proposals of the present Government had taken note of the opinions that the Society had expressed. He fully recognized that the new arrangements, with their numerous sub-committees, required the time and energy of a substantial number of scientists, and for many who were still active in research this involved difficult decisions in determining the allocation of their time and energy—decisions which, as Sir Richard Clarke's Stamp Memorial Lecture on December 1 suggested, are not limited to the scientists or technologists.

It is interesting to note that at the time when the organization of British science is being reviewed, and when special consideration is being given to the channels through which Government is to be advised on problems pertaining to science, an *ad hoc* group of scientists and engineers has been formed by the U.S. National Academy of Sciences to advise Congress. The group will be under the chairmanship of Dr. G. B. Kistiakowsky (professor of chemistry, Harvard University, and chairman of the Academy Committee on Science and Public Policy). An initial survey is being undertaken jointly by the Academy Committee and the House Sub-committee on Science, Research and Development, under the chairmanship of Congressman Emilio Q. Daddario, to determine the areas in which the Academy is best equipped to give advice (see *Nature*, 204, 229; 1964).

Effective organization is an important factor in the wise use of scientific and technical manpower. 60 per cent of Britain's research and development effort is financed by the Government and one-quarter of the qualified manpower is in Government establishments, while 350,000 of the 750,000 people in Britain who have completed full-time courses of higher education are engaged in teaching: we can ill afford to neglect the efficiency of organization and utilization of such a precious resource. Sir Howard was right to remind the Royal Society that the supply of competent advisers and administrators is limited, although its Fellows would welcome the establishment of a body or bodies commanding their confidence and on which they could rely to see that matters of organization were kept under constant review and that big decisions on the deployment of resources for scientific and technological purposes were only taken after the best possible advice had been given to the politicians responsible.

Sir Howard showed that he was well aware of what was involved if the Fellows of the Society accepted the view that it was their duty to do all they possibly could to assist the Government in this somewhat critical stage of Britain's development. He recognized that the establishment of the Ministry for Education and Science had broken the direct link of the Society with the Treasury which had existed for more than a century, but he did not suggest that collaboration would be any the less friendly or

effective. He did suggest, however, that one of the services the Society could render to science just now would be to initiate an examination of how scientific opinion is brought to bear on Government so as to provide a clearer guide through these complexities by which opinions are formed and decisions reached on scientific matters.

The Science and Technology Bill\* now before Parliament and the statements regarding ministerial responsibilities in this field made by the Prime Minister and the Secretary of State for Education and Science in the House of Commons on November 26 are appropriately considered in the light of Sir Howard Florey's remarks. To a certain extent the Government has followed the recommendations of the Trend Report, but no clear principle can be discerned, and while the general arrangement could fairly be described as patchwork, Lord Acton's dictum is worth bearing in mind: "Freedom provokes diversity; diversity preserves freedom". The Road Research Laboratory, for example, is scheduled to go to the Ministry of Transport, while the research functions of the Nature Conservancy are to go to the Natural Environment Research Council under the Ministry of Education and Science, along with the other Research Councils, and not to the new Ministry of Land and Natural Resources.

Whatever reservations may be held as to the structure and placing of the Ministry of Land and Natural Resources, it is, at least at first sight, a Ministry in which it would seem that the internal structure for scientific enquiry and advice should be strong, and this would appear to override any advantage which a research council might gain from its independence of an allied ministry. In any event, the proposed arrangement should provide some evidence on this point, and experience with the Agricultural Research Council under the Ministry of Education and Science instead of the Ministry of Agriculture should also be instructive. How far the arrangements proposed for the Natural Environment Research Council to take over the Geological Survey and Museum, the Hydrology Research Unit, the National Institute of Oceanography and the functions of the Development Commission in relation to marine and freshwater biology and fishery research will meet the views of the Slater Committee or of the Brundrett Committee is not quite clear. So far as both the new Research Councils are concerned, however, the advice of the Trend Committee is largely followed.

It will be noted that the functions taken over from the Department of Scientific and Industrial Research by the Science Research Council in respect of research grants and postgraduate training awards do not extend to awards within the fields of the other Councils. Since the University Grants Committee now falls within the sphere of the Ministry of Education and Science, it remains to be seen in practice how far the principle of diversity of support for research can be maintained: much will depend on the extent to which the reconstituted University Grants Committee preserves its autonomy. The Science Research Council is also to take over the activities of the National Institute for Research in Nuclear Science: under Clause 3 of the Science and Technology Bill, which dissolves the Council for Scientific and Industrial Research and the Department, the activities of that Council are to be distributed among the appropriate Research Councils and other Government departments. The Radio Research Station is to go to the Science Research Council, along

with the Royal Observatories and the scientific space research programme, so also the responsibility of organizing Britain's participation in the European Space Research Organization and the Centre for European Nuclear Research (CERN).

Apart from the Road Research Laboratory and the Tropical Products Research Institute, the remaining stations of the Department of Scientific and Industrial Research go to the Ministry of Technology, which will also be responsible for the National Research Development Corporation and the Atomic Energy Authority. The powers of the Atomic Energy Authority are extended under the Bill to permit the Authority to undertake research and development outside the nuclear field. It is clear, both from the Bill itself and the statements made in Parliament on November 26, that there is a good deal yet to be worked out in respect of responsibilities in such fields as water supply and conservation. This may well appear in regard to building research and some other fields, especially where responsibilities for overseas research are involved.

One conspicuous example of lack of foresight appears to be in the field of information and library services. There is no reference to the position of the National Reference Library for Science and Invention, but the function of the Department of Scientific and Industrial Research in disseminating technical information is to be transferred to the Ministry of Technology, while responsibility for the National Lending Library of Science and Technology, the more general information work, and control of the scientific attachés goes to the Ministry of Education and Science. This is illogical and not easily defensible. The opportunity to develop, under the latter authority, a really comprehensive national library and information service seems to have been missed. Such a plan would seem to be practicable now that the Minister for Education and Science holds so large a share of ultimate responsibility for the provision of the Nation's library services, including those of the universities and technical colleges as well as public libraries. The omission is the more surprising in view of Lord Shackleton's remarks on the need for national organization to deal with scientific information and documentation, at the thirty-eighth annual conference of the Association of Special Libraries and Information Bureau on September 28 (*Nature*, 204, 1025; 1964), and of Lord Mountbatten's announcement at a dinner of the electronics industry on November 10 of a new lending library for research workers in that field in which papers on electronics research will be handled by computer. Lord Shackleton maintained that the Government should accept full responsibility for establishing the necessary national organization for handling information and documentation effectively, and he made a pointed reference to the use of mechanization. Lord Mountbatten made it clear that the imaginative scheme proposed for the electronics industry could be developed to serve all branches of science and industry, and the presence on that occasion of Sir Solly Zuckerman, who also spoke on the information problem, should ensure that these possibilities are kept before the Government, in spite of the division of responsibility between the two Ministries.

How far such misgivings are justified has yet to be seen, and they could well be removed by wise administration. It should be noted, moreover, that the authority given to the Secretary of State for Education and Science and to the Minister of Technology, to incur expenditure in connexion with scientific research, expressly covers research and development in any of the sciences, including

\* *Science and Technology Bill*. Pp. 11 + 12. (London: H.M. Stationery Office, 1964.) 1s. 6d. net.



the social sciences, or in technology. It would seem that the Bill provides sufficient flexibility to enable further changes to be made if necessary, and although the arrangements now proposed do not entirely dispose of objections to the Trend Committee's proposals which were raised at the time (for example, by a Committee of the Geological Society which advocated an Earth Sciences Research Council, and by several Fellows of the Royal Society, who feared the proposals might lead to dangerous fragmentation of marine science and suggested a Marine Sciences or a Marine and Freshwater Sciences Research Council), it should not prove too difficult to meet the objections if the division between the two Ministries is not too rigid. For practicable administration, there must be a limit to the number of independent research councils.

The real dangers lie rather in the pursuit of preconceived ideas; new Ministers, as new as their advisers, have constantly to be on their guard lest either professional tradition or political dogma or administrative convenience lead them astray. That is why it is so essential that critical but constructive thinking about the mechanism of scientific and technological advice should continue in the light of whatever fresh experience can be gathered. This is the only firm basis for an impartial assessment of the arrangements proposed by Mr. Wilson's Government, whatever justification there may or may not be for describing them as 'presidential government' in view of real or apparent similarities to the arrangements in the United States. The justification of any particular structure must rest simply and solely on its appropriateness to the needs and circumstances to be served, when full account has been taken of the implications.

If an end is called for to tendentious or captious criticism, such as has come even from such a body as the Institution of Professional Civil Servants, there can only be welcome for attempts to think out first principles such as have come from Dr. S. Toulmin or more recently from Prof. B. R. Williams and Dr. C. F. Carter in their paper on "Government Scientific Policy and the Growth of the British Economy"\*. These are all concerned, however, with the content of policy rather than the mechanism of advice or the formulation of policy. In the present context the proposal of the Parliamentary and Scientific Committee for a new Select Committee which should examine the annual reports of the Research Councils, of the Atomic Energy Authority, the National Research Development Authority and similar bodies, and also consider such scientific and technical matters as are being considered by Government Departments as a basis for legislation or the formulation of policy, is more relevant. The proposal contemplates that the Committee would call experts as witnesses and that an expanded library and reference service with scientific service with scientific staff would be at its service.

The proposal obviously involves the Government's departure from the steadfast refusal of the Executive in recent decades to provide the House of Commons with adequate services, and its effectiveness will also depend on the recruitment to the House of Commons of an increasing number of Members with scientific and technical background. Apart from the fact that this is essentially long-term policy, something more is required, and in this connexion Mr. R. H. S. Crossman's article, "Scientists in Whitehall", in *Encounter* for July 1964 is pertinent.

Mr. Crossman discusses more especially this question of scientific advice, recognizing that Government must see that professional scientific advice is available in every Ministry wherever it is required and that, as a long-term policy, a steadily increasing proportion of posts at Assistant Secretary and Permanent Secretary level are held by Civil Servants with a scientific background.

So far, as already noted, the present Government has not pursued a consistent policy on the first point, but Mr. Crossman does not suggest that all that is needed is to put more scientists into key positions. Like Sir Henry Tizard, he sees it as ultimately a matter of education and a proper balance between science and arts. Meanwhile the problem is to secure such marriages between the permanent Civil Service and outside expertise as was achieved so successfully during the Second World War, and this means that as a matter of deliberate policy such devices as are immediately open to us must be used. Mr. Crossman suggests, for example, a sharp increase in the size and status of the Scientific Civil Service and a full recognition of the vital role of the outside specialist on temporary assignment to Whitehall. These would provide the type of manpower required for the clear definition of the targets to which the effort of the community should be directed and the systematic use of scientific methods in selecting the aims and in working out the plans to achieve them. Specifically, Mr. Crossman refers to the need to ensure that the chief scientific adviser in each Ministry has direct access to the Minister and that there is sufficient steady movement between Government, university and industry to ensure flexibility of procedure.

Meanwhile, rather more light on the spirit in which the Government proposes to use the new organization, once it has been approved by Parliament, is to be found in the debate on technological development which was opened in the House of Lords by Lord Todd on December 2, particularly in the speeches of Lord Snow and Lord Bowden. Lord Todd in a wide-ranging speech pointed out that financial and social barriers were sometimes more important barriers to technological innovation in industry than scientific or technological obstacles. Moreover, although he admitted that the traditional industries presented the greatest obstacle to development, he questioned whether Britain's past policy towards the research associations had enabled her to get the best value out of what he regarded as a sound idea. He also maintained that we should concentrate our efforts in development on industries with a high content of technological skill and cease to prop up industries that had no real future. With reference to co-operation between industry and the universities, particularly in engineering and at the post-graduate level, he suggested the experiment in some university of a five-year minimum course for an honours degree in engineering, one of the final years being in industry on a sandwich arrangement.

Lord Todd also referred to the need to have enough supporting technicians; he was a little concerned at the prospect of separate Ministries for science and technology as well as a third for aviation, which will also be largely concerned with science and technology. Above all, however, he stressed the need for a unified scientific policy, and it was to this plea that Lord Snow, the Parliamentary Secretary to the Ministry of Technology, responded not simply with a reasoned defence of the division into two Ministries, but by outlining how it was proposed to mitigate admitted drawbacks in the proposed system by common membership of the various councils and

\* "Government Scientific Policy and the Growth of the British Economy" (reprinted from the *Manchester School of Economics and Social Studies*). By C. F. Carter and B. R. Williams. Pp. 20. (Manchester: *Manchester School, Department of Economics, The University*, 1964.)

committees and other formal and informal links for consultation. Besides the use of the development contract and an intelligent adaptation of the purchasing technique used by Mr. Robert McNamara in the United States, Lord Snow said that the Ministry proposed to make extensive use of listening. He stressed the value of Prof. P. M. S. Blackett's appointment on the Advisory Council on Technology, and agreed with Lord Todd's comments on the traditional industries and the research associations, also stressing the need to improve the status of the engineer.

Lord Snow's speech was generally welcomed in the debate, particularly by Viscount Mills, but although both the Earl of Halsbury and Lord Llewelyn-Davis welcomed the establishment of the Ministry of Technology they, as well as Lord Peddie, supported much that Lord Todd had said, as did the Earl of Bessborough. Lord Bowden, who replied on the debate as Minister of State for Education and Science, dealt more specifically with the educational aspect, and more especially with the question of attracting students to courses in subjects such as engineering or mathematics where Britain was experiencing a serious shortage of trained manpower. He welcomed the Earl of Bessborough's reference to the use of development contracts in the American universities, and assured the House that the two Ministries were as close together as it was possible for them to be. He also welcomed Sir Howard Florey's announcement regarding new fellowships of the Royal Society in applied science, and hoped that this would have its effect in the universities and in industry. The feature of both Lord Bowden and Lord Todd's speeches is the extent to which agreement on the organization of Civil Science and on the content of scientific policy cuts right across the bias of party politics. There may be disagreement as to timing or as to the measures to be used to effect particular purposes, but the extent to which agreement is shown to exist should itself encourage the response from scientists and technologists which Sir Howard Florey urged in his anniversary speech to the Royal Society.

## FIRST EDITION OF THE ORIGIN OF SPECIES

### On the Origin of Species

By Charles Darwin. Facsimile of the First Edition with an Introduction by Ernst Mayr. Pp. xxvii+ix+502. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1964.) 48s. net.

THE *Origin* is one of the most important books ever published, and a knowledge of it should be a part of the intellectual equipment of every educated person. Darwin had hoped to produce his book in a leisurely manner. He had completed much of the work and the *Origin* seemed to be shaping into a large volume when, in the third week of June 1858, the postman delivered to him a letter which had been posted some weeks earlier in the Celebes Islands. In this, Alfred Russel Wallace, a travelling naturalist, had forestalled Darwin, for here was a sketch of his theory. The shock to Darwin was great; Lyell and Darwin suggested that an abstract of the theory from Darwin and Wallace should be communicated, and the two papers were read before the Linnean Society of London on July 1, 1858, but were received without great stir.

Darwin then commenced to abstract and condense what he had written, and after another year's work the first edition of the book was published with the sub-title "An Abstract" on November 24, 1859. The publishers

did not expect a great demand for the book and printed 1,250 copies, which sold quickly. A second edition of 3,000 was produced in 1860, a third in the following year, a fourth in 1866, a fifth in 1869 and a sixth and final edition in 1872. Darwin lived for another ten years; but he had so thoroughly sifted and reconsidered his data and inferences that he made no further changes in the book. Many reprints, translations and editions by others have appeared in a period of nearly a century, but almost invariably Darwin's own sixth edition has been used. Prof. E. Mayr, the author of a first-rate book on the *Methods and Principles of Systematic Zoology*, has provided a useful introduction and a new index to a facsimile reprint of the first edition. Thus, the student will be able to follow modifications in Darwin's thought over a period of more than a dozen years. In the earlier edition there is a forthrightness of assertion and, as in other editions, little technical language or professional jargon, so that the work is remarkably accessible to the layman. Darwin's power of marshalling his facts and systematizing the results of thousands of observations is manifest on every page.

There is little wonder that most of the philosophers did not love Darwin, for without recourse to metaphysical ideas he had invaded and conquered much of their territory. As Prof. Mayr points out, Darwin thought in terms of populations rather than of classical Platonic ideas, for, in the latter, the type (eidos) is real and variation an illusion. Thus, Darwin opened the way to the statistical concept which has proved so fruitful in research in biological and other sciences in the twentieth century. Prof. Mayr's introduction leaves us wishing for more. It could be read in conjunction with that of the late Sir Arthur Keith to the sixth edition (Everyman), in which are also printed the additions and corrections to the text in successive issues of the book, some notes on foreign editions and a historical sketch of the progress of opinion on the origin of species, previous to the publication of the first edition of this work.

In spite of Darwin's use of plain words and the tangible rather than the abstract, the book is not easy to read because of its concentration. It was indeed an abstract of a much larger work which was brought to a stop by Wallace's letter. The book will endure in future ages so long as a knowledge of science persists among mankind.

It remains to be said that the edition here reviewed is very worthily produced and contains a little-known picture of Darwin.

W. L. SUMNER

## CHAPTERS OF QUATERNARY HISTORY

### Animals and Plants of the Cenozoic Era

Some Aspects of the Faunal and Floral History of the Last Sixty Million Years. By Dr. Ronald Pearson. Pp. vii+236. (London: Butterworth and Co. (Publishers), Ltd., 1964.) 55s.

DR. PEARSON'S book can perhaps be more accurately described as a series of essays on the history of the mammals and some groups of flowering plants in certain areas of the globe during the past sixty million years. Taken as essays, the chapters give useful summaries of some fields of research that are under active investigation, but there is all too little cross-linking from chapter to chapter.

We are told (p. 26) that a flora with tropical characteristics found at Comstock, Oregon, is of particular interest because it is associated with a marine fauna of known Eocene age. Marine faunas of the western United States are discussed on pp. 102-105, but there is no cross-reference to the plants of the region. The London Clay flora is discussed, but all we are told about its interesting vertebrate fauna is that "the animal remains, with the exception of the Mollusca, are regarded as showing tropical

affinities" (p. 19). There is a very good section (pp. 21-47) describing the Tertiary floras of the western United States from the Eocene to the Pliocene, but beyond two brief separated paragraphs there is no attempt to equate the plants of the landscape with the animals that lived in it. However, there are tables listing fossil mammal localities in the same area and belonging to the same periods. A synoptic table showing both botanical and zoological localities, together with the ecological interpretations that could be drawn from the lists of fossils recorded, would have been of great interest and value.

In the Quaternary section the early Dutch deposits are described, Danish work on the Eemian is discussed, but the German deposits are not referred to at all. The English deposits are well covered, but though the title of the section refers to the British Isles, Jessen's important work on the Hoxnian deposit at Gort in Ireland is not mentioned. Similarly in the Late- and Post-Glacial section the account is heavily centred on England, and reference is scarcely made to the much broader picture of the European woodlands given by Firbas. A considerable amount of North American information is now available, and this too could have been used. Iversen's attempt to trace climatic changes from the varying abundance of pollen of ivy, holly and mistletoe at different stages of the post-glacial period is summarized, but it must be remembered that this work was largely completed before it was fully realized how profoundly man's interference with the woodlands must have affected the growth of these plants.

Radiocarbon datings may have begun about 1940, but uranium/lead datings of igneous rocks were first determined by Boltwood in 1907, and much work on these lines had been done by Holmes and others long before anyone ever thought of the atomic bomb. Emiliani's interpretation of the temperature fluctuations of the surface waters of the oceans which he claimed to have recorded has now been severely criticized by Ericson and others.

The chapters on mammalian palaeontology are very different from those that precede them. Here we have detailed accounts of modern work on the origin and evolution of the different groups, and these will be of great value to vertebrate palaeontologists. In no more than seven pages Dr. Pearson gives a very skilful account of the tangled interrelationships of the numerous proboscidean groups.

As a guide to recent advances in studies of some animals and plants of the Cenozoic Era, Dr. Pearson's book will be of considerable value, but the full account of the natural history of the era must await a great deal of further work.

G. F. MITCHELL

## ISLAND BIRDS

### Birds of the Atlantic Islands

Vol. 1: A History of Birds of the Canary Islands and of the Salvages. By Dr. David Armitage Bannerman. Pp. xv + 358 + 17 photographs. (Edinburgh and London: Oliver and Boyd, 1963.) 84s.

FOR more than forty years Dr. Bannerman has been producing an almost continuous succession of generously proportioned, illustrated works on the bird faunas inhabiting the West African region, Cyprus and the British Isles; with indomitable industry he has now completed 25 volumes. The latest, covering the Canary archipelago and Salvage Islands, is to be followed shortly by two more on Madeira and the Azores.

His first landing on the Canaries was in 1904; and from 1908 until 1913 he made visits there every year, some lasting several months, while he systematically explored the avifauna of the different islands. The First World War put a premature stop to the survey, and, in place of the book he had been meaning to write, he had to be content with a partial presentation of the data, in a systematic

paper in *Ibis*. This ran notwithstanding to seven instalments in 1919-20, and filled almost 300 pages. He was back in the Islands again in 1920, this time writing a descriptive book, *The Canary Islands* (1922). Thereafter he had to turn his attention elsewhere, and the definitive work on the birds has, in the end, had to bide its time for half a century.

The main body of the book is a systematic account of the life-histories of 61 species of breeding birds. This extends and greatly enriches the outline of status and distribution given in the *Ibis* papers, and incorporates material compiled from published work (some of it very valuable) by other authors since 1920. There is naturally a general air of reminiscence, of naturalists active in the early years of the century and of conditions unlikely ever to return to the Islands because of growing population pressure and changes in land-use. So greatly has the vegetation been altered this century that some of the endemic insular bird stocks are now in desperate straits.

The evolution of island faunas is a subject of perennial interest. Endemic species in the Canaries include two sympatric laurel pigeons (both now extremely scarce), the famous blue chaffinch, Meade-Waldo's chat, and Berthelot's pipit. (The canary itself, which of course takes its name from the Islands and not *vice versa*, is an endemic sub-species of the widespread serin.) Bannerman restores the island blue tits to full specific status (*Parus teneriffae*); like many other resident birds, they have distinctive sub-species on different islands.

The Canaries are volcanic, and believed to date from the Pliocene. In spite of being separated by only 60 miles of ocean at their nearest point from the African coast, the affinities of the land-bird fauna are much stronger with western Europe than they are with north-west Africa. This can be presumed to reflect the strong tidal flow of northern migrants through the Islands each autumn and spring. No less than 144 species of non-breeding or off-season visitors are included in a special section on migratory birds, comprising pp. 308-343.

This is a handsome book, with large pages and 13 fine coloured plates of which all but two are the superb work of D. M. Henry. The same artist has also provided many charming and accurate black-and-white portraits of birds which embellish the text; to these the publishers have added several older drawings by Roland Green and Grönvold. Bannerman's style is, as always, lively, personal, unhurried and spacious; in great contrast with most present-day systematic works in which compendiousness usually demands as high a concentration of facts as possible, and no digressions, his book is suited not merely for consultation as a reference work but equally for enjoyable reading—from cover to cover if leisure permits—by any professional or lay ornithologist fortunate enough to turn his attention to these Atlantic isles.

V. C. WYNNE-EDWARDS

## MECHANICAL PROPERTIES OF MATTER

### The Mechanical Properties of Matter

By Prof. A. H. Cottrell. (Wiley Series on the Science and Technology of Materials.) Pp. x + 430. (New York and London: John Wiley and Sons, Inc., 1964.) 70s.

THIS is a remarkable book. In the lucidity with which the material is presented, in the width of matter dealt with and in the originality with which the whole is woven into a coherent unity it breaks completely new ground. Differing as much from the conventional treatment of properties of matter as it does from that of strength of materials, it welds both together on the basis of the atomic structure of the various states of matter. It gives to materials science a reality never before achieved.

What the author has set himself to do is best described in his own preface. "I have tried to give an explanation, in terms of atomic behaviour, of the mechanical properties



of solids, liquids and gases; the traditional approach, by contrast, usually limits such explanations to the kinetic theory of gases and deals with solids and liquids in a severely empirical way. This atomistic approach has led me to discuss the structure of matter, particularly solids, at some length, but I cannot see how to understand the mechanical properties of matter in any other way. Finally, I have also interpreted the subject fairly broadly, so that at several places the text overlaps into neighbouring domains of physics, chemistry, metallurgy and engineering, in order to obtain an integrated view of the whole field as both a pure and an applied science." This is an ambitious programme and could easily become a mass of ill-digested snippets and, to use the author's own words, "a mathematical jungle". That he has escaped both dangers speaks well for his erudition, skill and ingenuity.

Starting with a discussion of the perfect gas, he then deals in succession with condensed states of matter and crystal structure. Three chapters are concerned with elasticity and the propagation of waves and vibrations in solids, and here it should be remarked that they are, to the best of our knowledge and belief, real solids and not the hypothetical homogeneous material of the classical treatment. Fluidity, viscosity and fluid mechanics are considered in Chapters 7 and 12. Surface phenomena are dealt with in Chapter 8, while Chapters 9 and 10 are devoted to plasticity. Finally, in Chapter 11 consideration is given to the problems associated with the fracture of solids. Although the treatment throughout is concerned with the principles involved, a glance at the index will reveal the extent to which these principles are related to actual phenomena. Abrasives and autofrettage, fatigue and brittle fracture, grain growth and the Reynolds number illustrate this, a treatment which will appeal to the reader concerned with the urge to know "what is the use of it all".

Although this work will appeal to very many more, it has been written mainly "for students of physical science and engineering who are at a fairly early stage of their university courses". With this end in view the text has been kept "simple and introductory", the only background knowledge assumed being ordinary "school science and some elementary mechanics and calculus", including some acquaintance with partial differential equations. According to the publisher's jacket, "the treatment is elementary", a claim which would not be accepted by all the students to whom it is directed. In places only the best will be really at home, but even the average student can deal with at least 90 per cent of the material and will benefit enormously thereby. The best will enjoy every word.

Few authors can be so certain that all the work put into a volume has been so well worth while. F. C. THOMPSON

## MOLECULAR ORBITALS

*Molecular Orbitals in Chemistry, Physics, and Biology*  
Edited by Per-Olov Löwdin and Bernard Pullman. Pp. xiii + 578. (New York: Academic Press, Inc.; London: Academic Press Inc. (London), Ltd., 1964.) 157s.

AN anonymous writer in the *University of Chicago Reports* (11, No. 5, 1961) wrote of R. S. Mulliken, "his playing field has been the molecule; the ball has been the electron. In this arena, pure elements recombine into materials and substances which man knows with his five senses. The agent of change is the electron, the negative charge of electricity that locks atoms together or keeps them apart". These words, which reflect Mulliken's attitude to science, are quoted in C. A. Coulson's contribution to this volume written in tribute to Mulliken on his retirement from his chair at the University of Chicago.

Mulliken has been involved in almost every stage of the development of molecular quantum mechanics and molecular electronic spectroscopy. The range of his

interests is easily seen from an examination of the articles in this book. For example, we find papers on molecular self-consistent field theory, group theory, shapes of molecules, molecular spectroscopy, the Huckel theory of  $\pi$  electron molecules, semi-empirical theories of  $\pi$  electron molecules and a final set on the theory of chemical reactions and biological processes. These articles, while not reporting Mulliken's work, discuss those fields in which he has worked.

*Molecular Orbitals in Chemistry, Physics, and Biology*, edited by Per-Olov Löwdin and Bernard Pullman, is indeed a fitting tribute to the pioneer. Its thirty-three papers are the work of some forty-six authors. The authors themselves are drawn from the United States, Europe, Asia and Australia. Some of them have been active for as long as Mulliken. We find papers from Eyring, Pauling and Slater, three of the workers who also pioneered in this field of investigation from the beginning. There is a paper by C. A. Coulson, who since the mid-1930's has been engaged in investigations almost as wide in scope as those of Mulliken. To list the rest of the authors would be rather pointless; let it be sufficient to say that almost all the foremost workers in molecular quantum mechanics have contributed to this volume.

The contents of the book, however, require some comment. Not very much of the work reported here is new, although it is useful to have it collected together in one volume. The opening article by C. A. Coulson, entitled "R. S. Mulliken—His Work and Influence on Quantum Chemistry", is very good indeed. This appreciation of Mulliken's work is itself a pocket-sized review of the work done in this field. J. C. Slater contributes the only other non-technical paper. It is a pity that this contribution is so short, because what there is makes good reading. Slater states that he has just published a volume on molecular structure and that the bibliography has three pages devoted to the work of Mulliken. The quality of the remaining papers is high, but one is left wondering if such detail really ought to be included in such a volume as this. I myself regret that the editors have not published any of Mulliken's work. So many of Mulliken's papers have become standard works that the editors would have rendered a definite service if one or two had been included. Although several of the authors are former students or collaborators of Mulliken there is no contribution from his laboratory. Again, this is a pity.

It may be, as the editors say, that a second volume may be required in a few years time to cover the new contributions from Mulliken. This book, however, is a suitable way to mark the retirement of a great scientist. It is very well produced, and the articles are relatively free from error either of the factual or of the printing variety. Unfortunately the book is very expensive. I cannot see it finding its way into many private libraries. This is unfortunate since it is the sort of book one likes to have. The publishers have done a good job both with printing and binding. Finally, the editors are to be congratulated on assembling such an interesting set of articles by so many front-rank theoretical chemists.

T. E. PEACOCK

## INTRODUCTION TO THE STUDY OF ORE DEPOSITS

### Ore Deposits

By Prof. Charles F. Park, jun., and Prof. Roy A. MacDiarmid. (A Series of Books in Geology.) Pp. x + 475. (San Francisco and London: W. H. Freeman and Company, 1964.) 70s.

IT is a good many years since a text on ore deposits for students (other than translated works) was last published in the English language, although several noteworthy books on this topic have lately appeared in French, German and Russian. Recent advances in know-

ledge have made such a text overdue; and in their chapters (pp. 15-205) on the origin and migration of ore-bearing fluids, on physical and chemical controls of ore deposition, on wall-rock alteration and gangue, on paragenesis and zoning, and on geothermometry, Profs. Park and MacDiarmid convey to the senior undergraduate who will use this work a judicious appreciation of the investigations of the past two decades. The remainder of the book (pp. 207-456), concerned with the classification of ore deposits and the description of well-chosen typical ore-fields, closely follows the system of Lindgren "which represents a fundamental standard by which most United States geologists attempt to classify ore deposits". Perhaps the main demerit of this system, as presented here, is over-insistence on the assumption that hydrothermal fluids "arose directly from an underlying magmatic source", a hypothesis which seems of doubtful validity in connexion with the great stratiform deposits which now furnish a very large part of the metal production of the world. Recognition that igneous intrusions and orogenic activity can stimulate the circulation at depth of intra-stratal brines and other groundwaters, which in turn can derive metals from the rocks which they traverse, seems in many cases to provide a channel between the Scylla of plutonism and the Charybdis of neptunism, on one or other of which hazards, according to the personal predilections of the professional reader, some of the authors' conclusions may be held to founder.

In their introduction, the authors emphasize that "the study of ore deposits and of ore genesis is not an exact science". On the mode of genesis of nearly all the great ore-types of the world—such as gold-uranium bankets, copper shales and red-bed coppers, noritic nickel ores, jaspilite iron deposits, and titanium ore-bodies in anorthosites—there are two antagonistic schools of thought; and in description of these debatable mineralizations an attempt has been made to give both sides of the issues, to a degree which may frustrate the mediocre student who likes cut-and-dried explanations but which will inspire the good student to think for himself. A few factual errors common in foreign text-books have been perpetuated. Thus the statement (p. 388) that the alum shale of Sweden carries as much as 0.5 per cent uranium is analogous to saying that chalk carries as much as 99 per cent silica—this high tenor of uranium occurring in the diagenetic nodules of kolm is altogether different from the alum shale, much as flint is different from chalk. Again, the observation (p. 454) that "platinum is found as well-defined nuggets in the Witwatersrand" is scarcely in keeping with the facts that the principal platinoid present is osmiridium, that it rarely if ever exceeds 0.1 mm in grain size, and that the tenor in the Witwatersrand ores (only four times the Clarke) is comparable to that in many gold-uranium veins of undoubted hydrothermal parentage. But these criticisms are very minor ones. In a text for undergraduates one would have welcomed a little about non-metallic ores—minerals such as diamond, magnesite, asbestos, and most other non-metallics are not dealt with. Despite this, however, the book fills a present need. It will be warmly welcomed and will deservedly have a wide circulation. C. F. DAVIDSON

## CORRELATION BY FOSSILS

### Time in Stratigraphy

By Dr. Alan B. Shaw. (International Series in the Earth Sciences.) Pp. xiv + 365. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1964.) 10.50 dollars; 81s. 6d.

THE valuable part of *Time in Stratigraphy* consists of the exposition and illustration of a new method for improving stratigraphic correlation by fossils. By applying his method to certain strata in Texas, Dr. A. B. Shaw demonstrates that it is possible to subdivide the succession

much more finely than by the present method of zoning. As a consequence, different sections may be much more accurately correlated with one another than has hitherto been possible. Should trial establish the validity of the method, its application to a variety of successions could lead to a breakthrough in various evolutionary and sedimentation investigations.

The method involves the determination of a standard succession of ranges of fossils, measured in units of length through a particular suitably chosen reference-section. The ranges are those not only of fossils occurring in that section, and extended where evidence from other sections shows an earlier entry or a later disappearance, but including also points of entry and disappearance of other species interpolated from other sections. The procedure used in interpolation from other sections into the standard is the essence of the method. In comparing another section with the standard, two processes are involved: (a) the differing thicknesses of strata corresponding to equal intervals of time and arising out of the different rates of accumulation of the two sections must be allowed for; (b) the two successions must be correlated. Both processes are carried out at the same time by a statistical method which can take account of all the different range limits common to both sections. In practice, not all range limits are used in the calculation, as some can be shown to be more valuable than others in particular cases.

By the successive incorporation into the standard of data from a series of sections, the quality of the standard is continuously improved. When all available suitable sections have been used, it is possible to go back over the sections and compare them successively with the improved standard which is thereby continuously further improved. The revision can be carried on along these lines until no further improvement results.

The statistical procedure used in interpolating data from other sections into the standard can now be applied for the comparison of any other section with the perfected standard. Thus the best possible correlation can be obtained.

It can be seen that this method of correlation is superior to present methods, in that it makes use of far more data and because it is much less arbitrary and subjective. The statistical technique is simple and the calculations can be made readily on an ordinary desk calculating machine.

On theoretical grounds, the method may be criticized for its assumption that rates of sedimentation are reasonably uniform throughout a given section. It would not be difficult to investigate this assumption by dividing up various sections and testing the variation in rate of accumulation in each part. Indeed, this ought always to be done before correlations are attempted if there are marked changes of lithology or other grounds for suspecting lack of uniformity during deposition.

The price of the book is obviously a consequence of the large number of complicated tables. These are absolutely necessary in the main, though there is some avoidable repetition and the columns of standard equivalent levels in Tables A-4 and A-6 are unexplained and confusing. It would seem that an attempt has been made to make the price more palatable by throwing in something extra, namely the first seventy-one pages of the book, which are concerned with general principles of stratigraphy. On the basis of this first part of the book, it has been given the rather meaningless title *Time in Stratigraphy* which suggests that the book is a contribution to general stratigraphy. Unfortunately there is insufficient reference to actual examples to make the discussion of principles worth while, especially where these principles are either new or formulated in a new way. In any event, all this is unnecessary, as the later part of the book does not depend on any new principles or new application of old principles. The book is only of real value for its new method of correlation, and it is not to be recommended to general students of stratigraphy. S. SIMPSON

## Organic Chemistry

By Dr. I. L. Finar. Vol. 2: Stereochemistry and the Chemistry of Natural Products. Third edition. Pp. xii+690. (London: Longmans, Green and Co., Ltd., 1964.) 50s.

THE third edition of Dr. Finar's book brings an already popular and successful text up to date, with references to the 1963 literature. The overall scope is unaltered, approximately one-third being devoted to stereochemistry and the remainder to natural products. In the main the revision consists of insertions into the old text, but Chapter 3 ("Nucleophilic Substitution") has been reconstructed and now includes a concise account of neighbouring group participation. Chapter 8 introduces the concept of bridged (that is, non-classical) carbonium ions, which is utilized in later discussions.

Dr. Finar's book presents a lucid, well-illustrated account of stereochemistry and a panoramic survey of the chemistry of natural products. Indeed, the reader has at hand, in one volume, such topics as carbohydrates, steroids, purines, porphyrins, alkaloids, flavonoids and terpenes. Such a menu cannot fail to attract customers, and at this price it is a bargain. As may be expected, such a compilation of material is only made possible by condensation, and there is a tendency to present facts without explanations or life-giving commentary. Thus the subtleties of the patulin molecule are glossed over, the stereochemical implications of the biotin synthesis are lost, the chemistry of flavonoids is treated too factually and the teaching potential of terpene chemistry is only hinted at. On the other hand, a real attempt is made to comment on the underlying principles of the Woodward sterol synthesis, and elsewhere the author makes commendable use of cross-references where space considerations forbid a full discussion.

Some readers will feel disappointed that, in spite of the references on the cover, and in the index, to infra-red, ultra-violet and nuclear magnetic resonance spectroscopy, the application of these techniques is not really demonstrated in the text. I could find only one case (p. 383) in which spectroscopic data were given. Other entries consist of a phrase such as, "Infra-red studies showed the presence of two double bonds".

With these reservations this is a valuable book which provides a useful basis for further reading. It has a very comprehensive index, formulae and diagrams are well reproduced, and the cross-indexing is excellent. The use of a larger page size in this edition is also welcome.

G. L. BUCHANAN

### Etude Critique des Bases de l'Interprétation Actuelle de la Mécanique Ondulatoire

Par Louis de Broglie. (Traité de Physique Théorique et de Physique Mathématique, Nr. 21.) Pp. ix+93. (Paris: Gauthier-Villars et Cie., 1963.) 28 Francs.

THE metaphysical interpretation of quantum mechanics will probably continue to worry philosophically-minded scientists and scientifically-minded philosophers for many years to come. The essential question seems to be: Do things really happen when physicists are not making measurements, or is the detailed history of the world merely a vast collection of possibilities and probabilities? One feels that it should be possible to answer this question in a way which satisfies common sense, but the combined efforts of physicists and philosophers of science have so far failed to do so. It is therefore a philosophical event when the father of wave mechanics gives us his mature reflexions on the paradoxes of quantum theory.

Louis de Broglie's book is short and meaty. It is written with a clarity of style which is all too rare even in the works of literary men, and he lays before the reader simply and cogently the paradoxes of the orthodox

theory of measurement. He states fairly and exactly what has become known as the Copenhagen interpretation of quantum theory, but shows that it leads to assertions which are very difficult to reconcile with the doctrine of physical realism. He does not question the correctness of wave mechanics but merely its completeness, and the reader begins to wonder whether de Broglie may be about to present a satisfying escape from the famous paradoxes.

But that would have been too much to hope for. What de Broglie offers us is, in fact, his old theory of the 'double solution' in which the particles of wave mechanics are somehow guided by the waves representing them. de Broglie recognizes that he has not demonstrated the existence of the 'double solution', or its consistency with the general postulates of quantum theory. In this he is admirably honest, and even if one doubts the value of this attempt, one must admire and respect the complete intellectual integrity with which it is put forward and criticized.

*Etude Critique des Bases de l'Interprétation Actuelle de la Mécanique Ondulatoire* is an object lesson in clear, imaginative and honest thinking about physical reality, and is to be heartily recommended to anyone who is seriously interested in the relation between physics and metaphysics.

H. C. LONGUET-HIGGINS

### Insect Pathology

An Advanced Treatise. Edited by Edward A. Steinhaus. Vol. 1: pp. xvii+661; 22 dollars, 157s. 6d.; Vol. 2: pp. xiv+689; 23 dollars, 164s. 6d. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1963.)

VOLUMES 1 and 2 of *Insect Pathology* comprise a collection of papers written by 35 international authorities, edited by Edward A. Steinhaus.

Volume 1 deals with physical injuries; chemical injuries; nutritional diseases; genetic diseases and aberrations; tumours; the micro-organisms of healthy insects; the pathogens of vertebrates and plants as pathogens of their acarine and insect vectors; immunity in insects; physiopathology and histochemistry; predispositions and interrelations in insect diseases; the nature of infections caused by nuclear-polyhedrosis viruses; the nature of nuclear-polyhedrosis viruses; the cytoplasmic virus diseases; the induction of virus infections; granuloses of insects, and rickettsiae and rickettsioses.

Volume 2 contains chapters on the taxonomy of entomogenous bacteria; diseases caused by certain spore-forming bacteria; the milky diseases; non-sporulating bacterial pathogens; *Coelomomyces* infections; entomophthorales infections; diseases caused by hyphomycetous fungi; *Gordyceps* infections; sporozoan infections; infections caused by protozoa other than sporozoa; nematode infections; pathologies caused by insect parasites; the epizootiology of infectious diseases; microbial control; the commercial production of insect pathogens; the background for the diagnosis of insect diseases, and techniques in insect pathology.

This list of chapters indicates the comprehensive scope of the volumes. The subject is of obvious interest to those in the life sciences concerned with entomology, either directly as entomologists or indirectly as virologists, botanists, parasitologists, chemists, pathologists. It is now realized that the control of insect-borne infections in man, animals, plants and crops must not be undertaken with unthinking enthusiasm, but must be directed to conservation and the appropriate exploitation of Nature by using sophisticated measures. For these measures, a detailed knowledge of insect pathology is essential. *Insect Pathology* is both instructive and educative, and it is therefore essential reading for those involved in advising on control measures against insects or the organisms they transmit.

W. E. KERSHAW



## THE WORLD, THE FLESH AND THE METAL\*

## THE PREROGATIVES OF SYSTEMS

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## The Seminal Idea

COMMANDING concepts arise in science which seem to roll up the universe into a ball, declaring: it works thus. I am thinking of such notions as inertia and entropy. The names of these concepts do not denote any discoverable thing out there in the world at all. They denote sophisticated ideas that scientists have about the way the world is arranged, or perhaps read into the world. What is out there is a manifestation of the idea; and that manifestation is pervasive, once the idea is sufficiently clear to be called obsessive.

The old way of talking about these concepts was to legislate about them, and to set up Laws of Nature. Who passes these laws is not clear, but certainly God alone may repeal them. So clustering about the concept of inertia are the laws of motion—together with a considerable body of amendments which encompass relativity; and clustering about the concept of entropy are the laws of thermodynamics—together with special versions of the enactments for other territories, such as statistical mechanics and information theory. These laws seem to be very unusual ones indeed; for there is no way of repealing them—once the Deity is defined in their own terms as a store of negentropy.

Now concepts of this kind are very difficult to understand, because there is no ostensive definition. Hunting down inertia is like hunting down the snark; the only one who actually saw it “softly and suddenly vanished away”. The result is it takes a long time before the concepts which are most potent in the scientist’s interpretation of the universe are manipulated with ease in our thinking. As evidence of this contention, consider the adjectival form of the terms already mentioned. Physics acquired the concept of inertia in 1687, through the *Principia*. But it was a hundred and sixty-two years before anyone invented the adjective, and spoke of ‘a weight of inertial resistance’. Where would science be without that word to-day, a mere hundred years later? As to the word entropy, the centenary of the birth of which falls in 1965, we are still so unsure of ourselves that there is no official record that the adjective has been coined yet. I have in fact coined it myself, and offered such phrases as ‘entropic drift’; but no one seems to want them.

The point of all this is to suggest that the concept of system is another such seminal notion, which we are only now beginning to understand. This word has also been used since the seventeenth century; and is certainly well understood in the rather special sense of an assemblage which is usually small, usually connected together deterministically, and usually well defined. But we cannot roll up the universe into that sort of ball, nor declare it works thus. Patently the universe is not, nor is it compounded of, that sort of system. Yet a system in another sense it clearly is, and any part of the universe is clearly a system in that same sense. Secondly, the syntactical clue to our unease in the manipulation of this wider concept is again present. For the adjective which means ‘pertaining to a

system’ is not ‘systematic’ (which means something quite different) but systemic; and that is a word one seldom hears. It took a hundred and eighty-four years to achieve the adjectival form this time, even in a limited physiological context. As an adjective of general relevance, the word systemic has been with us for only a hundred years, and its use is now marked ‘rare’ in the dictionary.

In short, we do not think about the systemic nature of things, we prefer to consider them in isolation. Stepping smartly down from these philological clouds, I point to a number of practical examples. The brain, a company’s profitability, a computer and the National Health Service are all integral systems. But we talk gaily about the motor cortex as if the rest of the brain were irrelevant to movement. We talk about the cost of making a product as if it did not matter what opportunities to make other products were foregone in making this one. We talk about the reliability of an electronic component as if this meant something independently of the failure rates of associated components in an operational ensemble. We talk about the cost of prescriptions under National Health as if this were independent of the doctors’ attitude to whether the patient bears a charge or not.

The seminal notion of system-as-pervasive in science is really very new, and we are not trained to think in systemic terms because our scientific approach has been analytical to the ultimate degree. We took things apart, historically, and described the atomic bits. We did our experiments, historically, on these bits—deliberately holding invariant the behaviour of other bits with which in fact they were systemically interacting in real life. Now advances in statistical method have enabled us to be more ingenious than this to-day; we can afford to let a number of things change at the same time, and sort out the meaning of the experiment by multivariate analysis. But this is a mastery of technique, not a change in methodology. The result is that our only approach to system is through aggregating bits. No wonder that we do not advance in understanding really big systems, then, and that the very connotation of this word system includes such question-begging epithets as small, determined and well defined.

The concept of system in its widest meaning is a cybernetic concept. Big systems are the topic of cybernetics. The systemic character of the world is the clue to its control; just as the inertial character of the world is the clue to its movements, and the entropic character of the world is the clue to its energies. Thus the contention is that the ‘laws of control’ are the prerogatives of system—wherever and whenever it is defined. And this is why I have taken liberties with the catechism in the title. For the prerogatives of system are pervasive, whether we speak of the world at large, or the flesh of our own bodies, or the metal of the ironmongery we invent as engineers.

## Methodology of Models

If this concept of system is so new and so important, how shall we attack it, how comprehend and manipulate it? In a paper written five years ago<sup>1</sup> I gave a philosophical approach to this problem. To-day the approach will be essentially mathematical.

\* Substance of the 1964 Stephenson Lecture delivered before the Stephenson Engineering Society, University of Newcastle upon Tyne, on November 26.

When an engineer looks at a big system (as already defined here) and recognizes its systemic character, he may well perceive its structure in terms of servomechanics. He draws rings around various activities, or sub-sets of events, in his mind, and observes that they are linked together by messages. That is to say, that the input to one sub-set is the output of one or more other sub-sets. For example, production is an activity which responds to a demand expressed by a market, while selling is an activity responding to a production output. The engineer may then recognize that what happens in each box of the system may be regarded as a transfer function applied to the signals that connect it to other boxes. If the macrosystem he is thinking about can once be defined as closed, as in the case of the marketing-production-marketing example, he is in business as a model-builder. The process soon becomes exciting. In order to attain to an ever-more-accurate account of what the big system is doing, the model-builder may insert all sorts of boxes in his design, until the model is very complicated indeed. Very soon he will see the need for local regulatory devices in the microsystems which comprise the macrosystem. This will lead to the insertion of closed-loop feed-back circuits, in which outputs are monitored by comparators, and inputs modified by some function of the error signal thereby formed. It is often possible to obtain a strikingly good analogue, expressed in servomechanical terms, of a fairly big system of world events in this fashion.

In fact, this is one example of the way operational research works. The scientific model conceived by an operational research team will depend on the kind of system being studied and on the versatility of the team itself. We are all conditioned by our training and experience. It can scarcely have been fortuitous that Prof. M. J. Lighthill chose to represent the flow of traffic with a model from fluid dynamics, or that Prof. A. Tustin sought to model the economy by just the kind of servo-system that I have been describing. This is not because the big systems of real life are 'really' hydraulic or electrical in character, but because Lighthill and Tustin are the distinguished men they are, and because the systemic character of the world is pervasive. This is why an operational research group is supposed to be interdisciplinary. It is my job to hold back a colleague who is determined to model a management structure by an anthropological model taken from the tribes of the Western Pacific, if this turns out to be rather unhelpful. Equally I expect my colleagues to intervene if my own habit of modelling company organizations on neurophysiological systems runs away with me. They always do intervene, as it happens; but I still think that the brain, supported as it is by many million years of research and development, must offer good advice on how to make a control system viable. Which just shows how preconditioned I am. Anyone who wishes to examine more closely how operational research makes use of models from science may refer to another paper<sup>2</sup>, in which various examples are given—including the electrical circuit of a servo-model of an actual production system.

But, assuming that this talk of models is now understood in a general sense, I propose to examine rather more rigorously what is going on when a model of one activity is taken from another activity. Here it should be borne in mind that our comprehension of anything at all is likely to be expressed to ourselves in terms of something else which we know that we understand; which is another way of saying that everything is a model of something else. If you should ask: what constitutes the prior structure of which other things are models?—then maybe the answer belongs to psychoanalysis, and must be expressed in terms of elemental and traumatic experiences.

Consider a big system out there in the world. It may be a firm interacting with its environment of markets in a search for survival; it may be a company competing

with another company for a larger share of a zero-sum pay-off; it may be a capitalist economy in which a socialist chancellor sits like a Maxwell demon increasing the entropy of wealth. (Please note in passing how, simply in order to nominate three samples of big systems, I have already invoked three models: from ecology, from game theory, and from thermodynamics.) In trying to understand these things as systems, we must detect in them whatever is systemic. This sounds tautologous, but it is not really. For we do not have full knowledge of what these systems are like—they are immensely complicated; and we do not have full knowledge of what constitutes system anyway—science is not yet omniscient, and we ourselves are relatively ignorant.

Now I shall discuss this problem in the language of group theory, for reasons which will be made specific later on. The point is that we need as rigorous a statement of this complicated methodology of models as we can get. It is in fact high time that some such attempt were made, for the very word 'model' has fallen into an unhelpfully casual usage. Some people use the word as synonymous with 'mathematical equation'; others treat the word as if it meant 'hypothesis'. A model is neither of these things. It is something that opens up a virtually new way of doing science.

Thus if we call the set  $M$  of elements  $a$  the totality of world events which we propose to examine, then the systemic configuration of events which we know about is a sub-set  $A$  of set  $M$ . If we call the set  $N$  of elements  $b$  the totality of systemic science, then the configuration of system which we ourselves understand is a sub-set  $B$  of set  $N$ . The process of creating a systemic model may then be described as a mapping  $f$  of  $A$  into  $B$ . By this I mean that for every element  $a \in A \subset M$  there exists a corresponding element  $b \in B \subset N$ , and thus  $b = f(a)$ . The image of the sub-set  $A$ , namely,  $f(A) \subset N$ , is the model. If we are able to exhaust the elements of  $A$  and to nominate their images in  $B$ , we have every hope of creating an isomorphic model. This means that there exists a complete inverse image of  $B$  under mapping  $f$  in  $M$ , so that  $f(A) \subset N = f^{-1}(B) \subset M$ . This is the state of affairs, expressed group-theoretically, which the operational research man is trying to reach.

Now an isomorphism is important because it preserves the structure of the original group in the mapping. Typically, if it is possible to perform additions inside set  $M$ , those additions will remain valid when the same operations are performed on the images of their elements in set  $N$ . It is this persistence of relationship when the mapping is done which makes a model operate as a model. So, if  $a_1$  and  $a_2$  when added together equal  $a_n$  in set  $M$ , it can be shown that  $f(a_1)$  plus  $f(a_2)$  must equal  $f(a_n)$  in set  $N$ . Now comes the interesting comment. The conditions can be set up in which the same answer  $f(a_n)$  in set  $N$  is obtained from the mapping  $f$  whether the transformation is effected before or after the mapping occurs. That is to say, we may either add the original elements in  $M$  and transform the answer under  $f$ , or we may transform the original elements first and then add them. The result will be the same. Formally:  $f(a_1 + a_2) = f(a_1) + f(a_2)$ . When one group is mapped into another group and this condition is generally fulfilled, the mapping is called homomorphic.

These elementary definitions are included so that the argument can be made quite clear. Because it is possible to coalesce elements of  $M$  before transforming them, without losing the capability of a mapping to preserve structural relationships as discussed, it is clear that a homomorphism may have fewer elements than its inverse image. In the case of the model, then, the mapping of  $A$  into  $B$  turns out to be a mapping *onto* a sub-group of  $B$ . Isomorphism turns out to be a special case of homomorphism, in that  $f(A) \subset B$  turns out to mean  $f(A) = B$ : the one-one correspondence of elements with which we begin is maintained. But for any other sub-group of  $B$

other than  $B$  itself, homomorphism involves a many-one correspondence, and the inverse mapping  $f^{-1}(B)$  will not exhaust the elements of  $A$ .

It is suggested, then, that the models of big systems that we entertain are homomorphisms of those systemic characteristics of the big system that we can identify. The homomorphic group  $f(A) \subset B \subset N$  is the particular model we use. It is in practice extremely difficult to include in this model all the features recognized in  $A$ , and typically we do make the many-one reductions mentioned. Thus, for example, we undertake production costings as if the behaviour of all three shifts in a works were indistinguishable, and as if two similar products were identical, and as if materials were consistently uniform—although we actually know that none of these simplifications is true. Then the effectiveness of the model as predictive depends on the choice of an effective transformation by which to map. If we add up the outputs of three shifts and then transform the answer by some mapping into the model, it is no use supposing that any calculation, comparison or prediction undertaken in the model can be worked backwards through an inverse mapping which will distinguish between the shifts. On the other hand, it is necessary to handle only a third of the elements we know about inside the model. A definite choice has been made to jettison modelling-power in favour of economy in the recording and handling of data. This is acceptable, so long as the choice is deliberate rather than accidental, and so long as it is remembered as a limitation in the model.

Secondly, however, there is a further loss of modelling power in the facts that  $A$  is a sub-set of  $M$  and  $B$  is a sub-set of  $N$ . Now an interdisciplinary team of scientists can minimize the losses of modelling power due to  $B \subset N$ . Because such a team can examine all the major sub-sets of  $N$  before deciding to use one specific group  $B$ ; it may even experiment with other groups too. But the losses due to  $A \subset M$  are more serious, and may be disastrous to the exercise. For if what we recognize in a big system is not what is really important about its systemic character, the ability to predict  $A$  may not help much in  $M$ . In other words,  $A$  is itself a homomorphic mapping of  $M$ , and one which by definition we cannot properly specify. Remember that  $M-A$  was acknowledged to be systemically unrecognized from the start. We may know that our knowledge of a big system does not exhaust it, without having the faintest idea of the character of the knowledge that is missing.

It is hoped that this attempt somewhat rigorously to formulate what goes on in model-building will prove helpful in pin-pointing what we can and cannot do. The ordinary operational research exercise works, and we can see why. It is possible to advance what we understand about a stock-holding system, for example, to the point where  $A$  approaches  $M$  asymptotically. It is possible to examine most  $B$  of  $N$ , which is to say most scientific approaches to the scientific totality of understanding about such systems. If we know what the stockholding system has to do, if (as the operational research man would say) we can define its criteria of success or objective function, then we can define a homomorphic mapping  $f$  of  $A \simeq M$  onto  $B \subset N$  which preserves the stochastic relationships in which we are interested. More especially, we can do this in such a way that the inverse image of  $B$  under mapping  $f$  yields a set  $f^{-1}(B)$  of elements in the real system  $M$  which are useful.

The difficulties about doing successful operational research in various circumstances can now be made quite specific. First, the modelling will not on the average work well if  $N-B$  is large: this happens if the operational research team is not corporately versatile. Secondly, the modelling will not work at all unless  $f$  is well defined: this entails good empirical research into what the system really has to do. Thirdly, the predictions of the model will be of no actual use if a modelled outcome  $\varphi(b_1, \dots, b_n)$

turns out to have a pragmatically indiscriminating inverse  $f^{-1}(\varphi)$  in  $A$ . This also entails good empirical research into the forms of many-one reduction. Fourthly, the modelled predictions though useful will not exert what could be called control unless the  $M \rightarrow A$  homomorphism captures the systemic character of the big system *in extenso*. This again appears to be a matter for good empirical research, although there is more to say.

Contrary to increasingly current belief, then, operational research is empirical science above all. The mathematical models dreamed up in back rooms are useless unless they can meet the four kinds of difficulty enumerated, and this cannot be done remotely from the world. But that is by the way.

### Basis of Systemic Control

If the ordinary operational research study works for the reasons just given, it is easy to see why we encounter difficulty in extending its scope to the really big system. It is not the difficulty of procuring the homomorphic mapping  $A \rightarrow B$ , which practice and scientific skill can encompass. It is the difficulty of procuring the homomorphic mapping  $M \rightarrow A$ . Now if we rightly called the mapping  $f(A) \subset B \subset N$  the model, it is the complete inverse image of this model in  $M$  that constitutes a sentient control. The point is one of some subtlety, and repays a little thought. It says that deliberative control can be exerted only through the systemic connectivity of the big system that we recognize; and, moreover, that we are enabled to exert that control only via our model of how it works, with an efficiency that depends on the relevance of the mappings  $f$  and  $f^{-1}$ .

Interestingly, this deliberative control is not primarily what does control any big system. It is not adequate to the task. No one imagines that the set of actions open to being taken by a Chancellor of the Exchequer constitutes the control of the economy; it constitutes at best an error-controlled feed-back loop of either positive or negative sign. And the managing director who believes that he constitutes the control of the firm is deluded for the same reason. The reason why this must be so is given by the volume of information defining the behaviour of a big system. In cybernetics, we call the number of distinguishable states that a system may take up its variety. Clearly, the big system proliferates variety; and clearly this variety is not readily handled by a control system which cannot absorb it—which is choked by it, or confused by it, or otherwise overwhelmed. I have already said that ideally  $A$  should tend to equivalence with  $M$  under some homomorphic transformation. This was meant to ensure that the systemic structure of the big system would be preserved in the understood version of its nature which was to be modelled. What we now go on to say is that the measure of variety evinced by the big system must in some sense be paralleled in whatever is actually controlling it.

Examine this proposition in terms of the thesis already developed. We want a model of the big system  $M$  competent to determine its actual rather than deliberative control mechanisms. Since we do not, by definition, know what these are, we need to preserve a one-one correspondence of elements. What then is isomorphic to a big system? It is inconceivable that we should go away and construct another system of equivalent variety—for this really is a big system, and our model would have to be as large. The group-theoretic formulation gives the clue to the answer. Cayley's theorem declares that every finite group is isomorphic to a certain group of permutations of itself; and it is part of the proof to show that one of these is the identical permutation. In other words, the isomorphism we seek for the big system is itself. Hence the big system  $M$ , to which is clamped another system  $A$  which is in fact itself, is to be called controlled.



Do not imagine that this conclusion is vacuous. It is, after all, the realization that an isosceles triangle  $ABC$  is congruent with the identical triangle  $CBA$  that enables us to prove, without construction, that the base angles are equal. If we try to give material substance to the mathematical abstraction of the big system's isomorphism with itself, we shall, so to speak, have to think of cleaving the system in half through its plane. Then every element is twinned, and the idea of what constitutes effective control suddenly becomes blindingly clear. Every player is marked by another player; or, to use another metaphor, half the population are policemen keeping one-to-one guard on the other half.

The law of control which has emerged is, I believe, a new formulation of Ashby's law of requisite variety<sup>3</sup>. It says, in effect, that control can be exercised only by a controller having at least as high variety as the system to be controlled. This is what happens in big systems, because it must. Two halves of the system are monitoring each other; though woe betide any glib observer who imagines he can determine the boundary between them. It is not as easy as that. The two sub-sets are interpenetrated; and, one suspects, elements move in and out of both sub-sets continually. Requisite variety in a controller is the power to absorb the variety proliferated by what is to be controlled, but this power is disseminated throughout the system rather than being concentrated in a control box.

However, there is better news—if we are cautious. Electrons are continually being exchanged between any object and its surroundings, but this does not in practice deter us from identifying objects and declaring that they persist. If we had sub-atomic magnitude ourselves, it would matter very much: doubtless we should not be able to identify these objects at all. If, then, we look at our big system as compounded of sub-systems which exhaust the whole, we may well make arbitrary rulings about what constitutes one part and what another, but we shall have preserved requisite variety. For although there is no means of saying exactly what is included in any sub-system, and although we may be quite unable to analyse the relationships which subsist between the elements of these sub-systems, we shall still be able to talk about control. Control is precisely the stable state of the variety interactions between the sub-systems nominated.

How does this account differ from the original one in which the engineer looked for the servo-system within the big system? In the first place, it is exhaustive—by logical definition. But, more important, it does not see communication as a signal passing from one transfer function to the next in a thin white line. It sees communication as interpenetrative between the sub-systems which are richly interconnected—(in the limit) element by element. Yet, more important still, it sees systemic stability itself as the object of the system, and not the holding steady of an arbitrarily assigned output—which is the usual criterion of control engineering.

These outcomes of the discussion give most of the leads which are required to answer the initial question: how do we attack, comprehend and manipulate the systemic character of the universe? They involve, I fear, a revolution in thinking for the trained scientist. No longer do we aggregate bits of the system, which have been adequately investigated, into even larger well-understood systems, which eventually (but when, indeed?) ought to approximate in size and scope to the big system under investigation. Instead, we begin by carving up the not-understood big system into interacting sub-systems, and we ask behavioural questions about the resulting homeostats—which are the stably interacting parts. This can be done, and can result in useful statements about the big system itself. Comprehension begins with observations about the way in which two halves, or better still  $n$  ths, of the big system interact, although there is virtually no knowledge

about the contents or internal structure of those halves or  $n$  ths. Comprehension is extended by resolving (in the optical sense) these structures to an ever-increasing degree. But we should not imagine that we shall ever reach the atomic parts and their relationships—which are the very entities with the close examination of which orthodox science is accustomed to begin.

Anyone who finds this too puzzling should forget that he is a scientist for the moment and remember that he is a man. How is it that we can make useful predictions about the way each other's brains work? We do a reasonable job; for people do not constantly surprise us, rather the dispiriting reverse. Perhaps we do it by drawing inferences from other people's behaviour, their responses to our stimuli, over a period; perhaps we argue by analogy with our own responses; perhaps we do both. In any event we do it by making models, by mapping behavioural structures on to each other; by juxtaposing; by interacting. What we quite clearly do not do is get down to that mess of pottage, a brain newly excavated from its cranium, with a microscope. If we did, and escaped hanging, we should still not learn enough to make predictions about people's behaviour. The classical method of enquiry would encumber us with the investigation of the basic nerve cell, the atomic component, called the neurone—from which we would work outwards to the big system, the cerebrum. According to the American cybernetician McCulloch (who very possibly knows) the transfer function of a neurone is to be defined only in terms of an eighth-order non-linear differential equation. There is no guarantee that any two neurones have an identical transfer function, and the brain contains ten thousand million of them. Next, we should need to know how they arranged and interconnected. The fact that all our lives we lose about a hundred thousand a day (they just pack up) is another complication. No: it just will not do.

Return then to the notion that requisite variety is supplied in natural systems, and that deliberative control is based on a model of a sub-set of the actual control mechanisms. What the would-be controller, or manager, has to do is to intervene in these natural processes rather than to impose some control arrangement on them. Good managers intuit that this is their task. The trouble is that so many of the 'experts' who seek to advise them do not always understand this point, unless the big system is a person. In that special case, human prerogatives are invoked to say that people ought not to be controlled by the imposition of restraints on their freedom: I speak of an ethical imperative. Certainly, some restraints are imposed, in the interests of the community at large; but if we want a particular man to do a particular act we do not contemplate using a pistol, or drugs or hypnosis. We have to organize the system of which the man is a part in such a way that he does what we want of his own accord. This is the right way to control any big system, such as a firm or an economy; not because of ethical imperatives but because of cybernetic laws. For the implicit control of the big system, which requisite variety guarantees, is carrying the system to a more stable condition naturally.

The management task is to arrange its pay-off objectives in relation to the system's own built-in objective of stability. The best way to express this rule is to say that the system has an informational entropy which carries it towards a more probable state. Management needs to arrange the system so that the most probable state is the desired state. This means that the natural force of entropy is harnessed as a control force in the system. It also means that the system offers us an amplifier of our control signals free of charge, and a complete set of appropriate control circuits for distributing control signals to the right places in the system—even though we have no knowledge as to where the right places are.

If this sounds too good to be true, remember that the job can be done effectively only if we use a model which is

an appropriate homomorphism. The 'experts' of whom I complained just now are people who imagine that their heads contain this model, and that their own *savoir-faire* is a sufficiently good account of the inverse mapping. Why do economists disagree as to the correct governmental action in a given economic crisis? Surely it is because they pass off hypotheses about the way the country works as models with known mappings; so that any two of these non-models will read different deliberative control policies into the inverse image, which are quite likely to be inconsistent if not contradictory. The same is true of advisers to firms, transportation experts and so forth. The control of big systems demands a prior investigation of the natural control homomorph, and a scientific account of what entropies are involved. From this we can learn how to use the entropic drift as a control device. It is the only way to generate requisite variety in deliberative control for the inverse mapping  $A \rightarrow M$ .

### Systemic Control in 'The World'

When we come to consider applications of these arguments to the world in general it is natural to turn to national affairs. For here are the big systems with which it is most important that we learn how to deal. There are, of course, international affairs, if we wish to set the sights higher.

Now it is possible to argue that most of the troubles we encounter in deciding on policies at the national and international level derive from our habit of dividing the system, which is too big and too complicated to handle integrally by classical methods, and trying to compute with the pieces—or sub-systems. The reason why a world which is affluent in some places and penurious in others cannot find a method for controlling food supplies is surely thus. There is no need for a third of the world to be hungry, for a baby to die of starvation every forty-two seconds. But the boundaries drawn around the sub-systems, around each country and around political blocs, are too rigid. In terms of the entropies which system-oriented scientists perforce discuss, these sub-systems operate within virtually adiabatic shells. Hence there is no chance of levelling out food supplies, which constitute the energy of the total system, or (by a mapping) of levelling out human happiness and human misery. Nor is there any obvious political technique by which a Stephenson Lecturer can propose to start engineering with these conventional packages. In national affairs, there is. Let us consider some examples.

It is by now a platitude to say that the plan conceived for the future of the railways is probably wrong, but that if it is wrong the fault lies with a Minister and not with the Rail Board. This is said because the railway chiefs were given certain tasks concerned with railway profitability; they were not asked to take into account the integral system of national transportation. Here, I should agree, is a perfect example of what the logician calls *fallacia divisio*: separating components which ought to be kept together. Keeping those components together is indeed a systemic imperative. Where transport is concerned, we have most notably fallen foul of the classical mental discipline which tries to build large systems as aggregates of carefully studied elements. That people should in general be able to get from one place to another is important. But this aim has become subservient to bogus optima, whereby each element of the system must itself be 'economic'. On the railways and in the air-lanes, this criterion has been applied. So far as roads are concerned, attempts are often made to cost the journeys of the private motorist, and to show that it would be cheaper for him to travel by public transport.

Beginning with this case, we see how the systemic law is ignored. If a man lives in circumstances which necessitate his owning a car, then the car is part of his transportation system. To cost a journey which he could have

made by train on the basis that the capital cost of his car ought to be apportioned by mileage to this journey is a silly piece of accounting. He has this car anyway. The cost of his journey includes wear on the car perhaps, as well as running cost; but it certainly does not include a share of capital cost. On this basis, he will prefer to travel by road on economic grounds. Thus begins the destruction of that non-homomorphic model which calculates travel in terms of a fixed price per mile. The cost per mile of any kind of journey depends on the systemic context. It follows that the prices charged per mile by transport undertakings ought not to be invariant, as they basically are. For example, since the profitability of an air journey depends on the proportion of seats occupied, and since potential passengers are to some extent motivated by economic considerations, it follows that the price per seat for a given journey should vary with the bookings made. Thus the price paid for an air ticket reserved months in advance would include a component for the value to the passenger of a guaranteed seat. As the time of departure approaches, the price for a seat should fall. A passenger prepared to risk making no flight in exchange for a cheaper ticket would bring the plane's occupancy to an economic level. Note that a continuously computed fare based on these factors would at last begin to engineer with the uncertainty of demand, founding itself on high-variety systemic interactions between the air service and need, and between the air service and competition.

This kind of approach takes note of how the world is; it begins to construct a homomorphic model which can be used for deliberative control. At present, our controls are attempted impositions of rules which do not pay off because they struggle against the tide of entropy. In such cases the proper resort of management is to engineer with the structure of the system, until the entropies created in interaction with the world yield what the airline wants, namely, a profit. A similar argument may be applied to the railways, which have tried by advertising to condition the passenger to believe that 'it is quicker by rail'. Often, it is not quicker. Often, the potential passenger is less impressed by speed than by comfort, cleanliness and a sense of privacy. His selection entropies may then be drifting in a direction that does not conform to the systemic reference frame set up by the railway management. The battle for custom is then lost. If a passenger prefers to be fussed over by a pretty air hostess than insulted by a ticket collector, it is no good protesting that he should have more sense: and the best conclusion is not necessarily to close the railway line. It may be to abandon the attempt to match a high-variety demand with a low-variety service. Not even a monopoly can force through the repeal of the law of requisite variety.

Nor, indeed, can a whole powerful ministry repeal that law. The task of controlling road traffic, when attempted by policemen or by local regulators such as traffic lights, is a high-variety task unsuccessfully handled by low-variety control devices. It is quite obvious that requisite variety cannot be obtained to handle a problem of the magnitude now made manifest without massive sensory input to large-scale computing engines. Yet the use of computers is regarded, at worst, as a novel gimmick; and, at best, as an expensive innovation which perhaps must come as part of a technological upsurge. We never see the argument, based on fundamental thinking, that there are systemic imperatives which can be analysed and must be met. It is not surprising, then, that we see no attempt to derive the homomorphic model of national transportation which would serve as the basis for well-informed research into the deliberative control of movement. Such a model must be global, following the principles adduced in the preceding section. Ideally, we should use satellites to photograph the ebb and flow of traffic, in order to obtain the systemic conspectus that we need. There are less

ambitious ways of doing so. But the London Traffic Survey does not even have this aim. It represents the old-fashioned, non-systemic approach of trying to understand the elemental components of the system. Life is too short.

The result, moreover, is that we do not begin to approach the cybernetic problems of traffic control which really are important. If we had a homomorphic model of the interactions of sub-systems of traffic, we could begin to work on the serious issues that affect the dynamics of all big systems. (So far we have not discussed dynamics at all, only the structural aspects of systemic control.) Now the great problem in cybernetic dynamics is this. Every sub-system, however defined, is itself seeking a local stability. The big system as a whole seeks stability in terms of such local equilibria. But the homomorphic model of the big system, as expressed through the interaction of sub-systems, is difficult to express in homogeneous terms. The reason is timing. If a sub-system is to be regarded as having an input which is the output of another sub-system, then it is vital that the relaxation time be consonant with the periodicity of the perturbations arising from the input sub-system. Otherwise, the receiving sub-system will remain in perpetual uncontrolled oscillation. Moreover, from the point of view of deliberative control considered as an inverse mapping of the model, there is a problem of scale. One sub-system will see an interconnected sub-system as pumping variables into its equations of motion; another will see the same input as setting its basic parameters. Which is which will depend on the relative relaxation times of the receiving sub-system to the two input sub-systems. There is no need, surely, to press these problems to an audience of engineers. The point is that we have no idea how to handle them, because we have not even recognized that they exist, because we have not even begun to think out the model that reflects them.

They are, nevertheless, the problems which have to be solved: and if they exist for traffic management, they exist for economic management too. Moreover, if it is wrong to cut up the transportation system into separate units, it is wrong to cut up the national fuel system into separate units too. Who is there in the country at all who understands the systemic interaction between coal and gas and electricity and oil and atomic power? No one, so far as can be discovered, has begun to consider the homomorphic mapping of this integral whole. The examples are capable of indefinite extension. They seem to indicate a wholesale failure to understand anything at all about the prerogatives of systems. I hope it will now be appreciated why such care was taken to expound a methodology which can at least begin to discuss such matters, and why it is important to see that there has to be a reversal of the classical trend whereby science advances from the element to the system. It is because we have always done this, I repeat, that we have not to this day encompassed the really big system at all.

This difference of approach may be examined in the context of another big system which is particularly important to us all, namely, technical education. The future of this system and the demand on it were investigated in a famous White Paper, it may be recalled. The method of this enquiry, it will be no surprise to recollect, was to build up the 1970 requirement for technologists from the atomic components of employment as it existed in the past. There was no attempt to assess systemic interaction, nor the systemic trajectories of the future. In another paper<sup>4</sup> I have tried to expose the fault in this enquiry, and to show how the cybernetician would approach the task of modelling the actual control mechanisms involved.

There is one last point to make about the prerogatives of systems in the world at large, and it is this: Because of the habit of dividing, for administrative convenience, what should be kept together, we often identify as special

to a part what is really a function of the whole. This causes us to try to measure, as a sub-systemic variable, some factor which might best be understood as a behavioural (that is, output) characteristic of the entire big system. An example which springs to mind from recent work concerns marketing. Everyone who spends money on sales promotion would like a measure of advertising effectiveness. This is, inevitably, sought in the change in sales value that follows in the wake of a campaign. But it is notoriously difficult to measure this change and to relate it to the appropriation—because of systemic interaction. There are time-lags, competitive effects, changes in conditions, and so on. It has become increasingly likely, to me, that the desired measure is not only impossible to make but actually invalid. The flavour of the argument, which cannot be set out here at length, is obtained by asking where in the engine of a car is to be found the vehicle's speed.

This sort of consideration can be contemplated with competence from the vantage point of an effective homomorphic model alone. For example, mention has already been made of the National Health Service. This centres on a system of patients and doctors interacting, each set providing the other with requisite variety. But there are several sub-systems interlinked with this one, and interpenetrating with it element by element. Two most notable such sub-systems are the regional hospital organizations and the pharmaceutical profession. All these sub-systems seem often to be uncoupled in the administrative and indeed the professional mind. Then the interesting question is how to resolve such a problem as the appropriate payment of all concerned. Here perhaps is another example of a global behavioural characteristic, masquerading as an entire set of sub-systemic variables. Certainly it is a problem for engineering in large systems to resolve, rather than a matter of local accountancy.

### Systemic Control in 'The Flesh'

There are various levels at which we can examine the relevance of systemic laws to animate systems, considered as distinct from the world that is not aware. If we chose to discuss the individual living creature, we should find many examples to enlarge our cybernetic understanding. For *Amoeba proteus* and *Homo sapiens* have this in common: they are both very big systems compounded of sub-systems, and their character is systemic or it is nothing. Indeed, if we nominate viability as the objective of any organism, whether animate or pseudo-animate (as a firm or an economy), it is natural to turn to biology for advice. Cybernetics has sprung from biology, and has quickly involved physics and engineering, rather than the reverse, for perfectly sensible reasons. It is wise not to forget this, nor to ignore its lessons.

But the example of the operation of systemic laws 'in the flesh' that I shall take here comes from a different level of viable organization. I want to talk about the process of decision in a management group. Now decision theory, so-called, we have; it is an investigation appropriate to mathematical statistics. But decision theory assumes a uni-dimensional model of the world; it says that we know the terms in which we should rightly talk, and that we know the interactions that are important; it is not systemic. The process of decision in a management group is, on the contrary, systemic in character. The reason why it often takes many years to reach a complicated decision is because this is not understood.

Consider, then, such a decision. For example, where, and when, and how should the country add to its steel-making capacity by building a new plant? All manner of people will have views on this, arguing from every sort of expertise. The management problem is to find coherence in the spate of advice. Some of it will be factual; some of it will be inspirational masquerading as factual;



most of it will be contingently factual—that is, 'what I am saying is true, if something else is true, but not absolutely'. It is the contingent advice which specifies the system of decision; but unfortunately most of it is not recognized as contingent. It is here, then, that operational research is needed, so that the decision space and its configurations may be rightly understood. But operational research will not take this decision. It will be taken by a large number of people, each of whom is guaranteeing some sub-decision of the whole. The control problem for the highest authority is to monitor the decision-taking procedure to which all the sub-deciders are contributing.

The classical approach to this situation is to regard a very minor sub-decision as a basic component, and to build up a highly complex total decision from these elements. Hence some junior metallurgist somewhere may declare it obvious that a metallurgical reaction is best obtainable in a certain way, and begin to dictate the whole technology to be used. An economist looks at the siting problem in terms of the technology he knows about; and so on. But, according to the arguments in this article, we should not build up a very complicated specification from single components of decision. There is another way of looking at the matter. We do not really start with a set of elemental propositions, but with an infinitely large number of high-variety, integral alternatives. We do not really finish with a highly complicated specification for action, but with a unique integral answer. So the decision-taking procedure is not a process of building up more and more variety, but of eliminating more and more uncertainty. How can this procedure best be controlled?

We said that the decision space had to be defined and that it is composed of possible answers. That is to say, every alternative integral answer might be considered as a small square. The decision space would then be the totality of these alternatives—say, a million small squares. For convenience, we might consider them as arranged in a square array, or matrix. This decision space will be ranged over by any decision procedure, in a hunt for the right answer. A management group normally undertakes the task as a heuristic search, which goes on by elimination. The average number of steps taken before the right answer is recognized is evidently half a million, since there is no reason why the right small square should be encountered either sooner or later than any other. True, sub-sets of small squares may be eliminated at once if they share a recognized characteristic regarded as damning; on the other hand, there may be a great deal of wasteful hunting back and forth between two or more sub-sets of small squares, each of which looks as if it might include the right answer. Note that this procedure is precisely obeying the control law of requisite variety. The variety of the decision space (a million alternatives) is matched by a control process organized to examine (if necessary) a million squares.

However: the natural variety proliferation of the world can be matched by variety generation—which I define as a deliberative control device. The decision space in our example is a square array. If we can identify the co-ordinates of this matrix as logical variables, then we can specify any small square (including the right answer) as a logical couple. To do so, we must search the co-ordinates. But the variety of each co-ordinate is 1,000; so the search space is 2,000 (instead of 1,000,000), and the expectation is that we find the answer in 1,000 steps instead of in 500,000. Thus the variety generator fulfils the law of requisite variety (since it has the selection power to discriminate between all the alternatives) but is a deliberative controller five hundred times as efficient as the natural controller.

All this is said to demonstrate the point. In practice, there is no reason why the decision space should be two-dimensional. Indeed, if a given decision had only two

logical variables, it would not be a difficult decision. Then it has  $n$  logical variables, and the decision space is an  $n$ -dimensional space. Then the variety generator is yet more efficient. For the expectation that a set of integral alternative answers of high variety  $V$  will yield up the wanted answer by a general search is  $\frac{1}{2}V$ . Whereas the expectation for an  $n$ -dimensioned search with variety generators is  $n/2.V^{1/n}$ . We have found in principle a method of dealing with large-scale decisions which capitalizes on the systemic structure of the problem. It is just not true that decision-takers normally do take this advantage, by some analogue of this algorithm, as empirical research into actual decisions reveals. But it remains to show how we shall take advantage of it in practice.

First of all, it is necessary to identify the logical variables which are relevant. That is to say, we need to know all the dimensions of the decision: what sorts of things have to be specified to make the decision definite. This process is not one of listing all the statistical variables which affect the magnitudes eventually to be used: that side of the job is done by applied mathematics. We want to know what factors must be accounted for before the decision counts as a decision. The steelworks decision, for example, is not specified unless it includes the location of the works, its output of ingot tons, its range of products, and so on. These are the logical variables. The systemic interaction of these variables defines the configuration and dimensionality of the decision space. The statistical variables will eventually determine the optimal *modus operandi* of the steelworks decided on.

The number of logical variables is  $n$ , that is the dimensionality of the decision space. But the variety-generator notion enables us to observe that the  $n$ -space involved is not just a square, nor a cube, nor a hypercube: it has a configuration. Suppose, for example, that it is sub-decided for sufficiently powerful reasons to have a steelworks of given ingot tonnage, or at any rate within some range of tonnages. This automatically delimits a range of reasonable costs, a collection of possible sites, and so on. We do not yet know what the elements of these sub-groups are; but we can see that the decision space is inwardly contorted in various ways. There exist logical inferences, which we have not worked out, for any combination of logical values that may be fixed for any of the logical variables. In that case we must try to express the decision space in terms of its logical variables to give structure to the systemic configuration which the real world tends to proliferate.

The way to do this is through symbolic logic. This is the language of science for handling relatedness: conjunctions and disjunctions, implications and inclusions. The decision we are after has a known number of logical dimensions, for we have listed them. The systemic structure of the decision will be given by a logical formula stating how the logical variables are interrelated. Now this is a model in the sense defined. It requires a mapping of the way the external world works in relation to this decision. We have its dimensionality; we have its configuration. Next, we must measure its variety. In fact, this is not too difficult. Some logical variables are binary, in which event they offer a single alternative—a one-bit decision. For example, the steelworks must either be integrated with an ironworks or not. Other logical variables may propose a group of choices. For example, there is a finite number of steel-making processes between which, or between combinations of which, a decision must be taken. Suppose that, in a given case, there are eight choices. The variety of this decision is 8, not 2. This is a three-bit decision: it is composed of three binary decisions:  $2^3 = 8$ .

We have now discovered how to measure the variety of the decision, which is to say the uncertainty which has to be removed in searching for the completed specification. The measure is, of course, an entropy; not a thermodynamic entropy, nor the entropy met with in statistical

mechanics, but an informational entropy—an entropy of selection. But, however we view it, the statistic that computes its value is the same. It is:  $H = - \sum p_i \log_2 p_i$ , where  $p_i$  is the probability of the  $i$ th choice. (We can ignore Boltzmann's constant in this application.) For every logical variable in the logical formula, we must compute this entropy.

In the case where the choice is a straight alternative between equiprobable outcomes, the entropy is  $H = -(0.5 \log_2 0.5) - (0.5 \log_2 0.5) = 1$ . This is the classic one-bit decision, and it shows why we are accustomed in cybernetics to use logarithms to the base 2: the answer is measured in the units of information theory, namely, bits. Now, in any application, there may be reasons why one alternative is more probable than another, and this will disbalance the equation. For example, if a committee of ten people is divided nine-to-one on the first showing of hands, the uncertainty to be resolved is less than one bit: If we wish, we may use this kind of fact as a measure of probability. Thus:  $H = -(0.1 \log_2 0.1) - (0.9 \log_2 0.9) = 0.469$  bits. But in general, we may consider that all alternative choices are nominated as equally probable. In the case that there are  $m$  alternatives, the equation is  $H = -m \cdot 1/m \log_2 1/m = \log_2 m$ , which is, of course, simple to compute.

By summing the selection entropies appropriate to each logical variable, we obtain a measure of the uncertainty that has to be eliminated in reaching the decision. This quantified formula is now a control device of requisite—and measured—variety. For any attempted specification of the decision, put forward by any party to it, has a certain entropy that can be computed; and the difference between this number and the number of the logical formula measures directly the inadequacy of the proposed answer. Moreover, if we start with a given level of uncertainty, we may progress our decision-taking by computing the uncertainty eliminated by any agreed sub-decision. Evidently, when all the uncertainty has been eliminated, the entropy is zero—because the probability that this is indeed the answer is unity. In other words, the decision has been taken.

In practice, the rate at which uncertainty is eliminated by a decision procedure will be some kind of negative exponential decay function. Empirical research will establish the family of curves which it is appropriate to use as control optima, and fiducial limits can be set up. Then we have an error-controlled feed-back loop with which to monitor the progress of the decision-taking. In a particular sphere of decision, moreover, particular clues to the uncontrolled situation will emerge. For example, whatever the decay function for uncertainty, it will surely be monotonic-decreasing. Hence if, in the course of plotting the progress of the decision-taking, a sudden upsurge in the graph should occur, it is clear that something has gone wrong. Uncertainty is being imported into the system. This will usually be due to an unacknowledged change in the objectives of the decision. It is clear evidence that the logical formula ought to be re-examined, and perhaps subdivided. Here again is one of those control devices which operate on unanalysed systemic blocs, rather than on elemental components.

This procedure, which is here made public for the first time, seems to be a powerful tool. It is simple to use, though difficult to understand for anyone who has not undertaken the preliminary methodological thinking given in this paper. Its particular merit, I believe, is its capability to quantify a logical structure. Hitherto, it has been virtually impossible to disclose a mode of inference which would couple together, in one technique, the power of logic to specify structure (which is much greater than that of mathematics) and the power of number to compute comparisons and the parameters of control (which is much greater than that of logic). It is a tool that has special value in the context of national decisions, such as those discussed in the preceding section; because the

specification of each such decision is so great, and the parties to it so numerous and so dissociated organizationally.

### Systemic Control in 'The Metal'

Having discussed, however cursorily, the relevance of the pervasive notion of system and its natural laws to the world and the flesh, we finally reach the metal. The metal stands for machinery, for devices, for artefacts built by man. Here, surely, we do understand the laws of system; here, one might hope, there is nothing to say.

Unfortunately, this is not the case. Where relatively small systems are concerned, the laws of system as understood by control engineering are happily ascendant. But we make big systems, by the criteria used before, in hardware too. In particular, we make computers, and we sponsor automation. It would be uncouth to repeat now all that I have so often said and written before on these topics<sup>5</sup>. But a few points ought to be made in the terms established by this text.

First, a system of automation is a deliberative control, that is, as has been stated, an inverse mapping of an  $M$  on to an  $A$ . It is vital that the laws of requisite variety be upheld; yet no automatic controller yet devised begins to match the variety of an ordinary works. Consequently, the Buddhist principle of control has generally been applied. It is the central, perhaps the only, idea of Buddhism that since we can never reach our targets of desire (and are thus condemned to frustration) we should alter those targets to equate with whatever we in fact attain. If what we are and enjoy becomes our target, behold, we have attained it after all. This is exceedingly good cybernetics; requisite variety is ensured, not by generating variety in our lives, but by eliminating variety in our ambitions. It seems to me that our notion of automation depends on this rule: the factory must behave in the way we have engineered to control it.

The result of this is that automation has been applied only to relatively straightforward, mass production situations—which are low-variety worlds. The jobbing works, on which the British economy largely depends, has so far been immune—*qua* big system. Sub-systems have been automated, it is true; and the computer-controlled machine tool is an example. But if we rightly understand and apply the systemic laws of cybernetics, it will not prove impossible to make automation much more general. The secret is, of course, to use computers as variety generators. Operational research does precisely this with its simulations; but engineering seems to think more in terms of variety reduction.

Thus we have the spectacle of the logical space of a general purpose computer programmed down to low-variety control, as, for example, in its application to the cut-up line of a rolling mill. This seems to be as wasteful of decision-taking capacity as is the programming down of a high-variety human being to being a machine-minder. There are no ethical objections in the former case as there are in the latter; but there are much stronger economic objections. These are the source of the marginal pay-off from computer applications in real time, which in my opinion is in turn a major cause of the slow advance of automation itself. The on-line control computer ought to be sensorily coupled to events in real time, and ought to have a motor output similarly coupled. But in between it ought to be uncoupled to a greater extent than is required to undertake a few simple-minded calculations on its inputs. In particular, it should have the facility to simulate the outcomes of possible decisions and to choose between them. This is not at all difficult to do; it is not even difficult in principle to equip the machine with a learning facility through which to profit from its own experience. The trouble is that managements do not commission such applications, and engineers do not offer to make them. The reason is, I strongly urge, not the imagined difficulty, but a very real insensitivity

to the systemic rules of the game. As we are trained in the scientific methodology of analysis rather than synthesis, so we are trained in the Buddhist principle of control. We are variety murderers to a man.

An interesting example of this contention is found in the machine translation of language. A language is a high-variety system *par excellence*. The history of machine translation shows how science ignored this fact, and set out to translate automatically using low-variety control. It obtained, with chagrin but quite predictably, very bad translations. But a start had been made. Gradually the research began to acquire for the automatic translator some of the variety it had manifestly required from the outset. Crude syntactical paradigms were replaced by more elaborate ones; stored vocabularies grew. But the methodology is the old familiar one. We are aiming to reach requisite variety by the exhaustive enumeration of possible states, and we are still a long way off. Yet the computer as such is by no means short of variety; the inadequacy has been in the variety of the structuring of the decision space. We are not using variety generators, once again.

Surely the clue to this problem is the way in which people learn to translate (note the word 'learn'). We do not set out to programme children to be good translators. The trick is, rather, to give them a rudimentary grammar and vocabulary with which they are encouraged to experiment. They proceed to generate variety all right; the only trouble is that the output is unacceptable. So we monitor the output, using a skilled linguist (called teacher), who himself has requisite variety. He observes the defects in the homomorphic mapping of acceptable language on to the child's language. He then corrects the child by reinforcing successes with rewards and damping down failures with reproof. In systemic terms he uses what I have called the algedonic (pleasure/pain) loop. It is not difficult to devise an artefact of this loop inside a computer: all one needs is a simple conditional probability model to supply the criterion of retrieval. Instead of the rather naïve notion that a stored linguistic association is either present or absent, we want the stored material to be more or less likely to emerge—in accordance with a balance of systemically connected probabilities, gained by experience, that the answer will be praised or blamed.

I do not think we should be too diffident in seeking to imitate in such ways what are apparently human prerogatives, for they may be nothing more than systemic prerogatives—which is what this lecture is about. Before becoming emotional about words such as 'think' and 'create' it is well to define in behavioural terms what counts as thinking or creating. If this proves to be possible, it seems to me inevitable that science and engineering between them can devise a machine with comparable behavioural characteristics. This is all we need. It is interesting to ask whether such machines are 'really' thinking and creating, and if so how, and if so whether human beings do the job in the same way or some other. But to declare that machines can think, or that machines cannot think, seems as meaningless as it is pointless. I should refuse to mount the scaffold on behalf of any such proposition. But bear this in mind: whatever we may reckon as to genuinely human prerogatives, and as to the possibly divine nature of man, what we see men actually do is organized to be done by their brains: and the brain itself is a control machine.

A good and a final example of what I mean concerns the trick of procuring arbitrarily reliable outputs from a machine with arbitrarily unreliable components. Considered in engineering terms, the brain is a frighteningly defective apparatus, and yet it serves us well. It was because John von Neumann posed the question: "How do we retain any semblance of humanity when we have had too much to drink?" that cybernetics found out how the brain works this apparently impossible trick. As is now quite generally known, the answer is that it generates

the requisite variety both structurally and operationally through redundancy: redundant nodes, redundant channels, redundant control complexes, redundant calculations. We have the theorems by which all this works; we even have a special logic of neural nets by which to describe it. But, again, engineers do not want to use all this knowledge. They have been brought up to believe that components ought to be reliable, and they design as if they were reliable. But no one has ever seen an actual component that did not have a measurable, finite probability of failure: and in a big system, a complex ensemble of components, these probabilities are multiplicative.

The result is that we refuse to send one redundantly controlled rocket to the Moon to make a few observations; we cannot afford to accelerate 'unnecessary' componentry to escape velocities. It is apparently better to fire six rockets, beautifully engineered and shorn of redundancy, none of which gets there. Again, we cannot afford the high cost of redundancy in a railway signalling system. After all, if perchance it should go wrong, the accident inspector will declare that it was 'one of those things'—a one-in-a-million chance. Personally, I regard myself as one of several million passengers; and my ghost would doubtless take a poor view of these odds. Yet again, then, we cannot bear to tell industrialists that they should consider installing redundant automation, because the expense might frighten them off the project. We prefer that they should refuse to accept automation on the grounds that it might go wrong.

It does not, in fact, seem to be totally ludicrous that we should be thinking in terms of a new kind of systems engineering, in the metal—as in the world and the flesh. It would be an engineering appropriate to big systems, aimed at providing requisite variety for control, equipped with variety generators, redundant circuitry, and an interpenetrative connectivity. Consider the following vision. Here is a vast plant, a huge, complex interacting system. Its engineers have built control equipment into it, of a subtle cybernetic kind. The plant is therefore self-regulatory (we know that trick). It is to some extent self-organizing (we begin to know this trick too). The plant is adaptive to environmental changes (we have discussed how this could be done through algedonics). But the built-in control systems keep going wrong. The teeth of the cogs drop out, the transistors fail, wires come adrift, whole packages burn out. The engineers, however, have seen all this happen in the brain. They have noted that brains do not continually 'go off the air' for maintenance: and so they have copied the brain's trick for handling unreliability, no longer complaining that things sometimes go wrong when manifestly they cannot always go right.

This works continues to work, despite all. It is not attended by an army of white-coated technicians armed with soldering irons, as some prophets have supposed. For the built-in control equipment is designed to outlast the plant. Do not say this is impossible; you and I are the evidence that it is possible. Do not say that it is impracticable; we have not even tried, and the principles are known. Say, if you must, that you are not going to do it, that you cannot afford it, or that it is time to go home; let us know where the onus lies.

There are systemic laws to apply, which could change the face of Britain—as George Stephenson once changed it. Twenty years before the *Rocket* ran that illustrious man nearly dug out the brain drain; for he was disheartened, and contemplated emigration. Some of us here have done the same. May we instead learn from his example, and see the visions through.

<sup>1</sup> Beer, Stafford, "Below the Twilight Arch: A Mythology of Systems"; chap. 1, *Systems Research and Design* (John Wiley, 1961).

<sup>2</sup> Beer, Stafford, *The Cost Accountant*, 42, No. 6, June 1964 (also obtainable from SIGMA, Ltd., as a pamphlet).

<sup>3</sup> Ross Ashby, W., *Design for a Brain* (Chapman and Hall (London), 1952; revised edition, 1960).

<sup>4</sup> Beer, Stafford, *Operational Res. Quart.*, 13, No. 2 (June 1962).

<sup>5</sup> Beer, Stafford, *The Manager*, October and November, 1963; *Metra Journal*, 3, No. 1 (1964) (also obtainable from SIGMA, Ltd., as a reprint).



## SHORTAGE OF SCIENCE APPLICANTS TO UNIVERSITIES\*

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THAT members of the conference on the above subject have responded to an invitation to attend shows that they, like those of us in the University of Edinburgh, really care about what ultimately happens to those young people who pass through the hands of all of us. It also shows that we care that our institutions of education should provide, in sufficient numbers, the kinds of men and women who can promote the welfare and growth of the British economy. Now since the British economy is that of an industrialized society, in which we depend, in large measure, on the application of science, by way of technology, for industrial strength and expansion, we are vitally concerned with supplying that society with both managers and workers trained in science and technology. And when I mention people trained in science and technology I include mathematicians as well, so long as they are mathematicians who display vigour, even sometimes at the expense of complete rigour, in their approach to their subject.

Now our supply of people capable of adding to our scientific capital, by way of research, depends on education. Similarly, our supply of people capable of using technology, and of adding to it by design and development, also depends on education. But the education of people of the kind I have mentioned is conducted in two tiers, school and university, and both are represented at this conference.

Now it would be quite wrong of me to attempt to compare these two tiers of education—school and university—in importance. Yet I venture to point out that it is often at school that a student's interest in science is engaged and his enthusiasm for it fostered. Moreover, I imagine that most people are attending the conference because of the influence of some particular teacher in this respect. I certainly know that this applies to me.

Now there are certain careers for which it does not matter much what courses have been pursued at the university. We can all agree that one essential function of university education is to develop the student's capacity to learn, to understand and to think on his own. For that purpose alone the degree course taken is often said to be immaterial. Now this may be all very well for people who join, say, the administrative grades of the higher Civil Service, for they become trained on the job, so to speak. But it is quite different with scientists, technologists and mathematicians who join the Scientific Civil Service, or industry, as specialists. They cannot learn the elements of their trade on the job. They must be soundly versed in fundamentals at the start, even if, as happens frequently in industry, they require further initiation in the particular technology which is the concern of the firm they serve.

Now, with our present gearing of the two tiers of education, the decision that an individual is going to pursue a scientific or mathematical career has to be taken at school; and, with things as they are, there is something wrong, from the point of view of the country's needs, at the school stage of decision. Not enough people are deciding to prepare for a university career in scientific subjects the mathematical content of which is high; and not enough young people are deciding, on university entry, to choose the applied sciences as distinct from the pure sciences.

So one of the questions for us to-day is this: "How does it come about that engineering is relatively so unpopular?" Some people have said that one reason for this is that engineering is not a school subject—generally, that is. I put the question recently to the president of one of the engineering institutions—the big three: "Should Engineering be a school subject?" and his answer was: "Certainly not. Concentration there should be on mathematics and the basic physical sciences". From the school side it is said that university engineering is too difficult: the failure rate is high and this has seeped through to the schools. The same is said of university mathematics courses. "If you want a degree take something else."

Now this leads me on to observe that one effect of the publication of the Robbins Report has been to infect people with what I may call 'degree worship'. Universities were at one time enjoined by the University Grants Committee to attempt to increase their first-year science entries at twice the rate at which they increased their arts entries. But all that has now gone since the publication of the Robbins Report.

The provision of a university education is taken as an act of social justice, and universities must do the best they can to meet 'consumers' choice'. There are two consequences here. Young people tend to choose subjects for their degree which interest them—which is, at least, understandable. But what is really unfortunate is that many of them, with a degree alone in mind, choose subjects which they deem to be easy ones, regardless of the effect of this on their career prospects after leaving the university.

Now this situation of *laissez faire* cannot, in the country's interests, be allowed to continue. We must bring home to school pupils, to university students, that in so many cases the choice of leaving examination subjects at school, and the choice of a degree course at the university, will determine their prospects of employment hereafter.

To help young people, as well as parents and school and university teachers, there is a need for forecasts of future employment prospects. This applies especially to science and technology if we are to stress the attractions of careers in these subjects. Something of the kind is being done already by one or two professional institutions. But there is need for an official, central, employment forecasting agency to keep such matters under continuous review.

Take, for example, the newly instituted Ministry of Technology. Its objectives have been described but not, as yet, the methods whereby those objectives will be achieved. Exhortations from Whitehall will be useless unless someone, Government or industry, can specify the methods by which innovation can bring about industrial expansion and can indicate, to institutions of higher and technical education, the requirements in terms of scientific and technological man-power which these will entail.

Now I have only two matters further to mention. They are both concerned with what I have called the gearing between school and university. I have received evidence from some parents that, in certain cases, school pupils are entered for higher subjects in their leaving examinations merely because they are good at them, and can therefore swell the number of passes which the school can claim; and this is sometimes done without regard to the requirements of university entry and the pupils' future career.

\* Substance of the opening address delivered at a conference on the "Shortage of Science Applicants to Universities" held in the University of Edinburgh on December 12.

The other matter arises from the shortage of sixth-form scientific and mathematical teachers; and is expressed simply in the question: "Can the university take over some of the elementary science and mathematical teaching which is at present in the care of the school?"

We have all heard much about the difficulties impeding science teaching in schools—shortage of staff, lack of space and equipment. I appreciate that one reason for

the shortage of science and mathematical teachers is that there are, nowadays, so many other attractive careers, outside the schools, which are open to them. Then there is the unfortunate fact that many young graduates have come to regard teaching as a disgruntled profession and therefore an uninviting one. It may seem like that from the newspapers; it does not seem like that when you talk to a science master about his own pupils and their prospects.

## NEWS and VIEWS

### Engineering in the Massachusetts Institute of Technology: Prof. J. C. Keck

DR. JAMES C. KECK, an authority on high-temperature gases and plasmas, has been appointed, as from July 1, a Ford professor of engineering at the Massachusetts Institute of Technology, where he will establish a Laboratory for Atomic and Molecular Kinetics within the Institute's School of Engineering. Dr. Keck is at present a principal scientist at the AVCO Corporation's Everett Research Laboratory, Everett, Mass. The new Laboratory will be established within the Department of Mechanical Engineering, but its facilities will be available for teaching and research to staff and students from all engineering departments. Its main aim will be the application of basic knowledge gained from studies on the structure and behaviour of atoms and molecules to applied engineering problems of present-day interest, such as re-entry aerodynamics, heat-shield design, power generation and conversion, rocket propulsion, etc. Dr. Keck's own scientific interest in recent years has centred on basic studies of gases that are heated by shock waves, with particular reference to chemical reactions, radiation phenomena, and ionization processes that occur at the atomic and molecular levels in such gases. He was deputy director of the AVCO Everett Laboratory from 1960 until 1963 and has been chairman of the Laboratory's Atomic Physics Committee since joining AVCO in 1955. Dr. Keck gained a B.A. degree in physics at Cornell University in 1947 and a Ph.D. in physics, also from Cornell, in 1951. He was a research associate at Cornell in 1951–52 and a Senior Research Fellow at the California Institute of Technology from 1952 until 1955. At both institutions, he worked on problems in nuclear physics, particularly photonuclear reactions, using high-energy synchrotrons.

### Computing in the University of Leeds and Directorship of the Electronic Computing Laboratory: Prof. G. B. Cook

DR. G. B. COOK, senior lecturer in applied mathematics in the University of Leeds, has been appointed professor of computing in the Department of Mathematics and director of the Electronic Computing Laboratory in the University. Dr. Cook was educated at Owen's School and Selwyn College, Cambridge. He read the Natural Sciences Tripos Parts I and II and the Mathematical Tripos Part III, in which he shared the Mayhew Prize. He remained at Cambridge during the period 1950–53 as a research student in the Department of Theoretical Chemistry and the Mathematical Laboratory, and he was awarded the degree of D.Phil. in 1954. From 1953 until 1957 he was on the staff of the Basic Research Division of the Royal Armament Research and Development Establishment at Fort Halstead. He went to the University of Leeds in 1957 as lecturer in electronic computing and was promoted to the directorship of the Computing Laboratory in 1960. He relinquished this post to become senior lecturer in applied mathematics in October 1964. During his time in Leeds, he has had a close association with

Lyddon Hall, having been a sub-warden for part of the period. Dr. Cook has served on committees of the Scientific Advisory Council and of the British Standards Institution. Since 1963 he has been a member of the Council and of the Education Committee of the British Computer Society. Recently he was appointed a member of the Church of England Board of Education. He is also a Fellow of the Institute of Mathematics and its Applications and of the Physical Society. His research interests include computational problems in quantum theory and diffusion, and he has published several articles on these subjects.

### Theoretical Chemistry in the University of Bristol: Prof. A. D. Buckingham

DR. A. D. BUCKINGHAM, who has been appointed as the first holder of the new chair of theoretical chemistry at the University of Bristol, graduated in 1951 from the University of Sydney, with first-class honours in chemistry and the University Medal. He obtained his M.Sc., under Prof. Le Fèvre, before proceeding to Cambridge on a Shell scholarship, to work with Dr. J. A. Pople. He moved to Oxford in 1955 on an 1851 senior studentship and, in 1957, became a student of Christ Church and a University demonstrator in the Inorganic Chemistry Laboratory. His early association with Le Fèvre gave him a lasting interest in electromagnetic polarization phenomena. At Oxford he has carried out and directed both theoretical researches and experimental investigations on the optical, electrical and magnetic properties of molecules, and on intermolecular forces. Dr. Buckingham gave the Tilden Lecture of the Chemical Society for 1964, has served as visiting lecturer at Harvard (1961) and is shortly to spend a period at Princeton. As well as being a distinguished theoretician, he is a cricketer of some stature; he has played for the University of Sydney, New South Wales (although not in the Sheffield Shield series), the University of Cambridge and, for the past few years, in the Duke of Norfolk's XI at Arundel.

### Agricultural Economics in the University of Manchester: Prof. W. J. Thomas

MR. W. J. THOMAS, reader in agricultural economics in the University of Manchester, has been appointed to a newly established chair of agricultural economics as from January 1. Mr. Thomas was educated at St. David's College School, Lampeter, and the University College of Wales, Aberystwyth. In 1939 he was awarded a degree of B.Sc.(Wales) in agriculture, and in 1940 the honours degree of B.Sc. in economics with agricultural economics. After graduation he joined the staff of the Department of Agricultural Economics at Aberystwyth and was awarded the degree of M.Sc.(Wales) in 1945. During this period he also worked on the Scientific Food Committee and on several regional and national committees connected with the war effort. From 1946 until 1949 he was senior agricultural economist in the Ministry of Agriculture in London, the duties of this post involving the collection and collation of material relating to the economic position

of the agricultural industry and the use of this material for price review and agricultural policy. In 1949 he was appointed lecturer in agricultural economics at the University of Leeds, a post which he held until 1953 when he was appointed reader in agricultural economics and head of the Department at the University of Manchester. His own research work has been mainly in the field of agricultural policy and on the economics of grass-land production and utilization. He has served on various national and international committees on agricultural problems.

### Vulcain Reactor

IN reply to questions in the House of Commons on December 22, the Joint Under-Secretary of State for Education and Science, Mr. J. Boyden, said that basic engineering and physical work in the research and development programme for the *Vulcain* reactor was now also complete, and a *Vulcain*-type core would be loaded in the Belgian experimental reactor *BR3* in mid-1965 for irradiation to high burn-up. The cost to the Atomic Energy Authority so far was about £1.6 million. The decision to proceed with a prototype fast-breeder reactor must await the outcome of the present development programme based on the experimental reactor at Dounreay and particularly on the design of the fuel.

### The Museum of London Bill

AT the Committee Stage of the Museum of London Bill in the House of Lords on December 21 and in answer to questions from Lord Chorley and Lord Airedale regarding the position of staff transferred from Guildhall Museum and the London Museum, Lord Champion confirmed his assurance that there would be no down-grading of staff transferred from either Museum, and added that the Government would always be prepared to meet representatives of the staff associations to consider these matters. It was inevitable and natural that the terms and conditions of service of the staff of the new Museum should be nearer to local government than to Civil Service practice. The whole question had already been carefully considered, but final decisions could not be taken until the Bill became law and the Board of Governors was finally appointed. Fair and attractive terms would be offered to the staff and these terms would be maintained fair and attractive. There were at present about 70 staff in the London Museum and only seven in Guildhall Museum. While it was accepted that there must be negotiating machinery, it did not appear appropriate to insert a clause making arrangements for negotiation and arbitration statutory. (See also p. 237 of this issue of *Nature*.)

### Improvement in the Status of Technology

IN a written answer in the House of Commons on December 23, the Secretary of State for Education and Science, Mr. M. Stewart, said that, in accordance with the recommendations of the Interdepartmental Committee on Improvement in the Status of Technology, a working party had been set up under his Department's chairmanship, on which the Departments were represented and the Joint Council of Engineering Institutions had been represented at recent meetings. The working party would rely chiefly on continuing to stimulate the interest of the Press and television, and there were close consultations between the working party and television authorities, who were responding to the need for programmes of technological interest. A programme of special Government publicity, prepared with the aid of the Central Office of Information, would include films, exhibitions and publications, and an exhibition on the theme of 'Applied Science' would be held at the end of December in conjunction with the annual conference of the Association for Science Education at the Imperial College of

Science and Technology, South Kensington. The working party was reconsidering the Inter-Departmental Committee's recommendations regarding careers advisers in the schools, the provision of career literature and the promotion of films and broadcasts featuring technological subjects. The Committee's report had been considered by the University Grants Committee, which was taking up with the universities those recommendations that affected them, especially the question of flexibility in courses.

### Anglo-Soviet Co-operation in Agricultural Research

AN agreement was signed in London on January 6 giving effect to arrangements for future co-operation between the United Kingdom and the U.S.S.R. in agricultural research. The agreement, which runs for five years initially, provides for: (a) exchange of information and, as appropriate, of scientific material and visits between scientists and institutions of the two countries on some 61 specified research topics under seven general headings—crop production, soil science, plant protection, livestock production, veterinary science, farm mechanization, and water economy; (b) exchange of scientific delegations in 1965; (c) designated 'co-ordinating centres' in each country; (d) a further meeting of experts in London later in the year, and thereafter annual meetings, to keep the working of the agreement under review. At the signing ceremony, the Minister of Agriculture, Fisheries and Food, the Rt. Hon. F. Peart, M.P., paid tribute to his predecessor, Mr. Christopher Soames, who promoted the idea. On behalf of the U.S.S.R., the agreement was signed by the Soviet Ambassador, His Excellency Alexander Soldatov. Speaking to the Soviet delegation, Mr. Peart said: "Her Majesty's Government welcome the agreement we have just signed as a valuable extension of our friendly relations with your Government. It holds out the promise of really useful co-operation in a field where a glance at the agreement itself shows each of our countries has much to offer the other. . . . Our scientists and scientific institutions will, I know, be working very hard to gain the maximum benefit, for both countries, from the agreement, and in doing so they will have at all times the strong support of myself and my colleagues".

### First Report of the Expert Committee on Drug Toxicity

IN August 1962 the Association of the British Pharmaceutical Industry set up an 'Expert Committee' to advise on certain aspects of the testing of drugs for toxicity. The terms of reference of the Committee were: (1) To review laboratory testing procedures for the assessment of drug toxicity and make recommendations. (2) To make recommendations for promoting the exchange of, and the prompt utilization of, knowledge and experience in this field. (3) To advise on technical and scientific problems of toxicity testing. The first report of the Expert Committee on Drug Toxicity covers only the first term of reference, and does this very adequately (Pp. 24. London: Association of the British Pharmaceutical Industry, 1964). It explains the reasoning behind the toxicity studies which should be carried out before a new substance is given to man, and makes recommendations concerning the tests which should be performed. The report is clear and concise and should be read by everyone who is interested in the toxicity of drugs.

### Industrial Behaviour and Personnel Management

WITH the increasing pace of technological change, it is imperative to make people aware of the need to adapt. It is also necessary to develop specialists in the analysis of the social and psychological problems which accompany technical and economic change. Prof. T. Lupton, Montague Burton professor of industrial relations in the University of Leeds, has put forward his belief in a recent publication from the Institute of Personnel Management that the behavioural sciences are now sufficiently well



developed to make such a specialism possible (*Industrial Behaviour and Personnel Management*. (*Industrial Relations Series*.) By Prof. Tom Lupton. Pp. 59. London: Institute of Personnel Management, 1964. 7s. 6d.). If society as a whole is to reap the benefits of modern technology, social structures must be adapted rapidly and appropriately to meet its demands. Prof. Lupton doubts whether the problems we are facing will yield to "the mixture of philanthropic ideology and administrative technique which are the typical basis of much personnel management at present", and he sees a completely new role for the personnel manager. His booklet should cause many readers to re-examine cherished beliefs, and many personnel managers to consider their adequacy for their existing positions.

### Scientific Research in Schools

THE seventh annual report to the Council of the Royal Society of the Scientific Research in Schools Committee records that 108 science teachers are carrying out investigations with the assistance of the Committee and that the Council's grant of £2,000 has been supplemented by £1,000 from the Atomic Energy Authority for teachers wishing to pursue research of interest to the Authority (Pp. 16. London: The Royal Society, 1964). Further support has been received from 11 industrial concerns. Of the projects, 39 broadly fell into the field of biology, 37 of chemistry, 13 of physics, and 9 of geology. As an experimental extension to encourage the development of applied research in schools, a project was also supported for two school science teachers to join one of the research teams of the Proctor Department of Food and Leather Science, University of Leeds, for 4 weeks during the summer vacation. Besides details of the schools involved and the research projects, the report includes a list of publications in connexion with the projects.

### Choosing a Career

THE series of pamphlets issued by the Central Youth Employment Executive deserves the attention of all young people who have not decided what to do with their lives. The pamphlets cover a wide variety of possible careers, including the various branches of medical and veterinary work, various hospital services and social work, and careers in the arts, in business and in crafts ranging from plumbing to woodwork, stonemasonry and journalism. No. 1 in the series is a general booklet which discusses the points which should be considered before a choice of a career is made. No. 111, *Veterinary Science*, details the preliminary qualifications required for work in this field, and indicates its scope, which includes various kinds of laboratory and field work, as well as veterinary practice. The booklet (Central Youth Employment Executive. *Choice of Careers*, No. 111: *Veterinary Science*. Second edition. Pp. 28+9 photographs. London: H.M. Stationery Office, 1964. 1s. 3d.) also gives information about the available courses of training, their cost, and their location, with the addresses to which application must be made. The booklets can be obtained from H.M.S.O. or from booksellers and their prices vary from 6d. to 2s. 6d.

### American Expedition to New Guinea

IN 1959 the American Museum of Natural History sent an expedition to New Guinea consisting of Leonard J. Brass, leader and botanist, and H. M. Van Deusen, mammalogist. Although the sixth expedition to the Island under the Richard Archbold Foundation, it was the first to cover the north-east—that is, the trusteeship territory of New Guinea—and was based on Lae (*Bulletin of the American Museum of Natural History*, 127, Article 4: *Results of the Archbold Expedition*. No. 83; *Summary of the Sixth Archbold Expedition to New Guinea* (1959). By L. J. Brass. Pp. 145–216+plates 2–3. New York:

American Museum of Natural History, 1964. 2.50 dollars). J. D. Collins, owner of a young coffee plantation, joined them, and they were assisted by six regular employees recruited locally, some with experience of preceding expeditions. Although Brass discusses briefly his classification of vegetation types and compares it with that of Robbins, used as part of the land-use investigations begun in 1953 by the Australian Commonwealth Scientific and Industrial Research Organization, the expedition was essentially to make collections of mammals, amphibians, reptiles and insects, and of plants, for deposition in the American Museum of Natural History—consequently enriched by some 55,000 specimens. After a brief general description of the area, illustrated by 23 excellent photographs, Brass mentions previous collecting expeditions and then details the itinerary from March 22 until December 20. Extensive use was made of air reconnaissance, but there is high praise for the 'Land Rover' used to cover the main journeys. Half the report is then taken up by the description of the 14 collecting stations. In view of the intense activity of Australian scientists over the whole of the eastern half of New Guinea, it seems a little unfortunate that there was apparently little liaison, and five years seems a long time to have waited for the summary report.

### Information Bulletin of the Soviet Antarctic Expedition

PROMPTED by Dr. G. de Q. Robin's review of Volumes 1 and 2 of the Elsevier translations of *Information Bulletin of the Soviet Antarctic Expedition* (*Nature*, 204, 110; 1964) and the pending publication of Volume 3 (the three volumes will cover *Bulletins* 1–30), Dr. Waldo E. Smith, executive secretary of the American Geophysical Union, has written to the Editor as follows: "It is possibly of interest to your readers to know that the American Geophysical Union is continuing this series, more or less on a serial basis . . . Nos. 31–42, inclusive, are available on subscription at a price of 36 dollars and will be published under six covers. The first six of these numbers have already been produced in English edition. It is one of our purposes in undertaking this series to get these on a current basis in order that they might be of maximum use to those engaged in Antarctic studies. We anticipate the other six to be available during the next few months, ending perhaps in January or February. We have plans to continue the series at least through No. 52". Further information can be obtained from Dr. Waldo E. Smith, Suite 506, 1145 19th Street, N.W., Washington, D.C. 20036.

### Queen Elizabeth Forest Park

THE Queen Elizabeth Forest Park in Scotland was established by the Forestry Commission to commemorate Her Majesty's Coronation in 1953. The Park lies within easy reach of Edinburgh, Glasgow and much of industrial Scotland, and is on a well-known and much-frequented touring route. It includes Ben Lomond, the Trossachs, and the undulating forests of Loch Ard and Rowardennan, as well as many picturesque tree-lined lochs. Situated in its midst is the village of Aberfoyle which forms a convenient 'centre'. Within the Park are two distinct types of landscape. To the south and east lie the gently undulating valleys and fertile farmlands of the River Forth, while to the north and west lies the rugged scenery so characteristic of the Scottish Highlands. The park thus lies across the division between Highlands and Lowlands and is crossed by the Highland fault marking the geological division between the two. The *Short Guide to the Queen Elizabeth Forest Park* (Pp. 14+4 photographs and map. London: H.M. Stationery Office, 1964. 1s.) describes its topography, history, botany, forestry and animal life, as well as the literature associated with the Forest. Amenities include the David Marshall Lodge which was presented to the nation by the Carnegie United Kingdom Trust in 1961.

### Medical Subject Headings

It has been announced that *Medical Subject Headings*, the cross-reference guide to the *Index Medicus* and the medical subject heading authority list published annually by the National Library of Medicine, is to be significantly revised. The 1965 issues will contain 579 additional indexing terms, making an overall total of 6,380. The new terminology will be concerned with the following categories: anatomy (category A); diseases (category C); chemicals and drugs (category D); and analytical, diagnostic, and therapeutic technology and equipment (category E). These terms reflect the content of 222 journals which were previously not covered by *Index Medicus*. A total of 208 other journals are being deleted from the *Index Medicus* list. The newly indexed journals cover the fields of dentistry, chemistry, psychology, sociology and veterinary medicine. Indexing terms in *Medical Subject Headings* will continue to appear in alphabetical and categorical lists. Although the format will not change, the alphabetical list will be composed for the first time in upper and lower case letters by GRACE —'Graphic Arts Composing Equipment'—a component of the Library's computer system. *Medical Subject Headings* will be published as Part 2 of the January 1965 *Index Medicus*. Yearly subscriptions to *Index Medicus* are available from the Superintendent of Documents, Government Printing Office, Washington, D.C., at 40 dollars (9 dollars additional for foreign posting). Single issues of *Index Medicus* are available at 3.75 dollars and single copies of *Medical Subject Headings* at 2.50 dollars.

### Research on Immunology

ALL scientists are nowadays aware of the rapid growth and increasing range and importance of research related to immunology. World Health Organization Technical Report No. 286 records the views of the members of five international groups convened by the Organization to consider various aspects of this subject (*Research in Immunology*. Pp. 97. Geneva: World Health Organization; London: H.M.S.O., 1964. 5 Sw. francs; 8s. 6d.; 1.75 dollars). The views expressed do not necessarily represent the views of the Organization or its policy. The subject is discussed under the following five headings; immunoprophylaxis and immunotherapy; immunopathology; tissue antigens and transplantation; immunochemistry; a final section entitled "Programme in Immunology", which includes sections on the training of immunologists and recommendations for research.

### Programmed Instruction Book on Semiconductor Devices

A NEW linear programmed instruction book on semiconductor devices has been published by the Mullard Educational Service. It is based on information previously published by the Service as *A Simple Explanation of Semiconductor Devices*. The book treats the subject in a non-mathematical way, emphasis being given to the physical aspects of electron and hole conduction. A supplementary booklet containing coloured diagrams illustrating semiconductor action is contained at the back of the book. This supplement is referred to by the reader as he progresses through the programme. In a programmed instruction book of this kind the subject is broken down into many small numbered units called frames. Each frame requires from the reader a written response which is compared with the model answer given alongside the frame. The reader goes through the programme at the rate which he finds most natural—an advantage of this new system of learning. A short test included at the back of the book should be attempted before and after completing the programme. In this way the reader is able to assess how much he has learnt from the book. Copies of the book (price 3s. 6d. including supplement) are available from the Mullard Educational Service, Mullard House, Torrington Place, London, W.C.1.

### High-speed Photography

Barr and Stroud manufacture two ultra-high-speed cameras, the 'CP5 Framing Camera' and the 'CP6 Streak Camera'. The 'CP5 Framing Camera' uses the Miller system of optics. The image of the event formed by an objective lens is focused on the surface of a rotating mirror. The light reflected from the rotating mirror sweeps across a number of lenses arranged in an arc, which focus the image on the mirror on to a strip of film. Several different optical systems are available for different framing rates and numbers of pictures. One system, for example, enables 117 pictures, 8-mm diameter, to be taken at  $8 \times 10^6$  pictures per second with an exposure time of 0.12  $\mu$ sec per picture. Facilities are available for synchronizing the camera and the event and for terminating the exposure. A camera of this type was used for the work described in *Nature*, 202, 443; 1964. The 'CP6 Streak Camera' consists of an objective lens which focuses the image of the event on a narrow slit, which is focused by a second lens on to an arc of film via a rotating mirror, which sweeps the image of the slit along the film. The maximum writing speed is 22 mm/ $\mu$ sec with a recording time of 27  $\mu$ sec. Using a 0.05 mm slit a time resolution of  $10^{-8}$  sec is possible. The facilities for synchronization and capping are similar to those of the framing camera.

### Announcements

MR. AUSTEN ALBU, M.P., has been appointed as a member of the Medical Research Council. Mr. Albu is a Governor of both the Imperial College of Science and Technology and Battersea College of Technology. He has been chairman of the Parliamentary and Scientific Committee since 1962.

PROF. A. G. WARD, head of the Procter Department of Food and Leather Science in the University of Leeds, has been appointed chairman of the U.K. Food Standards Committee for a period of three years as from January 1 in succession to Mr. M. W. Perrin, who is giving up the appointment because of pressure of work and other commitments.

THE seventh Castner memorial lecture entitled "Some Achievements in Petroleum Chemicals" will be delivered in London by Dr. H. M. Stanley on March 8. Further information can be obtained from the Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "The Formulation of Pesticides", arranged under the auspices of the Pesticides Group and the Colloid and Surface Chemistry Group of the Society of Chemical Industry, will be held at the School of Pharmacy, London, during March 30–31. Further information can be obtained from the Honorary Secretary of the symposium, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

THE nineteenth annual symposium on fundamental cancer research on "Developmental and Metabolic Control Mechanisms and Neoplasia" will be held in the University of Texas during March 4–6. Topics under discussion will include: biosynthesis and control mechanisms; molecular basis of early development; molecular basis of later development and control; comparative studies on control mechanisms in normal and neoplastic tissues. Further information can be obtained from Dr. D. N. Ward, Department of Biochemistry, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston 25, Texas.

ERRATUM. The communication entitled "Iron-deficient Low-temperature Pyrrhotite", which appeared on p. 175 of the October 10, 1964, issue of *Nature*, is by F. J. Sawkins, Dr. A. C. Dunham and Dr. D. M. Hirst, and not F. J. Sawkins, Prof. A. C. Dunham, F.R.S., . . . as stated in the contents page of that issue.

## ADULT EDUCATION IN BRITAIN

IN opening a debate on adult education in the House of Lords on November 24, Lord Greenhill said there was a real increase to-day in the demand for adult education in Britain. There was, in spite of the expansion in publicly provided education, a still greater demand for advanced education, both technical, cultural and non-vocational, which we could not supply. He urged that additional accommodation, particularly in residential colleges, should be made part and parcel of Britain's normal provision of adult education. He was strongly supported by Lord Hill of Luton, who urged that this was one of the great tasks of to-day following the educational policies of the past twenty years. Those who sought adult education, in the sense in which the term was used by Lord Greenhill in opening the debate, must undergo a disciplined study under the guidance of a competent teacher. Television could play a part in providing this disciplined study only if other links could be created between the teacher and the taught. This would not be easy, but it would be worth while trying to take the fullest advantage of an immensely powerful medium of mass communication, without forgetting that the personality and power of the teacher were essential. Baroness Elliot of Harwood suggested that the adult education organizations should approach the question from a more modern point of view than in the past, seeking to introduce some modern ideas into the classes provided. She also suggested that the pamphlet on consumer education, recently issued by the Research Institute for Consumer Affairs, indicated exciting possibilities for the fusion of adult education and consumer education, starting from practical issues and indicating how adult education and consumer education could create the informed, self-respecting and 'concerned' persons whose influence could be so great on the mind of the society in which we lived.

Lord Chorley directed attention to three groups requiring support. First, there were the evening institutes, and the evening schools for technical education which had scarcely received the recognition they deserved; then there were the refresher courses which were becoming increasingly important in the professions and to which the Robbins Report devoted some attention; the third

group was the movement for bringing up to date the adult population, as a whole, in regard to knowledge—with-out it the modern world was incomprehensible and the adult citizen could not play a proper part in society. Besides this there was the general cultural education mentioned by Baroness Elliot, and Lord Chorley also supported Lord Hill's remarks concerning the need for proper teaching. Lord Francis-Williams thought that, contrary to the views of the Pilkington Committee, establishment of a fourth television channel entirely devoted to education would be useful. He thought we should consider more carefully the possible development of local radio and television linked to the universities, particularly the new universities, which might join with the local education authorities and local adult education associations and possibly with some of the existing correspondence colleges for the purpose. Baroness Gaiskell also believed that this idea of a university of the air would be worth exploring and could be more effective if combined with correspondence courses. She hoped that the Government would put adult education high on its list of priorities in education.

Lord Geddes suggested that a less academic view should be taken of some of the features of adult education, that the Albemarle Report should be taken more seriously, and that adult education for the less intellectual part of the population should be linked with some of the social service activities. He suggested that more research should be encouraged into the causes of anti-social behaviour, and that in this connexion we might consider the possibility of non-military national service which would cover, under disciplined procedures, many facets of social service, such as aid to the old, hospital service, service in under-developed countries and agriculture.

Lord Aberdare, replying to the debate for the Government, pointed out that a good deal had already been done for adult education in Britain: grants to adult education had more than doubled in the period from 1954-55 to 1964-65 and the number of full-time posts had increased from 254 to 311; he also directed attention to what had been done in sound broadcasting in the field of adult education.

## MUSEUM OF LONDON BILL

IN moving the second reading of the Museum of London Bill in the House of Lords on November 26, Lord Champion explained that the London Museum originated with the first Lord Harcourt, and was started in 1911 with private funds made available to form a collection of suitable objects. It soon succeeded in creating interest and attracting gifts and loans. King George V gave permission for the Museum to be housed in the State Apartments and other parts of Kensington Palace where the Museum was opened to the public in 1912. Kensington Palace was never intended to be more than a temporary home, and in 1913 Sir William Lever bought the lease of Stafford House and gave it to the Nation for the joint purposes of housing the London Museum and providing a setting for Government hospitality. The Trustees of the London Museum then placed their collections at the disposal of the Government for the term of the lease of Stafford House, and as long afterwards as the collection should continue to be exhibited there or in some other equally suitable building maintained by the Government. The Government accepted these offers, and undertook financial responsibility for maintaining the London Museum, and from that time onwards the Trustees, apart

from some *ex officio* ones, were appointed by the Prime Minister. The Museum was accommodated in Lancaster House, as Stafford House was re-named, from 1913 until the Second World War when most of the collections had to be put into safe storage and Lancaster House began to be used for international conferences. In 1940 the original lease of the building expired and a new forty-two-year lease was taken by the Ministry of Works from the Crown Estate. After the War the Government decided that the building would continue to be required for conferences and official hospitality and that another home would have to be found for the Museum. In 1949 King George VI gave consent for part of Kensington Palace again to be used by the Museum for a term of fifteen years from December 1950, and this term has been extended by Queen Elizabeth for a further five years to 1970.

Guildhall Museum was founded by order of the Court of Common Council in 1826. The first contribution to the collections consisted of Roman and other antiquities found during the excavations of the foundations of the new post office in St. Martins le Grand. The original concept of the Museum was simply that of an adjunct to



the Library where objects could be seen to illustrate books on the Library's shelves. In 1872, when the new Library wing was opened, the Museum was housed in the basement beneath it and opened to the public. It has continued to be closely integrated with the Library, and the Library Committee of the Common Council is directly responsible for its administration. Since 1921 the Museum has been supported financially by the City of London Corporation. All the objects in Guildhall Museum are connected with the City, many of them consisting of archaeological material, including much that has been unearthed in the course of excavations connected with the re-building of the City since 1946. Guildhall Museum has always been closely associated with the scientific examination of sites cleared for re-building, and it has been generally agreed that all objects found during excavation should be given to the Museum. During the past eighteen years the total number of specimens in the Museum has increased by more than fifty per cent. As a result of war and war-damage the Guildhall collection moved about, and eventually the exhibition, containing about 10 per cent of the collection, came to rest in the Royal Exchange; most of the collection was left in store at Guildhall.

Like the London Museum, Guildhall Museum is thus still waiting for adequate accommodation in a permanent home. Both are essentially local museums, although the London Museum obviously has a far more than local interest. From this situation arose the idea that the accommodation problem might be solved by bringing the

two Museums together under one roof with the cost shared between the Treasury and the County Council and the City of London. The essence of the scheme is that the two Museums will be amalgamated to form a new Museum of London, housed in a new building to be erected for the purpose on a site in the City of London. The capital and running costs will be borne jointly and equally by the Exchequer, the City Corporation and the Greater London Council, which has agreed to accept the obligations entered into by the London County Council in respect of the project. The Museum will be managed by a board of eighteen governors, six being nominated by each of the three authorities. An Interim Board of Governors was constituted on these lines in May 1962, and a Director and Deputy-director-designate were appointed and a firm of architects was invited to prepare a preliminary scheme and estimates. This scheme provides for the Museum building to be on six floors, arranged around a garden court, with its east side forming the base for a 200-ft. high office tower block which will be leased separately by the City Corporation and will be so arranged that some 19,000 sq. ft. of space can be made available for expansion of the Museum at a future date. The Bill provides for payment by the Treasury of a sum not exceeding £150,000 in respect to furniture and equipment, and for the staff of the Museum to be brought within the Corporation's superannuation scheme. Staff salaries will be determined by the Corporation after consultation with the Treasury and the Greater London Council.

## THE BATTELLE INSTITUTE, GENEVA

**I**NDEPENDENT consultative and sponsored research in industry has long been established practice in the United States, perhaps more often than not on a commercial rather than a strictly professional scale, as has been the general tendency over the years in Britain, although to this latter traditional precept there are to-day some noteworthy exceptions successfully established in Britain.

The original aim of the founder of 'Battelle', Gordon Battelle (Battelle Memorial Institute, Columbus, Ohio), was "... to contribute to industrial development by disseminating and using scientific knowledge for purposes of discovery and invention, thereby furthering technical progress". Battelle now has three research centres, at Columbus, Frankfurt and Geneva, with six branch offices in Washington, Los Angeles, Paris, London, Madrid and Gothenburg. It has thus established active and regular relations with industrial and scientific centres, as well as Government authorities throughout the western world; its activities are spreading to some of the so-called developing countries.

A booklet entitled *Ten Years' Research at the Battelle Institute, Geneva\**, has recently been published, marking the completion of ten years' work of this organization in Geneva; it presents a well-documented and illustrated picture of some of the many and varied activities of the

Institute at this particular centre. It is described as "... an invitation to European industry to visit the Institute". The impact of this well-designed and profusely illustrated publication can scarcely fail to impress the reader with the scale on which the Institute operates not only at Geneva but also internationally. A selection of some of the important disciplines covered by this centre includes: economics; applied mathematics; semi-conductors (solid-state physics); electronic paramagnetic resonance; optics; ultra high-vacua; metallurgy and physical properties of materials; refractory ceramic materials; electrochemistry; physical processes of measurement and analysis; electrical engineering and electronics; mechanical engineering; industrial high polymers and petrochemistry; foodstuffs; pharmaceutical and phytopharmaceutical products; chemical engineering; and mathematics and theoretical physics. In connexion with some of these subjects there have resulted "... a total of 252 inventions" in the past few years at Geneva, "... and 1,266 patent applications have been filed in different countries; 587 patents have so far been issued, 221 of them in countries where prior patentability studies are carried out".

The booklet includes 192 references to publications with which the work and staff of the Institute have been concerned in the course of the research projects referred to here. For obvious reasons much sponsored research is of necessity highly confidential as between client and the Institute: to this extent the full story of achievements of the Institute cannot be divulged.

\* *Ten Years' Research at the Battelle Institute, Geneva*. Pp. 71. (Geneva: Battelle Institute, 1964.) Copies are also obtainable from the Battelle Institute, Ltd., 15 Hanover Square, London, W.1.

## COSMOLOGY IN THE U.S.S.R.

**T**HIS account summarizes articles which have appeared in *Priroda*. A. G. Nikolaev and P. R. Popovich (1, 1; 1963) provide photographs of the Earth's surface as seen from a sputnik. G. A. Skuridin (1, 3; 1963)

discusses cosmic physics—the radiation belt of the Earth, interplanetary plasma, primary cosmic radiation, magnetic fields of the Earth and the Moon and short-wave solar radiation. V. V. Shuleikin (4, 53; 1964) gives an account



of experiments on a weightless fluid as observed in a cosmic ship and in a falling vessel. V. V. Kozlov and E. D. Suludi-Kondratiev (6, 44; 1964) discuss lunar geology and tectonics, and they suggest the name "lunite" as a name for the lunar prevalent pumiceous rock, which probably contains 60–65 per cent of silica. K. A. Kulikov (6, 51; 1964) also discusses the problems of the Moon. D. A. Frank-Kamenetsky (11, 17; 1963) suggests that the origin of elements in the solar system is best explained by a hypothesis already proposed by him, namely by their synthesis in a 'cold' gaseous plasma. Ya. B. Zel'dovich (6, 25; 1964) also applied the 'cold' process to the evolution of the pre-star matter consisting of pure hydrogen. N. A. Vlasov (9, 20; 1964) discusses antimatter in the cosmos, while the formation and evolution of organic matter in the cosmos are discussed by A. I. Oparin (6, 29; 1964), (2, 18; 1963) and D. M. Troshin (12, 89; 1963), life on Mars by V. G. Fesenkov (2, 22; 1963), and a review of recent books dealing with life in the cosmos is given by an anonymous reviewer (12, 80; 1963). A description of a newly constructed astrophysical laboratory at Pulkovo, near Leningrad, is given by L. A. Mitrofanova (8, 44; 1964).

I. S. Astapovich (7, 121; 1964) reviews a book on the Tunguska meteorite edited by G. F. Plekhanov and published by Tomsk State University in 1963, being a report on the 1959–60 expedition. The area of fallen trees measures about 2,000 km<sup>2</sup> with thermal effects in its centre. Meteorite fragments were not found. It is suggested that the meteorite did not fall on the Earth but passed through the atmosphere along a parabolic

trajectory. On the other hand Ya. Ya. Yakovlev (9, 113; 1964) describes minute globules of magnetic matter found in the soil in this region. He suggests that the Tunguska meteorite was really due to the explosion of the nucleus of a comet passing through the atmosphere. I. S. Astapovich (10, 87; 1963) reports that on July 12, 1948, a bolide was observed in south-western Turkmenia and that later fragments of iron were collected in the Karakorum desert, suggesting that the bolide was a manifestation of a iron meteorite or a meteoric shower. I. A. Yudin (3, 58; 1964) also reports that in the spring of 1961 a bolide was observed in the Kurgan district and that in July 1963 an iron-bearing olivine-pyroxene chondrite weighing 21.8 kg was found in Kargopolie of Kurgan district. This meteorite was named "Kargopolie". I. S. Astapovich (5, 84; 1964) provides a short review of the alleged falls and problems of ice meteorites. B. I. Vronsky and K. P. Florensky (3, 90; 1964) provide a review of recent work done in the estimation of the quantity of cosmic dust falling on the Earth. A. A. Yavnel (8, 112; 1964) presents a report of the eleventh Meteorite Conference held in Moscow during May 26–30, 1964, when, among other subjects, the origins of chondrites and carbonaceous chondrites were discussed. It was also suggested that the so-called Tunguska meteorite was really due to an exploded comet. K. Tucek (9, 19; 1964) presents a short report on a conference on 'Tectites' held in Czechoslovakia during June 6–7, 1964, mainly to study local tectites, formerly called moldavites and now called vltavites, after the river Vltava, which the Germans called Moldau.

## MAGNETIC EVIDENCE FOR TRINUCLEAR CLUSTERS IN RHENIUM(IV) CHLORIDE

By DR. R. COLTON and PROF. R. L. MARTIN

Department of Inorganic Chemistry, University of Melbourne

**R**HENIUM(IV), with an electronic configuration  $5d^3$ , usually shows a magnetic moment corresponding to three unpaired electrons in its octahedral halocomplexes of the type  $M_2ReX_6$  (ref. 1). In contrast, the magnetic moment of rhenium(IV) chloride appears to be anomalously low<sup>2</sup>, suggesting the presence of some unusual structural feature.

Recent determinations of the crystal structures of caesium tetrachlororhenate(III)<sup>3–5</sup>, rhenium(III) chloride<sup>6</sup> and certain other halocomplexes of rhenium(III)<sup>7</sup> have revealed that in all these compounds the basic structural unit consists of three rhenium atoms in a triangle with one halogen atom bridging each pair of rhenium atoms. The remaining halogens are arranged approximately at the corners of an octahedron around each rhenium atom.

It seems likely that such clusters may also occur in oxidation states other than rhenium(III) and indeed with other elements. Accordingly, we have re-examined the recently reported magnetic properties of rhenium(IV) chloride and find that they can, in fact, be nicely accommodated on the basis that the compound contains trimeric units,  $Re_3Cl_{12}$ .

The bonding and magnetism of rhenium(IV) chloride are tractable in terms of a molecular orbital (M.O.) description. We follow here an L.C.A.O.-M.O. treatment in the Hückel approximation of the type foreshadowed by Bertrand, Cotton and Dollase<sup>4</sup> to describe the bonding in the diamagnetic  $[Re_3Cl_{12}]^{3-}$  anion. We assume that the  $Re_3Cl_{12}$  molecule belongs to the point group  $D_{3h}$  and that the immediate environment of each rhenium atom is comprised of five chlorine atoms arranged at the corners of a slightly distorted octahedron (Fig. 1). The sixth lobe of

an octahedral  $5d^26s6p^3$  hybrid  $\sigma$ -set centred on each rhenium is then naturally orientated towards the centre of the  $Re_3$  triangular core, three such lobes forming a basis for M.O.s with the irreducible representations  $a_1'$  (bonding) and  $e'$  (anti-bonding) in the point group  $D_{3h}$ . Likewise, the residual set of  $5d$  orbitals of each rhenium atom forms a basis for M.O.s which can be classified in  $D_{3h}$  into the species  $a_1''(d_{yz})$ ,  $e'(d_{xy})$  and  $e''(d_{xz})$  (bonding) and  $a_1'(d_{zz})$ ,  $e''(d_{yz})$  and  $a_2'(d_{xy})$  (antibonding). It is sufficient for the present purposes to estimate qualitatively the magnitude of the splittings from the directional properties of the  $5d$  orbitals using the assumption that the splittings between a given bonding orbital and its antibonding counterpart are roughly proportional to the

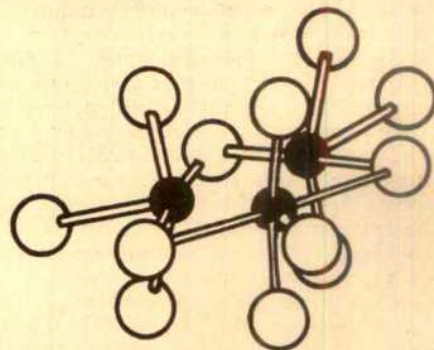


Fig. 1. Structure proposed for  $Re_3Cl_{12}$ .



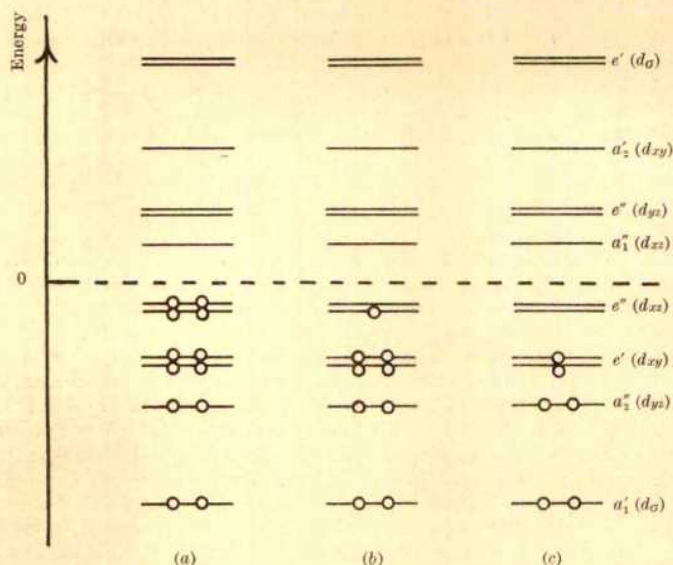


Fig. 2. Highest molecular orbitals for  $\text{Re}_3$  core of: (a)  $\text{Re}_3\text{Cl}_3$  and  $\text{Re}_3\text{Cl}_{12}^{3-}$ ; (b)  $\text{Re}_3\text{Cl}_{12}$ ; (c)  $\text{Re}_3\text{Cl}_{12}^{3+}$ . Not drawn to scale. Point group symmetry  $D_{3h}$ .

overlap integral. The bonding scheme which emerges for the  $\text{Re}_3$  core of the 'united' trimer is given in Fig. 2. For rhenium(IV) chloride, a ground state electronic configuration  $(a_1')^2 (a_2'')^2 (e')^4 (e'')^1$  should be associated with a single unpaired electron per trimer with a consequential magnetic moment of 1.0 B.M. per Re atom. Like  $\text{Re}_3\text{Cl}_3$  and  $\text{Re}_3\text{Cl}_{12}^{3-}$ , Re-Re metal-to-metal bonds cement the  $\text{Re}_3$  core in  $\text{Re}_3\text{Cl}_{12}$  into a structurally stable unit.

The variation with temperature of the magnetic susceptibility of rhenium(IV) chloride is given in Table 1. Extrapolation to infinite temperature of a plot of molar susceptibility against reciprocal absolute temperature shows the presence of a substantial temperature independent contribution to the paramagnetism of about  $230 \times 10^{-6}$ . A re-evaluation of the experimental data with the inclusion of this term reveals that the magnetic moment of rhenium(IV) chloride is 1 B.M. and virtually independent of temperature as required for the trimer model.

Table 1. MAGNETIC PROPERTIES OF RHENIUM(IV) CHLORIDE

Temp ( $^{\circ}\text{K}$ )	$\chi_M \times 10^6$	$\mu(\text{B.M.}) = 2.84[(\chi_M - 230) \times 10^{-6} T]^{1/2}$
304	654	1.02
275	695	1.02
255	730	1.01
240	766	1.02
215	807	1.00
189	888	1.00
158	1,016	1.00
143	1,132	1.02
123	1,311	1.03
114	1,418	1.04
96	1,693	1.06
92	1,840	1.09
87	2,054	1.13

Only one method of preparation of rhenium(IV) chloride has so far been reported, namely, the action of thionyl chloride on hydrated rhenium dioxide<sup>8</sup>. Unfortunately, when prepared in this way the material is amorphous and the highly desirable X-ray diffraction patterns cannot be obtained. However, strong corroborative evidence for the existence of metal-to-metal interaction in rhenium(IV) chloride is available both from its chemical properties and the magnetic properties of some of its simple derivatives. For example, when rhenium(IV) chloride is dissolved in aqueous alkali, a red-brown solution is obtained which possesses the same remarkable inertness to chemical attack as that exhibited by acidic solutions of rhenium(III) chloride. Furthermore, although exactly one-quarter of the total chlorine content of  $\text{ReCl}_4$  can readily be precipitated as silver chloride, the remaining chlorine can be

extracted only by fusion with alkali<sup>8</sup>. Such facts are consistent with a single labile chlorine atom attached to each rhenium atom of an extremely stable  $\text{Re}_3\text{Cl}_3$  core. The X-ray examinations of the rhenium(III) compounds noted earlier have revealed that each in-plane terminal Re-Cl band is abnormally long and chemically reactive<sup>3-7</sup>. It is also relevant that the monomeric phosphine derivative of rhenium(IV) chloride,  $\text{ReCl}_4(\text{PET}_3\text{Ph})_2$ , synthesized by indirect methods<sup>9</sup>, has a magnetic moment of 3.64 B.M. ( $20^{\circ}\text{C}$ ), which is normal for the  $d^3$  configuration of octahedral rhenium(IV).

An interesting feature which emerges from our simple L.C.A.O.-M.O. treatment is the prediction that a trinuclear rhenium(V) cation  $[\text{Re}_3\text{Cl}_{12}]^{3+}$  may exist which could be of comparable stability to the trimer structures already known.

The magnetic properties of this cation would decide which of the  $e'(d_{xy})$  and  $a_2''(d_{yz})$  bonding M.O.s lies lower since the alternative ground configurations  $(a_1')^2 (e')^4$  and  $(a_1')^2 (a_2'')^2 (e')^2$  are diamagnetic and paramagnetic, respectively. This question cannot be unambiguously resolved in the present approximation and we are therefore attempting to prepare and isolate such a species.

Finally we note that a valence bond description of the type originally proposed<sup>3</sup> for the  $[\text{Re}_3\text{Cl}_{12}]^{3-}$  anion in terms of a spin-paired  $5d^3 6s 6p^3$  hybrid  $\sigma$ -set for each rhenium atom is somewhat less satisfying for rhenium(IV) chloride. Thus the spin-paired configuration  $d^3$  leads to 1 rather than  $1/3$  of an unpaired electron per rhenium atom, although the latter result could be accommodated by the introduction of additional spin-spin coupling.

We thank Drs. C. G. Barraclough and R. D. Harcourt for their advice.

*Note added in proof.* Since this communication was submitted for publication, extensive chemical, spectrophotometric and X-ray structural investigations by F. A. Cotton's group<sup>10-14</sup> in the U.S.A. and J. E. Fergusson's group<sup>15</sup> in New Zealand have confirmed the pronounced tendency of  $\text{Re(III)}$  to form trimeric clusters. In our discussion here of the magnetic evidence for trimeric rhenium(IV) species, we inadvertently overlooked that the L.C.A.O.-M.O. treatment of the  $[\text{Re}_3\text{Cl}_{12}]^{3-}$  anion foreshadowed in reference 4 had in fact appeared in reference 13. The two treatments happily lead to the same order of energies for the bonding molecular orbitals of the  $\text{Re}_3$ -core (the respective reference sets of co-ordinate axes are rotated so that the present  $x = x, y = z$  and  $z = y$ ), but the more elaborate analysis of Cotton and Haas suggests a somewhat different order of energies for the antibonding species. These differences are of no consequence in the present discussion of the bonding and magnetism of rhenium(IV) chlorides.

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# AN EFFECT OF ETHAMBUTOL, 2,2'(ETHYLENEDIIMINO)-DI-1-BUTANOL-, ON THE STRUCTURE AND ACTIVITY OF ALCOHOL DEHYDROGENASE

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THE observation that the regulation of enzymatic reactions by certain substances is accompanied by changes in the molecular configuration of the enzyme has been reported by several investigators<sup>1-4</sup>. In recent investigations in these laboratories on dextrorotatory 2,2'(ethylenediimino)di-1-butanol-, ethambutol, a new antituberculosis drug<sup>5</sup>, it was observed that the drug could serve as a substrate for alcohol dehydrogenase<sup>6</sup>, and moreover that the compound served to stimulate ethanol oxidation by the enzyme. This article presents the results of the preliminary investigations of the stimulation of alcohol dehydrogenase by ethambutol, and evidence is presented which indicates that stimulation of the enzyme is accompanied by alterations in its molecular configuration.

Yeast alcohol dehydrogenase used in this experiment was obtained from the Mann Research Laboratories as crystals or as a crystalline suspension, and was dissolved in 0.05 M phosphate buffer, pH 8.0, prior to use. Ethambutol and its *l* isomer were prepared in these laboratories.

The assay system for alcohol dehydrogenase contained 3  $\mu$ moles DPN, 218  $\mu$ moles of ethanol, and from 2 to 8 units of alcohol dehydrogenase in a total volume of 30 ml. of pH 8.0, 0.05 M phosphate buffer. In some experiments the enzyme was pre-incubated in 1.0 ml. of buffer containing 72  $\mu$ moles of either ethambutol, its *l* isomer, or EDTA, for up to 120 h at 4° C, and 0.1 ml. of this enzyme solution was used in the assay; control enzyme was pre-incubated in the buffer under similar conditions without the test compounds. When the enzyme was not pre-incubated, 5.8  $\mu$ moles of ethambutol or its *l* isomer were added at the time of assay, and the assay systems of the pre-incubated enzyme contained 7.2  $\mu$ moles of the drug. Alcohol dehydrogenase activity was assayed spectrophotometrically by following increase in absorbance at 340 m $\mu$  in the Cary recording spectrophotometer at 25° C.

Gel filtration and sedimentation analysis were performed on control and drug-treated enzyme after incubation for

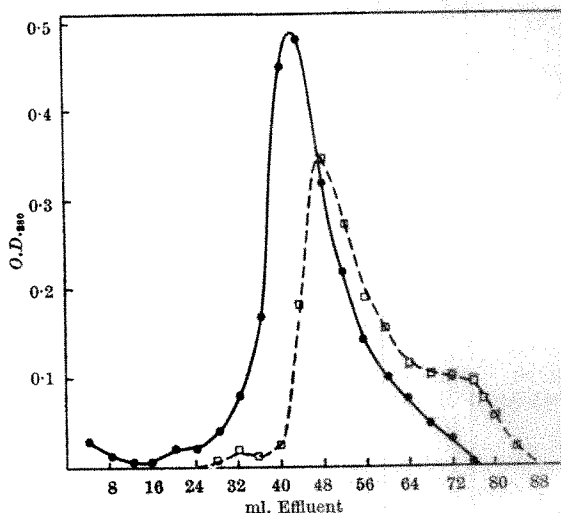


Fig. 2. Elution diagram of alcohol dehydrogenase from 'Sephadex G-200' in the presence of ethambutol. Control refers to enzyme in buffer alone. —, control; ---, ADH + ethambutol

24 h as described here. The gel filtration was performed on a 2 cm x 28 cm column of 'Sephadex G-200' gel at 4° C. The enzyme was eluted from the column with pH 8.0 phosphate buffer at a flow rate of about 2 ml./h, and 4-ml. fractions were collected for assay. Protein concentrations in the fractions were determined by measuring the absorption at 280 m $\mu$  in the Beckman spectrophotometer. Sedimentation experiments were performed in the Spinco model E analytical ultracentrifuge at a speed of 52,640 r.p.m. at 20° C.

The alcohol dehydrogenase catalysed oxidation of ethanol by DPN was stimulated by incubation of the enzyme with ethambutol or by addition of the drug to the control enzyme at the time of assay (Fig. 1). When the enzyme was incubated with ethambutol the extent of stimulation varied from 1.4 to 3.7 times that of the control rate depending on the time of incubation. Similar incubations of the enzyme with the *l* isomer or additions of the *l* isomer to the control enzyme resulted in stimulation of activity 1.6-1.7 times that of the control. Incubation of the enzyme with an equimolar concentration of EDTA did not stimulate ethanol oxidation, and instead depressed its activity slightly (Table 1).

No measurable production of DPNH could be observed with the amount of enzyme used in these experiments when it was presented with ethambutol or its *l* isomer as a substrate in the absence of ethanol (Fig. 1). Assay of the activity of enzyme incubated with ethambutol for 7 days and then dialysed against pH 8.0 phosphate buffer for 20 h at 4° C indicated that the stimulation of enzyme activity persisted. Although all the ethambutol was not removed, there was an indication that the continued presence of the drug at its initial concentration was not required for enhanced activity once the enzyme had been exposed to ethambutol (Fig. 1d).

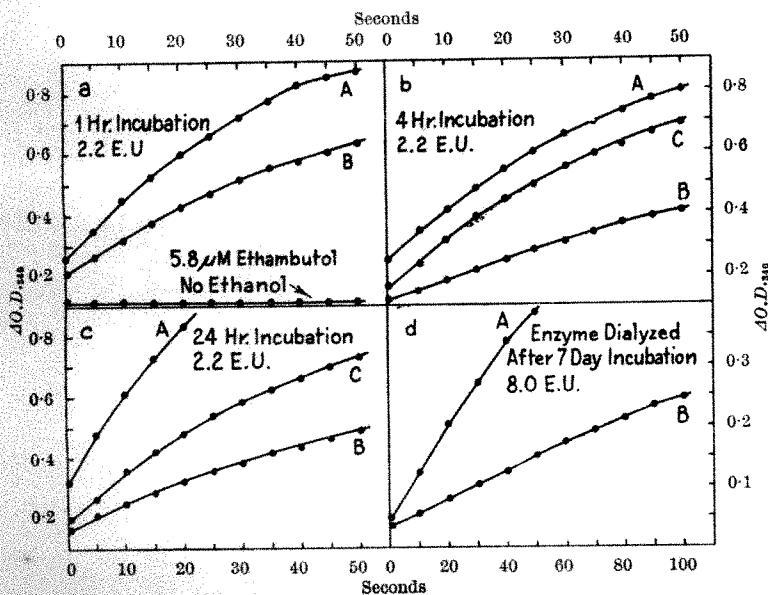


Fig. 1. Effect of ethambutol on alcohol dehydrogenase catalysed oxidation of ethanol. A, enzyme, pre-incubated with ethambutol; B, control enzyme; C, control enzyme to which drug had been added at the time of assay



Table 1. RATIO OF ACTIVITY\* OF DRUG-TREATED ALCOHOL DEHYDROGENASE TO CONTROL ENZYME

Time (h)	Pre-incubated enzyme		
	Ethambutol	<i>l</i> Isomer	EDTA
1		1.7	
1	1.4	1.7	0.74
3		1.6	
4	1.9		
24	3.7	1.7	0.88
120	1.9		

\* Activity is calculated as:  $\frac{[\Delta A_{340}/30 \text{ sec}] \times 2}{\mu\text{g enzyme}}$

In the absence of ethambutol, alcohol dehydrogenase was eluted from a gel column as a single peak trailed by a small amount of some component of lower molecular weight, estimated to be 8 per cent of the total enzyme. On the other hand, when ethambutol was present the elution pattern demonstrated that there was an increase in the amount of the secondary component which amounted to 22 per cent of the total enzyme (Fig. 2). Accompanying this increase in the amount of the secondary enzyme fraction was a concurrent decrease in that of the main component. The suggestion of a slight increase in elution volume of the enzyme from the column in the presence of ethambutol further indicated a change of the physical characteristics of the enzyme.

The results of the gel-filtration experiments indicated that in the presence of ethambutol there was an alteration of the molecular size of the enzyme since in principle the gel is able to distinguish between structures of different molecular weight. This change in the state of the enzyme could best be described as a depolymerization of a normally aggregated molecule into a disaggregated state.

The effects of ethambutol on the gross state of aggregation of the alcohol dehydrogenase molecule were further demonstrated by the sedimentation experiments. It was apparent that the enzyme consisted of aggregated and disaggregated components (Fig. 3A) sedimenting at faster and slower rates respectively, and that ethambutol caused a further disaggregation of the enzyme into its slower sedimenting sub-unit (Fig. 3B). The proportion of the enzyme sub-unit increased from 25 per cent of the total material in the untreated enzyme to 56 per cent when alcohol dehydrogenase was treated with ethambutol. The increase in the amount of the sub-unit was accompanied by a decrease in that of the aggregated enzyme. The sedimentation coefficient of the sub-unit of the enzyme in both the untreated and the drug-treated enzyme was calculated as 2.8 S (this value uncorrected for viscosity and concentration).

These experiments indicated that activation of alcohol dehydrogenase of yeast by ethambutol (or its *l* isomer)

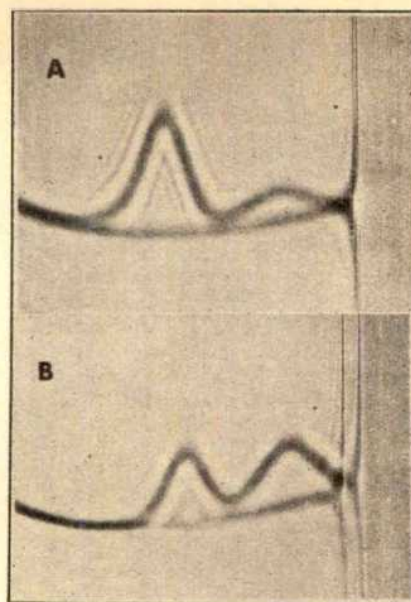


Fig. 3. Effect of ethambutol on sedimentation behaviour of alcohol dehydrogenase. A, control enzyme; B, enzyme treated with ethambutol. Sedimentation is from right to left.

was associated with changes in the state of aggregation of the sub-units of the enzyme. The stimulatory effect of the *l* isomer indicated that there was no stereochemical requirement for the action of the drug, and from the lack of stimulatory activity of EDTA it was concluded that chelation *per se* was not likely to be the primary mechanism in the dissociation of the enzyme. While the exact mechanism of stimulation could not be established, the possibility exists that the disaggregated form of the enzyme produced by ethambutol was much more active than the aggregated form.

I thank Drs. D. A. Buyske and J. R. Florini for their advice and M. C. Davies for performing the centrifugation analyses.

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## CHOLINESTERASE-CONTAINING PATHWAYS OF THE HINDBRAIN: AFFERENT CEREBELLAR AND CENTRIFUGAL COCHLEAR FIBRES

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IN a recent investigation<sup>1</sup> we showed that an extensive neuronal system, probably corresponding to the ascending reticular activating system, could be demonstrated in the rat forebrain by a combined surgical and histochemical technique for cholinesterases. We have since, in collaboration with Dr. Ann Silver<sup>2</sup>, compared the acetylcholinesterase and choline acetylase content of fibres supplying the hippocampal formation, and the changes which occur after lesions of the fornix. The correspondence was such as to prove beyond reasonable doubt that the cholinesterase-containing fibres which travel in the fornix and terminate on the hippocampus

and dentate gyrus are cholinergic. We conclude, therefore, that our method is likely to be valid for cholinergic pathways in the central nervous system generally, and we have now extended it in the rat to cover the hindbrain.

Stereotaxic lesions, produced by radio frequency current, were placed in selected regions of the medulla, cerebellum and cerebellar peduncles. Most of the animals were killed after four days, which we have found to be the optimum time in the forebrain for enzymatic pile-up. Some were allowed to survive longer in order to demonstrate enzymatic loss. After sectioning, the brains were treated by the thiocholine technique. The response to



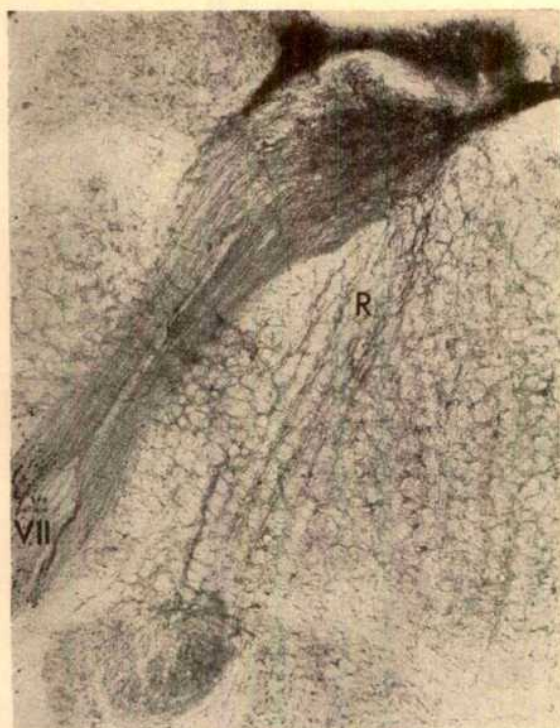


Fig. 1. Transverse section through the medulla showing acetylcholinesterase-containing fibres of the facial nerve (VII) and olivo-cochlear bundle (R)



Fig. 2

Fig. 2. Pile-up of acetylcholinesterase in fibres of the middle cerebellar peduncle. D, dentate nucleus



Fig. 3

Fig. 3. Pile-up of acetylcholinesterase in fibres of the inferior cerebellar peduncle

injury shown by cholinesterase-containing fibres was essentially similar to that observed in the forebrain. Thus, where the intra-medullary fibres of the facial nerve were involved by a lesion dorsomedial to the motor facial nucleus, acetylcholinesterase piled up in the interrupted axons on the cell body side. In another experiment involving the acetylcholinesterase-containing bundle which we have shown to consist of olivo-cochlear and salivatory fibres<sup>3</sup>, enzyme was lost from the cut ends of axons on the side remote from their cells of origin. We found that the proximal part of the olivo-cochlear pathway could be followed, through the superior olivary peduncle, back to its origin (from retro-olivary and pre-olivary reticular cells) in sections cut from normal fresh brain and fixed briefly in formalin before histochemical treatment (Fig. 1).

We investigated the course of reticular fibres by placing large lesions in the dorsomedial and ventromedial regions of the reticular formation of the medulla. In each case acetylcholinesterase was found to pile up in severed reticular axons, but only on the caudal side of the lesion. We have never found evidence of descending reticular pathways containing cholinesterase in the brain stem. We consider, therefore, that only ascending reticular fibres are cholinergic, and that descending reticulo-spinal pathways operate through some transmitter other than acetylcholine, possibly a mono-amine<sup>4,5</sup>.

Lesions involving any of the cerebellar peduncles invariably produced pile-up of acetylcholinesterase on the side remote from the cerebellum, and the response in the middle peduncle was no less extensive than that which occurred when either of the other peduncles was damaged (Figs. 2 and 3). We conclude from these findings that a significant influx of cholinergic afferents reaches the cerebellum by all three peduncles, and not mainly by the superior and inferior peduncles as has been suggested<sup>6</sup>. The cholinesterase-containing afferents in the middle peduncle, in spite of their fine calibre, can be recognized in normal material. Those of the superior and inferior peduncles become more apparent after the fibres have been interrupted, probably because they are scattered among the numerically preponderant non-cholinergic fibres.

In addition to the peduncular pathways, we have found a fourth route by which cholinergic fibres may reach the cerebellum, namely, the dorsal longitudinal fasciculus of Schütz. Cholinesterase-containing fibres in this fasciculus ascend on either side of the mid-line in the floor of the fourth ventricle, then swing outwards to enter the cerebellum on the inner side of the superior peduncle. Interruption of the dorsal longitudinal fasciculus resulted in a loss of staining in the ventricular floor above the lesion, and also round the cells of the nucleus coeruleus which is interpenetrated by these fibres (Fig. 4). The dorsal longitudinal fasciculus has usually been regarded in the past as a purely descending pathway.

The origin of the cholinesterase-containing afferents to the cerebellum was investigated mainly in unoperated material. The fibres which travel in the middle peduncle arise in part at least from the nucleus reticularis tegmenti pontis. Some of the fibres of the superior peduncle appear to have a midbrain origin from the laterodorsal tegmental nucleus, and traverse the portion of the peduncle occupied by the unstained dentato-rubral fibres. Other, finer fibres

Fig. 4

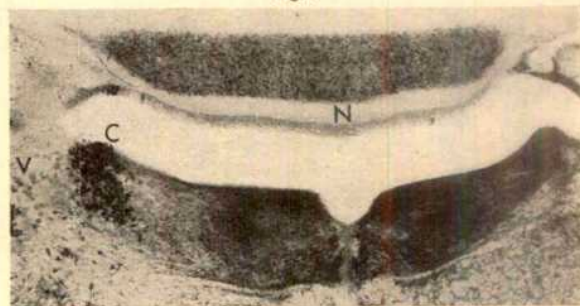


Fig. 5



Fig. 4. Loss of acetylcholinesterase from fibres of the dorsal longitudinal fasciculus on left side. C, nucleus coeruleus; N, nodulus; V, mesencephalic trigeminal nucleus

Fig. 5. Acetylcholinesterase-containing bipolar cells in the white matter of the flocculus



of the superior peduncle ascend with the unstained fibres of the ventral spino-cerebellar tract. The fibres of the inferior peduncle arise from cholinesterase-containing cells of reticular type situated among the cells of the lateral cuneate nucleus, and from similar cells in the spinal trigeminal nucleus and the cuneate and gracile nuclei. The dorsal longitudinal fasciculus conveys fibres from the nucleus eminentiae teretis, from the gracile nucleus, and from the paramedian reticular nucleus; they are joined by fibres from the nucleus coeruleus. We are thus able to confirm the earlier claim<sup>7</sup>, afterwards denied by various workers<sup>8</sup>, that the gracile and cuneate nuclei send projections to the cerebellum.

Experimental as well as normal material was used to determine the course and distribution of cholinesterase-containing fibres within the cerebellum. With the exception of the flocculus and paraflocculus, the lateral portions of the cerebellum were found to be much less richly innervated than the vermis. Many of the fibres derived from the superior peduncle were shown to decussate within the cerebellum. When a lesion was placed in the cerebellar white matter so as to interrupt these fibres, enzyme piled up on the medial side in fibres that had already crossed, as well as on the lateral side in uncrossed fibres. Other cholinesterase-containing fibres associated with the superior peduncle can be seen to cross in the superior medullary velum. Fibres from the middle peduncle supply preferentially the paraflocculus and the declival portion of the lobulus simplex (sub-lobules VIb and VIc of Larsell<sup>9</sup>). Some earlier investigations in the cat<sup>10,11</sup> suggest that the declive may be concerned with the functional organization of the head and neck. The role of the paraflocculus is uncertain, but it may regulate the activity of axial musculature<sup>12</sup>.

In addition to the paraflocculo-declival tract, there is another distinct bundle of cholinesterase-containing fibres to be seen within the cerebellum, which we have called the cochleo-flocculo-nodular tract. Its fibres run caudally from the region of the superior vestibular nucleus and are distributed to the cochlear nuclei and to the flocculo-nodular lobe. In an experimental animal in which the tract was partly divided, enzyme was found to pile up in the ends of damaged fibres rostral to the lesion and to be lost on the caudal side from terminals in the dorsal cochlear nucleus. Another animal, in which the floccular stalk was severed at its base, showed some evidence of enzyme loss in the fibres supplying the flocculus. There are, however, many cholinesterase-containing cells within the white matter of the flocculus which are not affected by severing its stalk. These cells tend to be fusiform and are apparently bipolar (Fig. 5): they resemble cells which we have found grouped on the course of cholinergic bundles in the forebrain, for example, in the nucleus of the ventral supraoptic decussation.

The cholinesterase-containing afferents to the rat cerebellum appear to terminate mainly within the granular layer of the cortex, although in some other genera many such fibres may reach the molecular layer<sup>13</sup>. They probably contribute to the glomeruli formed by the terminals of mossy fibres synapsing with the dendrites of the granule cells. The glomeruli, however, are not responsible for all the cholinesterase of the granular layer as has been stated<sup>14</sup>, nor is all the enzyme derived from afferent terminals, since acetylcholinesterase is also found in the bodies of Golgi cells<sup>15</sup>. These interneurons are somewhat larger than the granule cells, and have short axons which project on to the glomeruli. They are scattered throughout the granular cortex, but are much more prevalent in the flocculo-nodular lobe and in the roof of the postero-lateral fissure between the flocculo-nodular lobe and the uvulo-paraflocculus<sup>16</sup>, that is, in the regions of the cortex which receive vestibulo-cerebellar fibres. The acetylcholinesterase content of the Golgi cells is largely responsible for the greater concentration of this enzyme which characterizes the vestibular portion of the

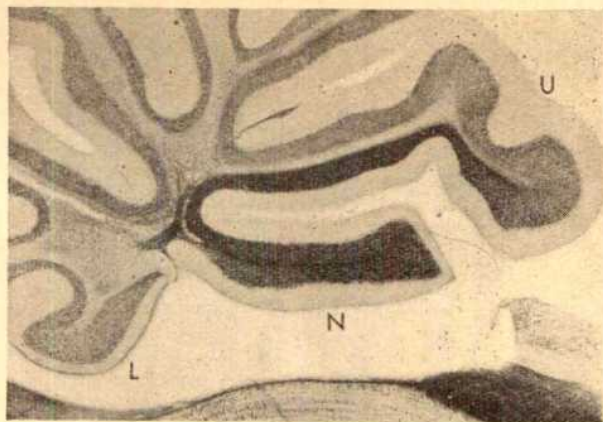


Fig. 6. Sagittal 100- $\mu$  section through the cerebellum showing concentration of Golgi cells in the vestibular receiving areas of the vermis. L, lingula; N, nodule; U, uvula.

cerebellum (Fig. 6). The lingula does not contain significantly more Golgi cells than other areas of vermal cortex, except in the region immediately adjacent to the nodule, which may receive an overspill of vestibular fibres. In animals where lesions had been placed in the cerebellar white matter, the Golgi cells of the overlying denervated cortex did not lose their enzyme.

Other cells which contain acetylcholinesterase are those of the dentate (Fig. 2) and other cerebellar nuclei. Here the enzyme is present in relatively small amounts, and in the rat there is not the preponderance of pseudo-cholinesterase which has been reported in the horse<sup>17</sup>. Unlike their cells of origin, the fibres of the dentato-rubral tract which comprise the greater part of the superior peduncle are quite devoid of cholinesterase. In view of this, and of the observation that the cerebellar nuclei contain less choline acetylase than does the superior peduncle<sup>18</sup>, we conclude that the dentate neurones and those of the other cerebellar nuclei are non-cholinergic, and that the cholinesterase, which is contained in their cell bodies and dendrites without extending into the axons, is postsynaptic.

Acetylcholinesterase is also found in small quantities elsewhere in the brain stem in the cell bodies of neurones which are not likely to be cholinergic, for example, in the trapezoid nuclei and in the mesencephalic trigeminal nucleus (Fig. 4). In general, therefore, one may say that the presence of acetylcholinesterase in the cell body of a neurone cannot be regarded as an indication that it is cholinergic unless the enzyme is also present in the axon and in the presynaptic terminals. In the case of neurones with short axons this may be difficult to determine, and in consequence it is by no means certain that the cerebellar Golgi cells are cholinergic. In view of the relatively low choline acetylase content of the cerebellar cortex<sup>18,19</sup>, it seems most likely that they are not. The presence of acetylcholinesterase may, however, indicate that they are cholinceptive.

In spite of the report that in cats the Purkinje cells are excited by cholinergic drugs while the granule cells are not<sup>20</sup>, the distribution of enzyme suggests that, in the rat at least, the main cholinceptive cells of the cerebellar cortex apart from Golgi cells are likely to be the granule cells through the glomeruli. In view of the absence of cholinesterase in their cell cytoplasm and the low cortical choline acetylase levels, the granule cells can scarcely be cholinergic. Where they have been interpreted as such<sup>15</sup>, the cholinesterase-containing fibres of the cerebellar white matter have been missed, and consequently the cholinesterase-containing axons of the granular cortex have wrongly been regarded as belonging to the granule cells. The apparent localization of acetylcholinesterase in the nuclei of granule cells, described by other workers<sup>20</sup>, we take to be a histochemical artefact.



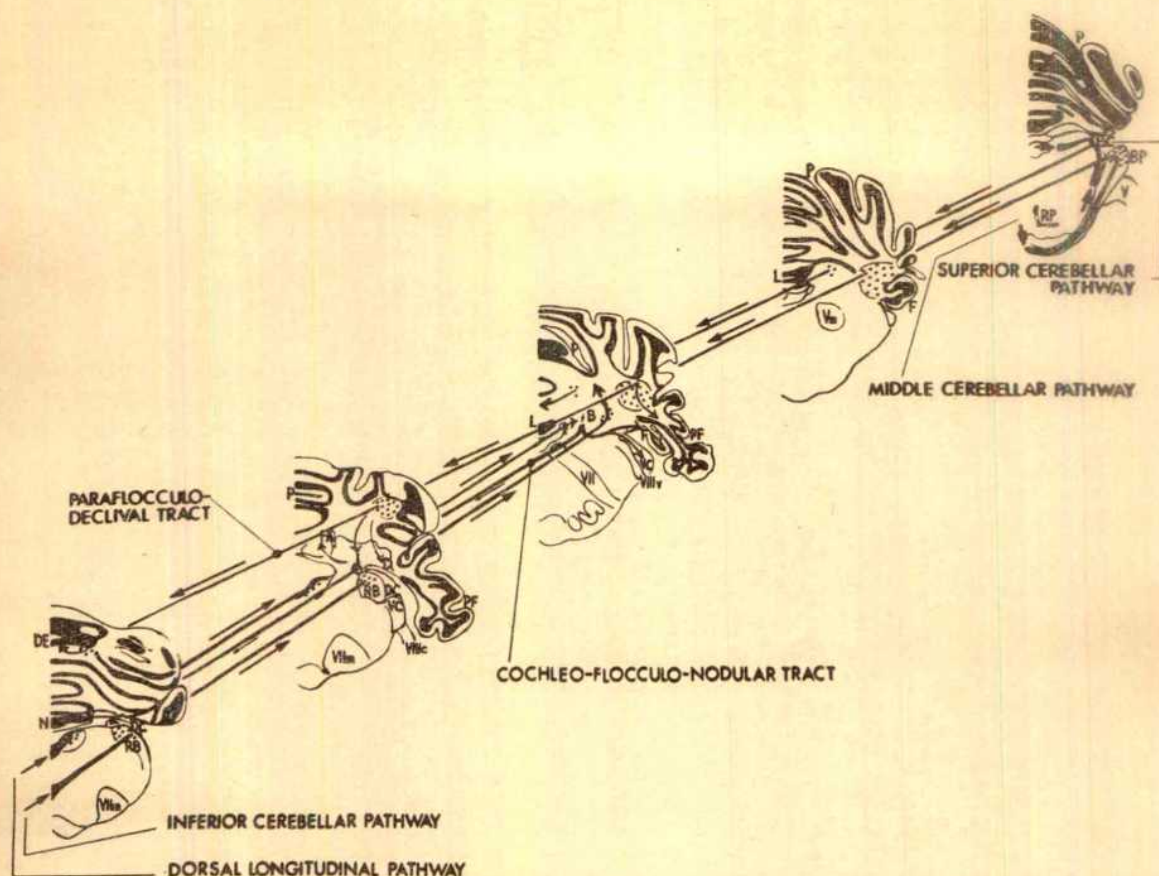


Fig. 7. Exploded diagram of hindbrain, showing the presumed cholinergic innervation of the cerebellum and cochlear nuclei. *B*, superior vestibular nucleus; *BU*, superior cerebellar peduncle; *BP*, middle cerebellar peduncle; *D*, dentate nucleus; *DC*, dorsal cochlear nucleus; *DE*, declive of lobulus simplex; *F*, flocculus; *FA*, fastigial nucleus; *I*, nucleus interpositus; *L*, lingula; *N*, nodule; *P*, fissura prima; *PF*, parafocculus; *RB*, inferior cerebellar peduncle; *RP*, nucleus reticularis tegmenti pontis; *V*, trigeminal nerve; *Vm*, motor trigeminal nucleus; *VC*, ventral cochlear nucleus; *VII*, facial nerve; *VIIIm*, facial nucleus; *VIIIc*, cochlear nerve; *VIIIe*, vestibular nerve.

We have made use of the data obtained from our experiments to construct a map of the main cholinesterase-containing pathways of the cerebellum (Fig. 7). This diagram consists of transverse sections of the brain stem taken at 1-mm intervals and set at ten times their natural distance apart. The superior, middle and inferior cerebellar pathways represent the fibres travelling respectively in the superior, middle and inferior cerebellar peduncles, and the dorsal longitudinal pathway represents the fibres of the dorsal longitudinal fasciculus. We believe that these pathways, as illustrated, constitute a cholinergic reticular activating system supplying the cerebellum and cochlear nuclei, comparable to the system which we have described as innervating cortical and subcortical structures in the forebrain.

Although the anatomical projections of the reticular formation to the cerebellum have been well worked out<sup>12</sup>, more clearly indeed than those to the cerebral hemispheres, much less is known of possible activating effects than in the case of the cerebral cortex. There is evidence that the lateral reticular nucleus which contributes fibres to the inferior cerebellar peduncle is fired by collaterals from spinal<sup>21</sup> and trigeminal<sup>22</sup> tactile pathways. With regard to the role of cholinergic neurones, the balance of evidence favours the view that the physiological effect of released acetylcholine on neuronal activity is likely to be facilitatory, especially in the cerebral cortex<sup>23</sup>. It has been suggested that certain centrally active drugs may produce their depressant or stimulant effects by respectively blocking or augmenting the release of acetylcholine<sup>24</sup>.

The possibility must also be considered that in some circumstances cholinergic neurones may have an inhibitory action, and so produce the filtering effect on sensory input which has been regarded as an essential

function of the reticular formation<sup>25</sup>. Acetylcholine has been shown to depolarize and depress conduction in non-myelinated fibres of the vagus<sup>26</sup>. It also depresses neuromuscular transmission in the frog, and the block is said to be partly presynaptic<sup>27</sup>. Conduction in the auditory nerve is depressed by high-frequency stimulation of the olivo-cochlear bundle<sup>28</sup>, and we have shown that this bundle contains fibres which may be presumed to be cholinergic. There is some reason for supposing, however, that the depression of auditory action potentials caused by olivo-cochlear stimulation may not involve release of acetylcholine, since the effect is not modified by eserine<sup>29</sup>, and occurs optimally under deep pentobarbitone or chloralose anaesthesia: conditions which do not favour cholinergic mechanisms<sup>24,30</sup>.

In an attempt to discover whether all the fibres of the olivo-cochlear bundle contain acetylcholinesterase, we have compared serial sagittal sections of the brain stem stained alternately by the thiocholine method and with silver. It was evident that, while the cholinesterase-containing fibres are aggregated into a compact bundle, there are many fibres devoid of cholinesterase which cross the midline adjacent to this bundle and might possibly be affected in stimulation experiments. Whether such fibres reach the cochlea is not known, although further evidence bearing on the problem may yet emerge from electron microscopy. Certain types of nerve ending have been seen in the organ of Corti which could belong to efferent fibres, and of these some connect with the bases of hair cells, while others end almost exclusively in relation to other nerve endings<sup>31</sup>. It is conceivable that, while the latter are inhibitory, the former are at least partly facilitatory, since stimulation of the olivo-cochlear bundle also results in a small increase in the cochlear microphonic potentials generated by the hair cells. It



would be illuminating to know whether either or both types of ending contain cholinesterase.

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## DISK ELECTROPHORESIS OF HUMAN SERUM LIPOPROTEINS

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SINCE the first successful isolation of a lipoprotein from horse serum by Macheboeuf<sup>1</sup>, considerable attention has been devoted to this class of conjugated proteins<sup>2</sup>. Recently, Barclay *et al.*<sup>3</sup> reported the presence of two new lipoprotein components in the human HDL<sub>2</sub> fraction ( $1.063 < d < 1.125$ ). Analytical ultracentrifugation and moving boundary electrophoresis are very precise tools, but they lack the resolution of the newly developed disk electrophoretic method<sup>4</sup>. For example, normal human serum is generally resolved into 3-5 components by these methods whereas over 20 components have been demonstrated by disk electrophoresis. Zone electrophoresis of serum lipoproteins has been examined by a number of investigators with a variety of media such as starch granules, polyvinyl resin, starch gel and paper<sup>5-7</sup>. However, it is not possible to vary the pore size of these media as readily as in the case of polyacrylamide gel, which is the supporting medium used for standard disk electrophoresis. Acrylamide-gel electrophoresis of lipoproteins has not been previously reported in the literature. In this article,

we would like to report disk electrophoretic experiments of the usual lipoprotein fractions (LDL,  $d < 1.063$ ; HDL<sub>2</sub>,  $1.063 < d < 1.125$ ; HDL<sub>3</sub>,  $1.125 < d < 1.21$ ) and to relate these data to the observed flotation patterns.

One pint of fasting blood was obtained from one of the authors (K. A. N.) and the serum derived from this blood was used for the isolation of lipoprotein fractions 1 (LDL), 3 (HDL<sub>2</sub>) and 5 (HDL<sub>3</sub>) according to the method of Havel *et al.*<sup>8</sup>. All lipoprotein solutions and the salt solutions used for dialysis contained 0.05 per cent versene. The procedure used for disk electrophoresis was a modification of the original disk electrophoretic procedure of Ornstein and Davis, and made use of 20 per cent sucrose mixed with solution B<sup>4</sup> as the anti-convection medium rather than sample gel. Prestaining of lipoprotein fractions or serum was carried out according to the procedure of Ressler *et al.*<sup>7</sup>. Tracking dye was not used, because preliminary experiments indicated that this dye also bound other proteins in addition to albumin and interfered with the visualization of the lipoproteins. Variations in gel concen-

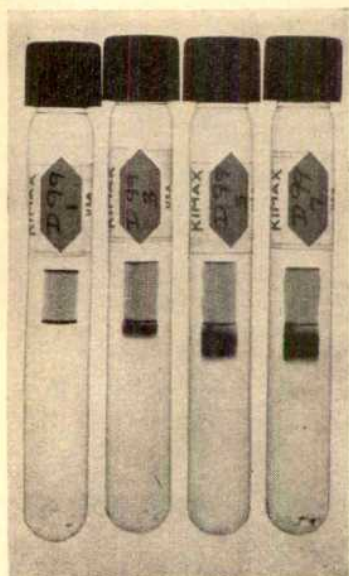


Fig. 1. Disk electrophoretic patterns. (Lipid prestain.) 7.5 per cent gel. LDL (D 99-1), HDL<sub>2</sub> (D 99-3), HDL<sub>3</sub> (D 99-5, 7), 30 $\mu$  each, 2.5 m.amp, 73 min for LDL and HDL<sub>2</sub> and 85 min for HDL<sub>3</sub>.

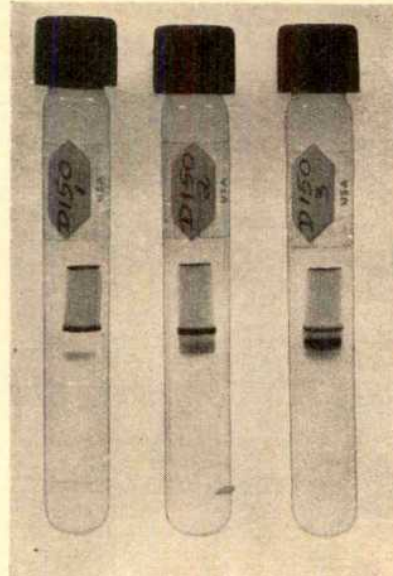


Fig. 2. Disk electrophoretic patterns. (Lipid prestain.) 3.75 per cent gel. LDL (D 150-1), HDL<sub>2</sub> (D 150-2), HDL<sub>3</sub> (D 150-3), 10 $\mu$  each, 2.5 m.amp, 34 min.



trations were obtained by suitable adjustment of the volume of solution *C* and water in the formula provided by Ornstein and Davis<sup>4</sup>. Analytical ultracentrifugation was performed with a Spinco model *E* ultracentrifuge at 52,640 r.p.m.

When the standard 7½ per cent polyacrylamide was used for the main gel, the prestained lipoprotein fractions resolved as shown in Fig. 1. The pore size of this gel has been estimated to be 50 Å and the large molecules (*LDL*, fibrinogen, 19 *S* γ-globulin) do not penetrate the gel<sup>4</sup>. *LDL* did not enter the main gel as seen in *D* 99-1. *HDL*<sub>2</sub> (*D* 99-3) and *HDL*<sub>3</sub> (*D* 99-5, 7) resolved into only 3 components. When a protein stain such as amido black was used a trace component having the mobility of albumin was also observed in all fractions. In these experiments, the lipoprotein fractions were used as such in their respective salt solutions without prior dialysis. In disk electrophoresis, which is conducted at very low ionic strength, the presence of even small amounts of salt in the sample could reduce the mobility of the protein constituents. The dark stain observed on top of the spacer gel was merely excess dye and does not represent denatured lipoprotein material. Proper separation was not achieved on 5 per cent polyacrylamide gel with samples of *HDL*<sub>2</sub> and *HDL*<sub>3</sub> which were dialysed against 1 M sodium chloride and undialysed *LDL*.

When 3.75 per cent polyacrylamide gel was used, prestained fractions (*LDL*, *HDL*<sub>2</sub> and *HDL*<sub>3</sub> dialysed against sodium chloride) resolved into several components as shown (Fig. 2). *LDL* entered the main gel but did

not resolve further. *HDL*<sub>2</sub> separated into 2 components near the top of the gel and 2 or 3 components in the lower part of the gel. Five components were observed with *HDL*<sub>3</sub>, but there was more of the fast and less of the slow components when compared with *HDL*<sub>2</sub>. When protein stain was used the patterns obtained with *HDL*<sub>2</sub> and *HDL*<sub>3</sub> were quite similar though not identical. With this stain, the slow components were resolved quite distinctly in both samples and, in the case of *HDL*<sub>3</sub>, 3 distinct fast components were observed (Fig. 3). Subsequent experiments have indicated that the faster of the slow components is *LDL*. From these data it would appear: (1) that *HDL*<sub>2</sub> isolated by standard preparative centrifugation contains small amounts of *LDL* (which is also present in trace amounts in *HDL*<sub>3</sub>); (2) that *HDL*<sub>2</sub> has a slower moving component (probably *HDL*<sub>1</sub>, *S*<sub>f</sub> 1.063, 0-3) not present in any appreciable amount in *HDL*<sub>3</sub>; and (3) that *HDL*<sub>2</sub> and *HDL*<sub>3</sub> both contain fast moving components of very similar electrophoretic mobilities. Moving boundary electrophoresis of *HDL* fractions was reported to have exhibited only one peak in all fractions and to have migrated with identical mobilities<sup>6</sup>. Starch-gel electrophoresis failed to resolve the *HDL* components<sup>6</sup>.

The flotation patterns of the three fractions are shown in Fig. 4. By analytical ultracentrifugation only one component was observed in the case of *LDL* and *HDL*<sub>2</sub>. It is possible there was a trace component floating ahead of the main peak in the case of *HDL*<sub>3</sub>. With *HDL*<sub>2</sub>, both at *d* = 1.125 and *d* = 1.21 (not shown in Fig. 4), at least 3 components were seen and their *S*<sub>f</sub> 1.125 (KBr-NaCl) values were observed to be 2.1, 14.3, and 18.4. These values were somewhat higher than those observed by Barclay<sup>3</sup> because of use of the KBr-NaCl medium rather than NaCl. It should be pointed out that the viscosity ratio of KBr solutions (over a range of 0.25-4.0 molar) to water at 25° is less than unity in contrast to most other salt solutions. If calculations are performed to determine the *S*<sub>f</sub> values at *d* = 1.125 (NaCl) of *LDL* (*S*<sub>f</sub> 3-10, *d* = 1.0635, NaCl) and *HDL*<sub>1</sub> (*S*<sub>f</sub> 0-3, *d* = 1.0635, NaCl) on the basis of their known partial specific volumes, the values obtained are respectively 17-58 and 0-16.5. Thus it would appear that the intermediate component seen in the centrifuge was apparently *HDL*<sub>1</sub> and was probably identical with the *HDL*<sub>2</sub> component possessing the lowest electrophoretic mobility. The fast component may represent a part of the spectrum of *LDL* species and may be tentatively identified as the faster of the two slow components observed by disk electrophoresis.

In a medium of density 1.21, *HDL*<sub>2</sub> exhibited a flotation pattern similar to that at *d* = 1.125. The *S*<sub>f</sub> value of the slow component of *HDL*<sub>2</sub> at *d* = 1.21 was 6.0 and was considerably higher than the *S*<sub>f</sub> value of *HDL*<sub>3</sub> (3.6), which is in agreement with the data of Lindgren and Nichols<sup>2</sup>. Therefore, it can be concluded that both *HDL*<sub>2</sub> and *HDL*<sub>3</sub> have components with very closely related electrophoretic mobilities but with different *S*<sub>f</sub> values.

Finally, as an application of this method, we have examined the disk electrophoretic patterns of prestained

sera from 6 individuals using 5 per cent and 3.75 per cent polyacrylamide gels. In Fig. 5, *D* 104-1, 2, 3 represent the sera obtained from coronary patients and *D* 104-4, 5, 6 represent the sera which we took from ourselves and which we presumed to be normal. The leading dense component in all cases was albumin, which appeared as a brown band and must be ignored for the present discussion. As before, gel concentrations of 5 per cent and higher did not permit the migration of *LDL*. In 5 per cent polyacrylamide gel, *HDL* separated into 2 components in all samples (Fig. 6). With 3.75 per cent gel concentration, *LDL* was better resolved but the high-density lipoproteins diffused rapidly. In the gel *D* 104-2, three

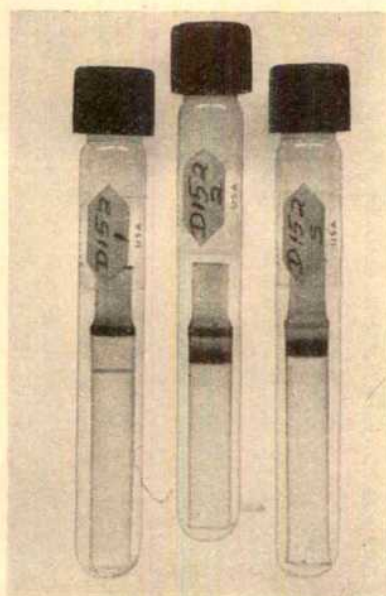


Fig. 3. Disk electrophoretic patterns. (Protein stain.) 3.75 per cent gel. *LDL* (*D* 152-1), *HDL*<sub>2</sub> (*D* 152-2), *HDL*<sub>3</sub> (*D* 152-3), 10% each, 2.5 m.amp, 39 min

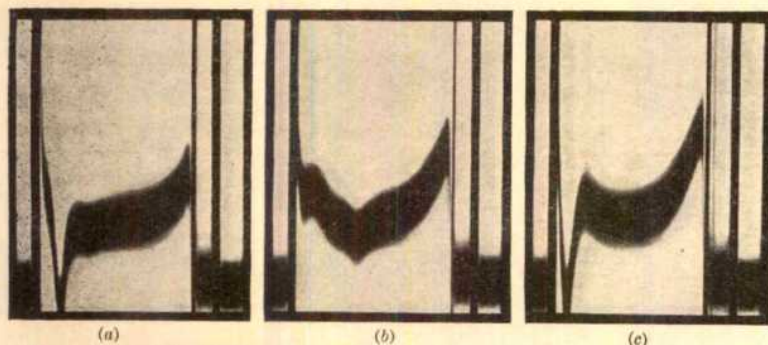


Fig. 4. Lipoprotein flotation patterns. Flotation left to right. (a) *LDL* in a medium of *d* = 1.063, 18 min after speed. (b) *HDL*<sub>2</sub> in a medium of *d* = 1.125, 19 min after speed. (c) *HDL*<sub>2</sub> in a medium of *d* = 1.21, 21 min after speed



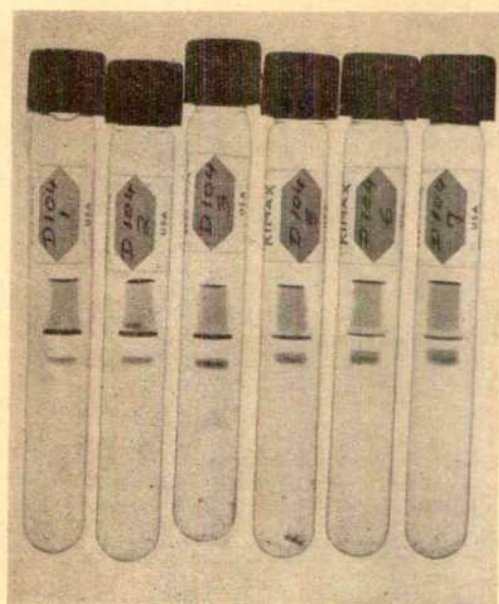


Fig. 5. Disk-electrophoretic patterns of human sera. (Lipid pre-stain.) 3.75 per cent gel. D 104-1, 2, 3 coronary samples, D 104-5, 6, 7 normal samples. 10% each, 2.5 m.amp, 28 min

components could be very clearly seen in the low-density lipoprotein region. The high-density lipoproteins tend to fade rapidly, particularly in the 3.75 per cent gel. Photography of the gels is a difficult problem, and therefore the faint bands of HDL and the partially resolved bands of LDL which were visible in the gels were not clearly defined in the photographs. The large difference in the amount of LDL and the relatively higher amount of LDL to HDL observed visually in the two groups are evident from Fig. 5. The increased ratio of serum LDL/HDL has been related to atherosclerosis<sup>8</sup>, and the present method which requires smaller quantities of serum may find application in this direction. In summary, polyacrylamide-gel

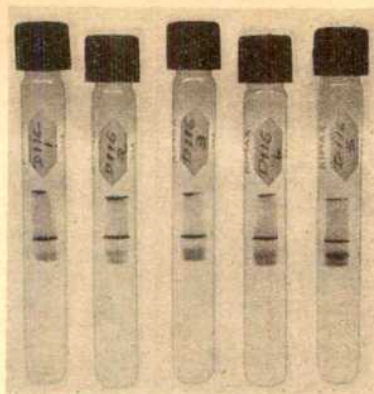


Fig. 6. Disk-electrophoretic patterns of human sera. (Lipid pre-stain.) 5 per cent gel. D 116-1, 2, 3 coronary samples, D 116-4, 5 normal samples. 10% each, 2.5 m.amp, 35 min

electrophoresis provides better resolution of lipoprotein components than ultracentrifugal or other zone electrophoretic methods.

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## A VITAMIN D<sub>3</sub>-DEPENDENT FACTOR INFLUENCING CALCIUM BINDING BY HOMOGENATES OF CHICK INTESTINAL MUCOSA

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AN earlier report by us<sup>1</sup> described findings of an effect of vitamin D<sub>3</sub> on radiocalcium binding by chick mucosal homogenates. It was apparent from those investigations that the supernatant from duodenal tissue homogenates derived from vitamin D<sub>3</sub>-treated chicks (in contrast to rachitic controls) had the ability to decrease radiocalcium uptake by the cellular debris. It was also noted that the observed decrease was not due to citrate levels, pH or the protein binding capacity of the supernatant. The direct addition of vitamin D<sub>3</sub> to rachitic supernatant was without effect. In particular, it was determined that this phenomenon is associated with a factor in the vitamin D<sub>3</sub>-supernatant and not with some property of the cellular debris.

Investigations by DeLuca and Engstrom<sup>2</sup> and Brierly *et al.*<sup>3</sup> showed that calcium is accumulated by mitochondria via a process dependent on ATP, Mg<sup>++</sup>, phosphate ion and oxidizable substrate. Engstrom and DeLuca<sup>4</sup> reported that the uptake phase was not influenced by vitamin D whereas the release of calcium was

vitamin D-dependent. In the present investigations, the actual fraction accumulating calcium in the chick homogenates, and the substrate requirements for this process, have not yet been precisely determined. Therefore, the relationship between the mitochondria investigations and the present homogenate-binding experiments cannot be delineated.

This article describes additional observations concerning the substance, which appears to be a protein or a polypeptide, in the vitamin D<sub>3</sub>-supernatant, responsible for the decreased uptake of radiocalcium.

Homogenates were prepared in the following manner. Four-week-old chicks on a rachitogenic diet (General Biochemicals, Inc.) were given 500 i.u. of vitamin D<sub>3</sub> at 72 and 24 h before use; controls were given an equal volume of the carrier alone (cotton-seed oil). After decapitation the duodenum was quickly removed and placed in ice-cold saline, then on to a glass plate where it was slit open and rinsed with cold saline. The mucosal cell layer was stripped from the muscle layers using a glass slide and placed in cold buffer (tris-HCl 1.37 × 10<sup>-3</sup> M, sodium chloride 0.119 M, potassium chloride

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$4.47 \times 10^{-3}$  M, glucose  $9.85 \times 10^{-5}$  M, pH 7.2-7.4). A 20 per cent (w/v) homogenate was prepared using either a Potter-Elvehjem system with 'Teflon' pestle or a Virtis homogenizer. In earlier experiments radiocalcium ( $^{45}\text{Ca}$  or  $^{47}\text{Ca}$ ) with carrier (about  $1 \mu\text{g}/\text{ml}$  homogenate) was then added, and the homogenate mixed and centrifuged for 20 min in a refrigerated centrifuge at 38,000g. In work on the properties of the supernatant factor, the centrifugation was performed before calcium addition; the supernatants or fractions therefrom were recombined with freshly prepared homogenate precipitates, mixed, radiocalcium added and then centrifuged. The recombined homogenates were at the same concentration (20 per cent w/v) as in the original preparation. If  $^{45}\text{Ca}$  was used, an aliquot of the supernatant was dried on a planchet and counted with a thin-window Geiger-Müller tube; when  $^{47}\text{Ca}$  was used, the supernatant and precipitate fractions were both counted with a sodium iodide crystal using a single-channel analyser set to eliminate any contribution from  $^{47}\text{Sc}$ . All operations were carried out at  $4^\circ\text{C}$ . Protein was determined by the method of Lowry *et al.*<sup>5</sup>.

Initially, the effect of the dilution of vitamin  $\text{D}_3$  supernatant with rachitic supernatant on radiocalcium binding by the test system was determined. The resulting mixtures were recombined with freshly prepared rachitic debris before radiocalcium was added. From Fig. 1, it may be seen that there was a linear relationship between the proportion of  $\text{D}_3$ -supernatant in the system and the percentage of  $^{45}\text{Ca}$  remaining in the supernatant. The curve does not intersect the axis at zero, presumably because of the existence of some binding-depressing capacity in the rachitic supernatant. This explanation is based on the observation that when *tris* buffer replaces supernatant, 98-99 per cent of the radiocalcium is taken up<sup>1</sup>. The linear form of the relationship indicates that the test system would be suitable for assay purposes.

Investigations were first undertaken on the temperature stability of the unknown factor at pH 7.2-7.4. It was found to be completely stable at room temperature for 72 h and up to 5 days at  $4^\circ\text{C}$ . On heating in a water bath for 10 min, no decrease in activity was observed at  $69^\circ\text{C}$ , a 33 per cent decrease was seen at  $79^\circ\text{C}$  and complete inhibition at  $86^\circ\text{C}$ . At  $69^\circ\text{C}$ , a considerable amount of protein coagulated and could be removed by centrifuga-

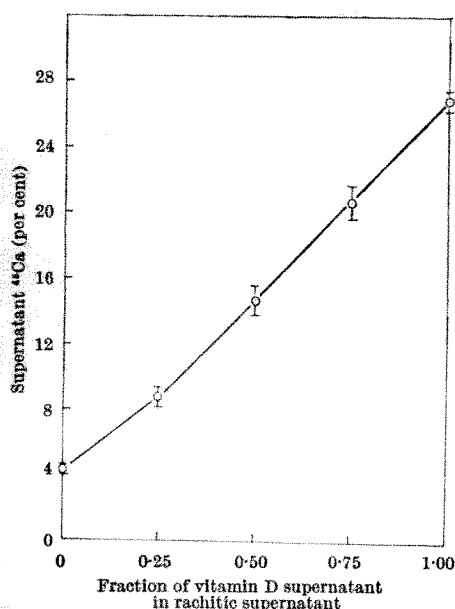


Fig. 1. Linear relationship between vitamin  $\text{D}_3$ -supernatant concentration and percentage  $^{45}\text{Ca}$  remaining in supernatant. The  $\text{D}_3$ -supernatant was diluted with rachitic supernatant in the proportions given on the abscissa. The mixed supernatants were recombined with freshly prepared rachitic debris to the original concentration (20 per cent w/v). The debris was re-suspended,  $^{45}\text{Ca}$  added, mixed and centrifuged at 38,000g for 20 min. The entire procedure was done at  $4^\circ\text{C}$ . The values represent the mean  $\pm$  S.E. of 4 determinations.

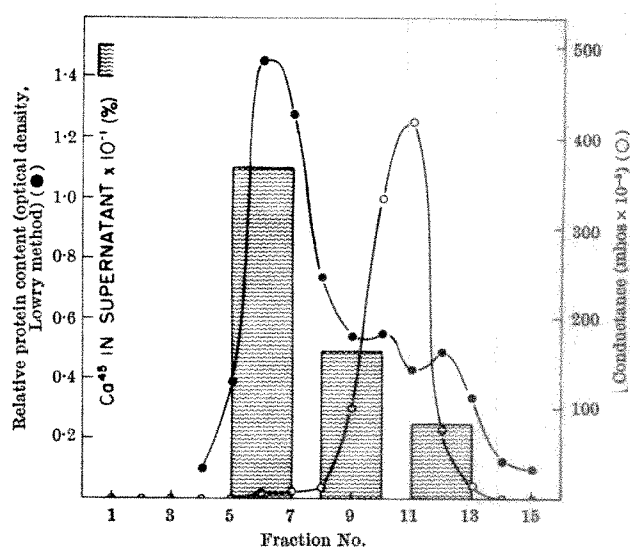


Fig. 2. Correlation of vitamin  $\text{D}_3$ -supernatant factor with protein in gel filtration fractions. Elution was performed with de-ionized water at a rate of 0.4 ml. per min through 'Sephadex G-25' ( $1 \text{ cm} \times 50 \text{ cm}$ ).

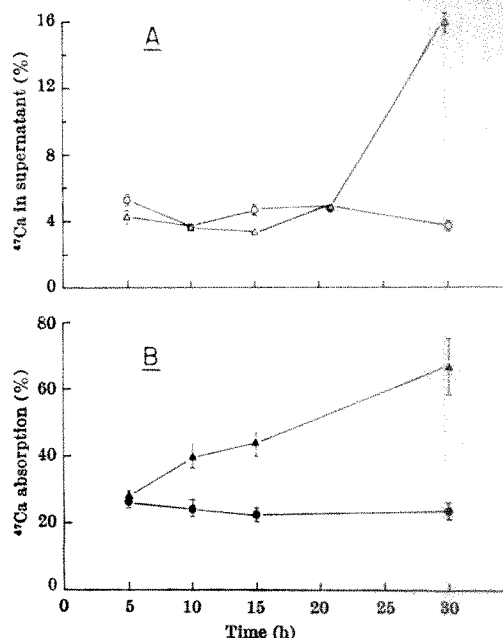


Fig. 3. Time relationship between appearance of supernatant factor and calcium absorption after vitamin  $\text{D}_3$  administration. A, percentage  $^{45}\text{Ca}$  remaining in supernatants from rachitic (○) and vitamin  $\text{D}_3$  (Δ) homogenates. *In vitro* test system as described in text. Values represent mean  $\pm$  S.E. of 5 determinations. B, absorption of  $^{45}\text{Ca}$  from ligated segments of chick duodenum *in vivo*. Period of absorption was 1 h. ●, rachitic; ▲, vitamin  $\text{D}_3$ . Experimental details given in ref. 6. Values represent mean  $\pm$  S.E. from 5 chicks.

tion; this affected about a 2-3-fold purification of the supernatant factor.

When the vitamin  $\text{D}_3$ -supernatant was dialysed against de-ionized water for 20 h, there was no appreciable loss of activity although some proteins precipitated from the solution. This indicated that the factor was of relatively large diameter or was associated with macromolecules.

Although the above evidence suggested that the factor may be protein in nature, additional information was sought by using gel filtration. Supernatant fluid was placed on a 'Sephadex G-25' column and eluted with de-ionized water. Pooled fractions were assayed for: (a) their ability to decrease radiocalcium binding by chick homogenates; (b) protein content; (c) conductivity (salt concentration). These results are depicted in Fig. 2. It may be seen that, as expected, the protein fraction preceded the electrical conducting substances. The supernatant factor was largely associated with the



protein or polypeptide fraction. As the protein concentration decreased, the concentration of the factor similarly decreased.

Since the presence of the supernatant factor in mucosal homogenates is dependent on vitamin D<sub>3</sub>, the question arises as to its importance in the gastrointestinal absorption of calcium. A time-dependent examination was therefore done to determine when the factor is formed in relation to when one sees a positive effect of vitamin D<sub>3</sub> on calcium absorption *in vivo*. Chicks were dosed with vitamin D<sub>3</sub> (500 I.U.) or carrier alone at periods of from 5 to 30 h before use. Half each treatment group was then used in a ligated-duodenum experiment<sup>6</sup> with <sup>45</sup>Ca to determine absorption *in vivo*. The other half were killed, and mucosal homogenates prepared for the <sup>45</sup>Ca binding examination as described here. Fig. 3 shows that the *in-vivo* effect of vitamin D<sub>3</sub> on calcium absorption is observable between 5 and 10 h after sterol administration, whereas the *in-vitro* system fails to show a response until 30 h.

This would suggest that either (a) the vitamin D<sub>3</sub>-supernatant factor does not play a primary part in calcium absorption, or (b) the factor may be initially in a bound form and not immediately released into the supernatant fluid. However, since the concentration of the unknown factor in mucosal tissue is dependent on vitamin D<sub>3</sub> and this factor does influence calcium metabolism, it may function in a more subtle manner on absorption than as determined here.

Recently D. Schachter (personal communication) observed that puromycin inhibited the vitamin D-stimulated oxygen-dependent uptake of calcium by rachitic rat duodenum. Edelman *et al.*<sup>7</sup> proposed the hypothesis that another steroid, aldosterone, "regulates active sodium transport by increasing *de novo* synthesis of enzymes involved in supplying energy for transport work"

in the urinary bladder of *Bufo marinus*. Olson<sup>8</sup> observed an antagonism by actinomycin D of the vitamin K-induced prothrombin formation and suggested the possibility that all the fat-soluble vitamins operated to control the synthesis of specific proteins and enzymes.

In this article the formation of a vitamin D-stimulated factor, presumably a protein or a polypeptide, is described which may have an important function in calcium movement. Whether vitamin D acts at the gene level or on some other phase of protein synthesis cannot yet be determined on the limited evidence available. Further, it is possible that the supernatant factor is an indirect consequence of the general influence of vitamin D on metabolism.

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*Note added in proof.* Pertinent here are the very recent observations of R. Eisenstein and M. Passavoy (*Proc. Soc. Exp. Biol. and Med.*, 117, 77; 1964). It was reported that actinomycin D inhibited the hypercalcaemia induced in mice and rats by large doses of vitamin D or parathyroid hormone, indicating that the latter agents may act by inducing the synthesis of new enzymes in bone through a DNA-directed RNA system. Present experiments in our laboratory also showed that actinomycin D prevented the stimulation of calcium absorption by vitamin D<sub>3</sub> in rachitic chicks.

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## INHIBITION INVESTIGATIONS WITH THE *Hi* AGGLUTINOGEN OF CHICKEN ERYTHROCYTES

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**A**N extract of *Pisum arvense*, the Canadian field pea, detects the presence of an agglutinin in chickens designated *Hi*. Normally, the *Hi* substance is found only in females which possess the *Hi* gene and which are sexually mature. The latter feature is due to the association of the *Hi* agglutinin with the female hormone, oestrogen. Injection of diethylstilboestrol into genetically competent males or young chickens results in the expression of the *Hi* substance in these birds as well<sup>1-3</sup>.

Progesterone, methyl testosterone or corn oil, the hormone solvent, injected at the same levels as diethylstilboestrol, did not stimulate the production of the agglutinin. Nor does testosterone have any residual effect, for diethylstilboestrol evokes the appearance of the *Hi* substance even when used after a course of injections with testosterone. Both males and females have been given continuous injections of a polyethylene glycol paste preparation of diethylstilboestrol subcutaneously, some for as long as 32 months. The titres of these birds, once they reached their maximum levels, did not diminish during this period, and these birds have been used as the standards for the experiments.

At the outset of these experiments, it was found possible to inhibit the agglutination reaction between the red cell and *Pisum arvense* extract. This discovery permitted examinations to be made on the nature of the *Hi*

substance and on the bonding between the agglutinin and the agglutinin.

Tests for inhibition were performed by mixing aliquots of lectin and the potential inhibitor and allowing these to incubate for 10 min at 22° C. An aliquot of a standard 2 per cent red cell suspension was then added and the mixture shaken and incubated for an additional 5 min. The tubes were then centrifuged (1,650g, 1 min) and scored macroscopically for the presence of agglutination; the score for maximum agglutination was four, with lesser scores for decreasing amounts of agglutination. All weak and zero readings were checked microscopically.

Tests for inhibition were conducted on more than 91 compounds. They included 21 amino-acids, 6 proteins, 13 hormones and more than 51 carbohydrates. Thyrotropin and a number of sugars were found to be inhibitory. All other substances tested failed to inhibit. Substances were tested for their inhibitory capacity using a 0.4 M solution. Those substances which when tested at this concentration resulted in 100 per cent inhibition were regarded as inhibitory; those substances which did not inhibit maximally at this concentration were deemed non-inhibitory. The smallest amount of a substance necessary for inhibition was determined by using successive dilutions of the inhibitor until inhibition could no longer be detected.

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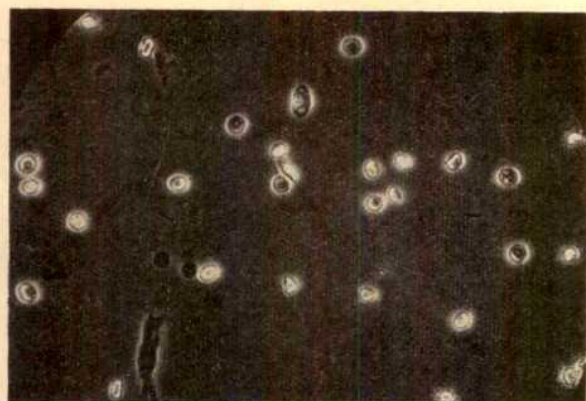


Fig. 1. Mixed agglutination of human and chicken erythrocytes

Human cells are completely cross-reactive with chicken cells in respect to *Pisum arvense*. This circumstance provided the opportunity for a mixed agglutination system in which human cell detectors form rosettes on chicken red cells coated with *Pisum arvense* (Fig. 1), the presence or absence of agglutination being detected by microscopic examination. The minimum amount of D-glucose<sup>4</sup> required for inhibition in this system was 0.13%, whereas the minimum amount in the conventional system was 2.1%. The use of the mixed agglutination procedure permitted an estimate of the amount of D-glucose<sup>4</sup>, a non-inhibitor, needed for inhibition. It was found that at least 90% of D-galactose was needed for inhibition.

The results of inhibition experiments with monosaccharides indicate that those in which the C<sub>3</sub> and C<sub>4</sub> hydroxyls are in the *trans* position have the capacity to inhibit the agglutination of *Hi* containing red cells with *Pisum arvense*. In the Haworth symbolism the C<sub>3</sub> hydroxyl is in the up position and the C<sub>4</sub> hydroxyl is in the down position. In this group are the sugars shown in Table 1. The amount indicated is for complete inhibition in the test tube, using 0.05 c.c. of agglutinin, 0.05 c.c. of the inhibitor solution and 0.05 c.c. of a standard 2 per cent red cell suspension. If the C<sub>3</sub> and C<sub>4</sub> hydroxyl groups are in the *cis* position and down, there is no inhibition. In this category are D-allose and D-altrose. If the C<sub>3</sub> and C<sub>4</sub> hydroxyl groups are in the *cis* position and up, there is no inhibition. In this group were D-galactose and  $\alpha$ -D-talose. A sugar in which the C<sub>3</sub> and C<sub>4</sub> hydroxyls were the reverse of that in D-glucose,  $\beta$ -L-glucose, did not inhibit.

Table 1. MONOSACCHARIDES WITH THE C<sub>3</sub> AND C<sub>4</sub> HYDROXYLS IN *trans* CONFIGURATION

D-fructose-( $\beta$ -D-fructofuranose) <sup>5</sup> —3.6 mg	2-deoxy-D-glucose—1.8 mg
1,5-anhydro-D-glucitol—1.8 mg	$\alpha$ -D-glucose <sup>4</sup> —1.8 mg
D-glucosamine <sup>6</sup> —3.6 mg	3-O-methyl-D-glucose—0.45 mg
N-acetyl-D-glucosamine <sup>6</sup> —3.6 mg	1,5-anhydro-D-mannitol—1.8 mg
methyl $\alpha$ -D-glucoside—1.8 mg	$\alpha$ -D-mannose <sup>4</sup> —0.9 mg
$\beta$ -D-glucose <sup>4</sup> —3.6 mg	methyl $\alpha$ -D-mannoside—0.9 mg

Inhibition experiments were carried out to determine which hydroxyl groups might be involved in combination with the agglutinin. Substitutions involving the C<sub>1</sub> hydroxyl did not alter the inhibition properties of the hexose. The following were tested and all inhibited: methyl- $\alpha$ -D-glucoside<sup>5</sup>, 1,5-anhydro-D-mannitol, methyl- $\alpha$ -D-mannoside, D-glucose 1-phosphate<sup>6</sup> and 1,5-anhydro-D-glucitol.

Substitutions involving the C<sub>2</sub> hydroxyl likewise did not alter the capacity of the hexoses to inhibit. Included in this group were D-fructose, D-glucosamine, N-acetyl-D-glucosamine and 2-deoxy-D-glucose. Substitutions for the C<sub>3</sub> hydroxyl also resulted in inhibition; the sugars tested were 3-O-methyl-D-glucose and D-glucose 3-sulphate.

The C<sub>5</sub> hydroxyl is not involved, since only the ring forms of the hexoses inhibit. D-Mannitol and D-glucitol<sup>4</sup>

do not inhibit, whereas the 1,5-anhydro derivatives of both do.

The C<sub>4</sub> hydroxyl group was substituted with the 4-O-methyl-D-glucose which failed to inhibit. Prior to testing with this sugar, we suspected that the C<sub>4</sub> hydroxyl was involved in the inhibition.  $\alpha$ -D-Glucose pentaacetate<sup>5</sup> and methyl  $\beta$ -D-glucopyranoside tetraacetate which possessed ring structures failed to inhibit; since we had already excluded the C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub> hydroxyls, then the fourth was implicated. The necessity for the C<sub>3</sub> and C<sub>4</sub> hydroxyls to be in the *trans* position likewise suggested that the C<sub>4</sub> hydroxyl was involved, and the inhibition experiment with 4-O-methyl-D-glucose confirmed this. More recently, another C<sub>4</sub> substituted hexose has become available, that is, dicyclohexyl ammonium salt of methyl  $\alpha$ -D-glucopyranoside 4-(dihydrogen) phosphate; this compound likewise failed to inhibit.

As a test of the influence of the C<sub>6</sub> hydroxyl, the disodium salt of glucose 6-phosphate<sup>6</sup> was used, and it failed to inhibit, implicating the C<sub>6</sub> hydroxyl group in the inhibition. Previous experiments with the barium salt of glucose 6-phosphate had been ambiguous; consequently, when the disodium salt became available, it was quickly tested. Solubility and pH of the prospective inhibitors were always matters of concern and occasionally limited for a time the testing of a particular compound. Confirmation of the role of the C<sub>6</sub> hydroxyl group came from tests with other C<sub>6</sub> substituted hexoses: 6-deoxy-6-O-nitro-D-glucose and dicyclohexyl ammonium salt of methyl D-glucoside 6-(dihydrogen) phosphate, and the same salt of methyl D-mannoside 6-(dihydrogen) phosphate; all these compounds failed to inhibit the reaction.

Pentoses had been tested in our initial survey, and had uniformly failed to inhibit the agglutination reaction. Their lack of a sixth hydroxyl group may be suspected as the basis for their uniform behaviour. Nine pentoses, as well as one tetrose and one triose, were tested and all failed to inhibit. These were L-arabinose, D-arabinitol<sup>3</sup>, L-arabinitol<sup>4</sup>, erythritol, D,L-glyceraldehyde<sup>6</sup>,  $\alpha$ -D-lyxose, 2-deoxy-D-ribose, D-ribose, D-xylitol, D-xylose, and methyl- $\beta$ -D-xyloside.

Based on the findings with monosaccharides, it was possible to make predictions regarding the inhibition with oligosaccharides. In order to inhibit, an oligosaccharide must possess at least one hexose residue with the C<sub>3</sub> and C<sub>4</sub> hydroxyl groups in the *trans* position and the C<sub>4</sub> and C<sub>6</sub> hydroxyl must be neither substituted nor involved in the linkage between residues. Oligosaccharides with more than one residue possessing the necessary properties would be expected to inhibit to a greater extent than the standard D-glucose. At the outset it was necessary to make one additional assumption based on our observations with sophorose, and that was that  $\beta$ -linkages result in a reduction of inhibition.

Listed next are comparisons between expectation and observation, with the reasons for the expectation given in parenthesis after each compound. The following compounds were not expected to inhibit and did not: cellobiose and epicallobiose (linkage involves C<sub>4</sub> of second residue,  $\beta$ -linkage); cellobiose (second residue is altrose,  $\beta$ -linkage); lactose<sup>4</sup> (first residue is galactose, linkage involves C<sub>4</sub> of second residue,  $\beta$ -linkage);  $\beta$ -gentiobiose (linkage involves C<sub>6</sub> of second residue,  $\beta$ -linkage); melibiose<sup>4</sup> (first residue is galactose, linkage involves C<sub>6</sub> of second residue); primeverose (first residue is xylose, linkage involves C<sub>6</sub> of second residue,  $\beta$ -linkage); the trisaccharide raffinose<sup>6</sup> (first residue is galactose, linkage involves C<sub>6</sub> of second residue,  $\beta$ -linkage between second and third residues); and the polysaccharides dextran (linkages between residues mainly involve C<sub>4</sub> with some C<sub>6</sub>); inulin ( $\beta$ -linkages between residues); starch (linkages involve C<sub>6</sub> for amylose and C<sub>4</sub> for amylopectin).

The following disaccharides containing two glucose residues were expected to inhibit to the same extent as the standard D-glucose, and this was found to be the



case with the reason for the expectation given in parenthesis: maltose<sup>4</sup> (linkage involves C<sub>4</sub> of second residue), sucrose<sup>4</sup> ( $\beta$ -linkage).

Because the following compounds contained two or more inhibiting residues and no prohibitive linkages, they were expected to inhibit to a greater extent than the standard D-glucose: trehalose (D-glucose, 1.35 mg; trehalose, 0.63 mg); turanose (D-glucose, 1.35 mg; turanose, 0.63 mg); melezitose (a trisaccharide possessing three inhibiting residues but also a  $\beta$ -linkage; D-glucose, 1.35 mg; melezitose, 0.45 mg).

The results of these comparisons confirm the results already obtained with monosaccharides. Mannose or a mannose-like monosaccharide is an important part of the agglutinin, and the linkage between it and *Pisum arvense* involves the C<sub>4</sub> and C<sub>6</sub> hydroxyl groups.

We thank the following for their contributions of monosaccharides and oligosaccharides: Dr. H. C. Fletcher and Dr. N. K. Richtmeyer (D-allose, D-altrose, L-arabinose, D-arabitol, cellobiose, cellobiose, dulcitol, epicallobiose, erythritol,  $\alpha$ -gentiobiose, 1,5-anhydro-D-glucitol, methyl- $\beta$ -D-glucopyranoside tetraacetate,  $\beta$ -L-

glucose, 2-deoxy-D-glucose, 3-methyl- $\alpha$ -D-glucose, inulin,  $\alpha$ -DL-lyxose, 1,5-anhydro-D-mannitol, methyl- $\alpha$ -D-mannoside, melezitose, primeverose, 2-deoxy-ribose, sophorose,  $\alpha$ -D-talose, trehalose, turanose, D-xylitol, L-xylitol, methyl- $\beta$ -D-xyloside); Prof. Fred Smith (4-O-methyl-D-glucose); Dr. Patricia Szabó (dicyclohexyl ammonium salt of methyl  $\alpha$ -D-glucopyranoside 4-(di-hydrogen) phosphate, of methyl glucoside-6-(di-hydrogen) phosphate, and of methyl  $\alpha$ -D-mannoside-6-(di-hydrogen) phosphate); Dr. F. W. Lichtenthaler (6-deoxy-6-nitro-D-glucose); Dr. J. R. Turvey (D-glucose-3-sulphate). We also thank Dr. D. T. O. Wong and Dr. N. K. Richtmeyer for their advice.

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<sup>4</sup> Pfahnstiel.

<sup>5</sup> Eastman Kodak.

<sup>6</sup> Cal. Biochem.

<sup>7</sup> Fisher.

<sup>8</sup> Pfizer, Charles.

<sup>9</sup> Concord Lab.

<sup>10</sup> North Eastern Utilization Research Lab.

## CLEARANCE OF PARTICULATE MATTER FROM THE TRACHEOBRONCHIAL TREE IN PATIENTS WITH TUBERCULOSIS

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THE importance of physiological mechanisms for clearing dust and other particulate matter from the lungs and tracheobronchial tree is illustrated by the fact that, while a coal miner may in his lifetime inhale 6,000 g of coal dust particles less than  $4\mu$  in size, only 100 g will be found in his lungs at post-mortem examination<sup>1</sup>. In a typical English industrial town, a person inhales about 100 g of carbon during a lifetime, but only 0.5–1.0 g are found in his lungs and bronchial glands at autopsy<sup>2</sup>. From experimental investigations of the behaviour of particulate matter in the respiratory tract, it is known that larger particles,  $10\mu$  or more in size, are trapped in the nose, while smaller particles penetrate farther into the lungs and settle on the mucous blanket lining the tracheobronchial tree<sup>3</sup>. About 90 per cent of particles less than  $1\mu$  in size penetrate through to the lungs.

One mechanism by means of which foreign particles are eliminated from the lungs is the activity of cilia which line the bronchial tree as far down as the respiratory bronchioles. Riding on the mucous blanket which covers the cilia, particles are propelled up to the oropharynx, where they are swallowed. A second mechanism is coughing. In the past, quantitative investigation of these mechanisms to determine their relative importance and degree of derangement under various physiological and pathological conditions has been difficult, particularly in human subjects. Recent advances in the field of external radiation detectors combined with the ability to label a variety of particles with  $\gamma$ -emitting radioactive isotopes have made possible a new approach to these problems. This article describes results obtained in the examination of patients with a chronic pulmonary disease, namely, pulmonary tuberculosis.

During the past few years we have used aggregates of human serum albumin labelled with iodine-131 as a type of particulate matter suitable for investigation of the phagocytic capacity of the reticulo-endothelial system<sup>4–7</sup>. Details of the method of preparation of the particles are given elsewhere<sup>4</sup>. In essence, aggregation of human serum albumin was produced by heating under controlled conditions of pH, temperature and shaking. The mean molecular weight of the final particle, measured by the light-scattering method, indicated that an average of 11 albumin molecules had aggregated, although the size

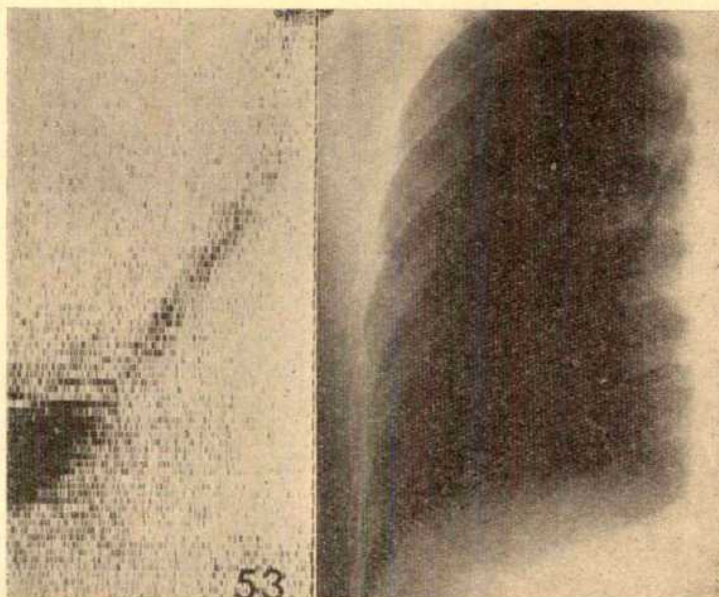


Fig. 1. Scan of the distribution of aggregated albumin particles 53 min after their injection into the lung through a catheter extending into the right main bronchus. On the right the catheter can be seen in the X-ray of the chest



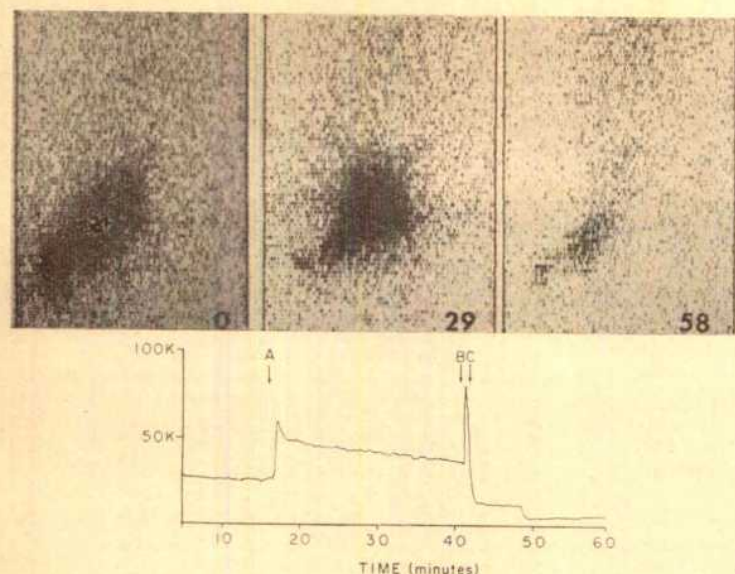


Fig. 2. Serial scans of the distribution of aggregated albumin particles. The numbers 0, 29 and 58 refer to the time (min) after injection of the particles into the right main bronchus. The area of increased radioactivity in the scan performed 29 min after injection of the particles was at the carina. The lower graph indicates the time course of radioactivity at the carina. At point A the patient coughed and the amount of radioactivity increased suddenly, presumably the result of particles being propelled upward to the carina. Thereafter the radioactivity decreased up to point B where the patient again coughed. This resulted in a further increase in activity; at point C the patient again coughed and the amount of radioactivity fell precipitously. The final decrease in activity occurred at 48 min and was also due to a cough.

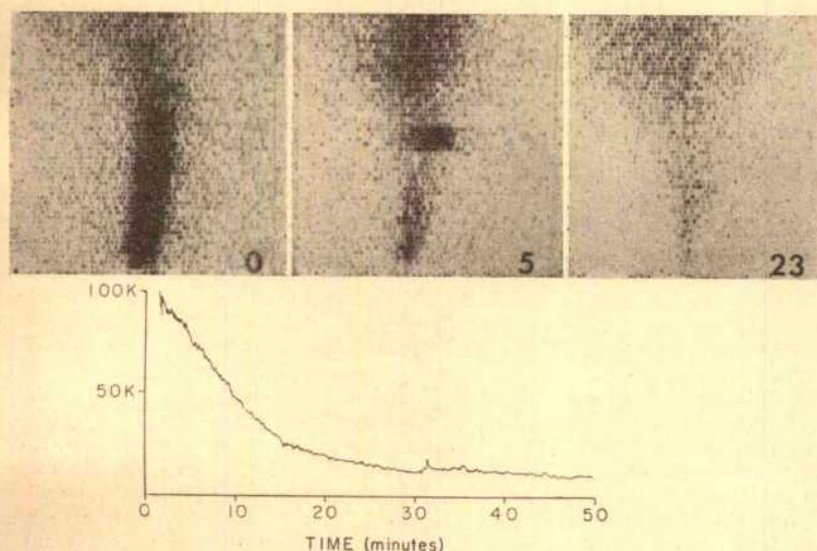


Fig. 3. Serial scans of the distribution of aggregated albumin particles after injection into the right main bronchus. The numbers 0, 5 and 23 indicate the time (min) that the scan was begun after injection of the particles. The lower graph is the time course of radioactivity as measured by a stationary radiation detector at the lower end of the area of maximum radioactivity.

of the particles was quite variable. Their mean size was about 100 Å. When injected intravenously, the albumin aggregates were removed from the blood by the reticulo-endothelial system, as evidenced by the kinetics of their removal from blood, their localization in the macrophages of the liver and spleen, and their effect in producing transient blockade of the reticulo-endothelial system. Large doses of these particles could be given repeatedly over prolonged periods of time to human subjects without evidence of hypersensitivity or excessive radiation. An advantage of albumin aggregates over the other colloidal particles, such as carbon, thorium dioxide ('Thorotrast'), saccharated iron oxide, radioactive chromic phosphate, colloidal gold or colloidal silver, is that these particles are metabolized after they are ingested by phagocytic cells

and therefore do not remain in the body for long periods of time. An additional advantage is that the appearance of radioactivity in the blood in the form of iodide ion can be used as an indication that the particles have been ingested by phagocytic cells.

In the work recorded here 0.1–0.3 ml. of a solution containing less than 0.1 mg of aggregated albumin labelled with 20–100 µc. iodine-131 was injected at various positions along the tracheobronchial tree in man by means of a fine radiopaque catheter inserted through a needle in the crico-thyroid membrane. Local anesthesia was used at the site of injection, but no other medication was necessary. The patients lay quietly in the supine position.

The rate and pattern of movement were measured in two ways, by means of serial radioisotope scanning of the chest<sup>8</sup> and by means of two stationary crystal scintillation detectors with slit collimators (3.0 × 0.3 cm). Fifteen investigations were carried out in afebrile male patients with chronic pulmonary tuberculosis in various stages of activity at the time of the work. The thyroid gland was blocked with 0.5 ml. of Lugol's solution because of eventual deiodination of the albumin aggregates.

Fig. 1 illustrates the position of the catheter in the right lung of one patient. The scanning image of the distribution of the particles was obtained in this work 53 min after injection of the albumin aggregate particles. The large area of increased activity at the most distal point remained unchanged for as long as 2 h, presumably because of the location of the particles in alveoli or terminal bronchioles where cilia are absent. The rest of the particles lining the bronchi were moved slowly toward the larynx at a rate of approximately 1–2 cm/min.

In many patients, foci of accumulation of the particles were observed to form as the particles moved. Most often these were at the carina, but also in other regions along the tracheobronchial tree. An example is shown in Fig. 2. The graph in the lower part of the figure represents the rate of clearance of the radioactivity, as measured by a detector at the carina. There was a net decrease in concentration of radioactivity as the particles were being moved by ciliary activity. At point A the patient coughed, with the result that the particles moved from lower in the tracheobronchial tree up to the carina; ciliary activity then moved the particles at a steady rate for approximately the next 20 min; then, at point B, the patient coughed a large number of particles up to the carina, followed by another cough at point C, which resulted in a great decrease in activity, related to movement of the particles up to the oropharynx. Serial scans performed at the times designated by the numbers (minutes after the injection of the particles) gave a visual image of the processes being monitored with the stationary detectors.

In the patient shown in Fig. 3 the second scan shows a typical focal concentration of radioactivity. The tracing at the bottom of the figure was obtained by a stationary detector over the lower end of the area of distribution of the albumin aggregate particles. In this patient there



was a continual decrease in concentration of the radioactivity, presumably entirely due to ciliary activity since the patient did not cough throughout the examination. In all subjects the radioactivity could be detected eventually in the stomach, arriving there when the particles in the oropharynx were swallowed. Insignificant amounts of radioactive iodine were detectable in the blood until after the particles had been swallowed, thus indicating that the phagocytic process did not participate significantly in the movement of the particles up the tracheobronchial tree to the pharynx.

LaBelle and Brieger have reported that the physiological events following the intratracheal injection of particulate matter are the same as those seen after the inhalation of the same particles<sup>9</sup>. Consequently we chose to use this simple method in our initial investigations. The effect of ciliary activity and cough could be readily quantified. Both were of considerable importance in clearing the lungs of this type of particle. Examinations of other types of particle are in progress.

The frequent finding of areas of decreased ciliary activity where the particles seem to gather is of interest since these may indicate areas where cilia or ciliated cells have been

destroyed or where changes of the type described by Hilding may be found. He reported areas of squamous epithelial cells scattered throughout the lower respiratory tract, particularly at the carina<sup>10</sup>. It has been known for many years that islands of metaplasia of ciliated columnar epithelium result from chronic inflammation. In these areas squamous epithelium replaces the ciliated, mucous-covered epithelium, and the regions of diminished motility may correspond to such areas.

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## REACTION OF THE RHODOPSIN CHROMOPHORE WITH SODIUM BOROHYDRIDE

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THE visual pigment, rhodopsin, is composed of a protein, opsin, bearing as chromophore the 11-*cis* isomer of retinal (vitamin A aldehyde, formerly retinene). Irradiation of this pigment isomerizes retinal from the 11-*cis* to the all-*trans* configuration, yielding as first product pre-lumirhodopsin ( $\lambda_{\max}$  543 m $\mu$ ). This is transformed in the dark via a series of transient intermediates, ending with the hydrolysis of retinal from opsin<sup>1</sup>. The transition up to this last step goes very quickly at room temperature, but can be blocked at any stage by cooling. Pre-lumirhodopsin is stable below -140° C; above this temperature it goes to lumirhodopsin ( $\lambda_{\max}$  500 m $\mu$ )<sup>2</sup>. Above -40° C lumirhodopsin yields metarhodopsin I ( $\lambda_{\max}$  478 m $\mu$ ) which, at temperatures higher than -15° C, enters into tautomeric equilibrium with metarhodopsin II ( $\lambda_{\max}$  380 m $\mu$ ), rise in temperature and acidity favouring the proportion of metarhodopsin II. Above 0° C, metarhodopsin II hydrolyses to a mixture of opsin and all-*trans* retinal ( $\lambda_{\max}$  387 m $\mu$ )<sup>3</sup>.

In rhodopsin retinal appears to be bound to the protein in a Schiff base linkage:  $C_{19}H_{27}CH=O + H_2N\text{-opsin} \rightarrow C_{19}H_{27}CH=N\text{-opsin} + H_2O$ . Evidence for this formulation has come from the work of Collins<sup>4</sup> and Morton and Pitt<sup>5</sup>. The latter workers have investigated the acid denaturation product of rhodopsin (the conjugate acid of N-retinylidene-opsin,  $\lambda_{\max}$  440 m $\mu$ ), a protonated Schiff base between retinal and denatured opsin ( $C_{19}H_{27}CH=N^+H\text{-opsin}$ ). Having found from model reactions that Schiff base formation does not occur readily at acid pH, they concluded that the Schiff base linkage had been present in rhodopsin itself before denaturation. Collins has shown that irradiation of a rhodopsin solution at pH 9 in the presence of excess formaldehyde results in the formation of the non-protonated Schiff base of retinal at the chromophoric site (N-retinylidene-opsin), all the other available amino-groups on opsin having been complexed with formaldehyde. This suggests again that the Schiff base was present in rhodopsin before irradiation. (Following Morton and Pitt, we will use the term N-retinylidene-opsin for the Schiff base derivative of rhodopsin in which retinal is attached to denatured opsin at its original site. Alkaline

indicator yellow is then the random collection of Schiff bases which forms in strongly alkaline solution between liberated retinal and all available amino-groups of denatured opsin.)

Morton *et al.* have also examined the properties of Schiff bases of retinal with a variety of amino-acids and amines<sup>6-8</sup>. The Schiff bases of retinal have  $\lambda_{\max}$  about 367 m $\mu$ ; in acid solution they are protonated to form the conjugate acids, with  $\lambda_{\max}$  440-450 m $\mu$ . Hubbard has proposed that in rhodopsin the Schiff base linkage is protonated, and that the further shift of spectrum toward the red in this pigment (to  $\lambda_{\max}$  500 m $\mu$ ) is associated with side-chain interactions between retinal and the chromophoric site on opsin<sup>9</sup>. Such exaltation of spectrum persists throughout the intermediates of bleaching of rhodopsin, to the stage metarhodopsin II; so that one should assume that up to this stage these pigments are not only protonated Schiff bases, but also exhibit the close fit between retinal and opsin that permits bathochromic interaction. In the transition metarhodopsin I  $\rightarrow$  metarhodopsin II, the proton is presumably lost from the Schiff base linkage, and the side-chain interaction of retinal with opsin also is greatly decreased.<sup>3</sup>

If the foregoing picture is correct, it should be possible to hydrogenate the Schiff base linkage between retinal and opsin to produce a secondary amine at the chromophoric site. The hydrogenated linkage should be stable to hydrolysis, and thus should enable one to isolate retinal still attached to the group to which it is bound in native rhodopsin.

The most appropriate reagent for such a reduction is sodium borohydride, first used in enzyme investigations by Fischer *et al.* to reduce the Schiff base linkage between pyridoxal phosphate and muscle phosphorylase<sup>10</sup>. The borohydride reduction has since been applied to the examination of another pyridoxal phosphate enzyme, glutamic-aspartic transaminase<sup>11</sup>, and to examine the site of binding of the substrate (dihydroxy-acetone) to transaldolase<sup>12</sup>, and of acetoacetate to its decarboxylase<sup>13</sup>. The reactions of borohydride with other linkages found in proteins have not been thoroughly investigated; but

it is known to reduce disulphide bonds and occasionally to cleave peptide bonds<sup>14</sup>.

Cattle rhodopsin, prepared by a modification of methods previously used in this laboratory<sup>3</sup>, was reacted with a thousand-fold molar excess of powdered sodium borohydride. The pH was adjusted to desired values with small volumes of 0.1 N NaOH or HCl, and the pH did not vary by more than 0.2 during an experiment. The reactions were run at constant temperature in the Cary recording spectrophotometer, and spectra were measured periodically.

Rhodopsin itself does not react with sodium borohydride, nor is its absorption spectrum changed by the addition of this reagent between pH 3 and 9. However, when rhodopsin (Fig. 1, curve 1) is irradiated in the presence of sodium borohydride, a new compound appears (Fig. 1, curve 2) with  $\lambda_{\max}$  333 m $\mu$ , near that of retinol (vitamin A,  $\lambda_{\max}$  328 m $\mu$ ). Like retinol, this product exhibits brilliant green fluorescence.

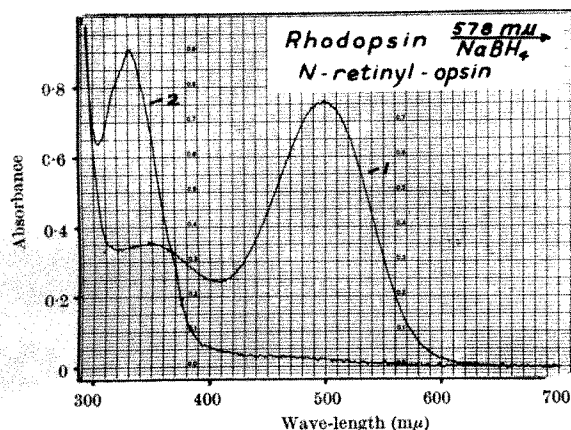


Fig. 1. The reaction of rhodopsin with sodium borohydride during its bleaching by light. Curve 1 is the spectrum of cattle rhodopsin in 2 per cent digitonin, M/15 phosphate buffer, pH 8. Curve 2 was recorded after the solution had been irradiated at 578 m $\mu$  in the presence of sodium borohydride.

A possible interpretation of these results might be that irradiation yielded free retinal which was afterwards reduced to retinol by the sodium borohydride (cf. ref. 15). To examine this possibility, methanol was added to the solution to a concentration of 60 per cent, to precipitate protein and liberate any carotenoid not covalently bound, and the mixture was extracted four times with petroleum ether, which should extract any free retinol. The petroleum ether extract was washed three times with water, transferred to chloroform, and the antimony trichloride reaction used to determine the amount of retinol present. Fig. 2a shows that no significant colour reaction was obtained. The reduction product therefore was not retinol.

This was shown further by a parallel control experiment in which rhodopsin was bleached by light and left 1.5 h in the dark to allow retinal to hydrolyse off completely. Sodium borohydride was then added to reduce the retinal to retinol, and the mixture treated with methanol and extracted as before. When this extract was mixed with antimony chloride, the deep blue colour and absorption band at 620 m $\mu$  characteristic of retinol were obtained (Fig. 2b).

These experiments show that the product of bleaching rhodopsin in the presence of sodium borohydride is not retinol but the reduced Schiff base in which retinal is still bound to opsin at the chromophoric site:



We shall call this compound N-retinyl-opsin.

The products of treating rhodopsin with strong acid or alkali are, respectively, protonated and non-protonated Schiff bases of retinal with denatured opsin (the conjugate acid of N-retinylidene-opsin, and alkaline indicator

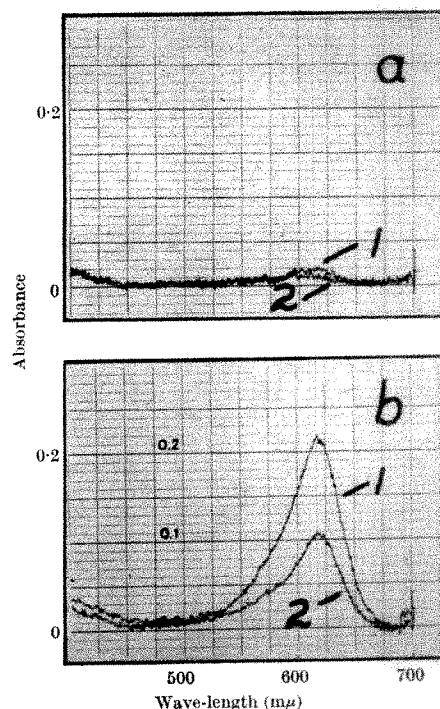


Fig. 2. Antimony chloride tests with reduced products of bleaching rhodopsin. (b) The retinal liberated on bleaching rhodopsin with light can afterward be reduced to retinol (vitamin A) with sodium borohydride. Such retinol is readily extracted with petroleum ether; mixed with antimony chloride it yields a blue colour owing to an absorption band with  $\lambda_{\max}$  620 m $\mu$  (curve 1 recorded 15 sec after mixing, curve 2 one min later). (a) Rhodopsin bleached in the presence of sodium borohydride, however, does not liberate retinol, as shown by the failure of this test in a similar extract of the solution that yielded spectrum 2 in Fig. 1. The reduction product in this experiment involves retinal still attached to opsin at the chromophoric site.

yellow)<sup>5</sup>. When sodium borohydride is added to alkaline indicator yellow, produced by irradiating a solution of rhodopsin at pH 9 (Fig. 3, curve 2), a derivative with  $\lambda_{\max}$  about 330 m $\mu$  appears immediately (Fig. 3, curve 3). Since alkaline indicator yellow is thought to be a random collection of Schiff bases between retinal and various amino-groups of opsin<sup>5</sup>, the reduction has probably fixed retinal on to several different amino-groups. Here again, the reaction mixture, extracted as described above, contained no free retinol or retinal, as shown by the complete absence of an antimony trichloride reaction. This was true also in all the further experiments to be described.

Fig. 4 shows the reduction of the product of acid denaturation of rhodopsin, the protonated N-retinylidene-opsin, in which retinal is thought to be still attached to

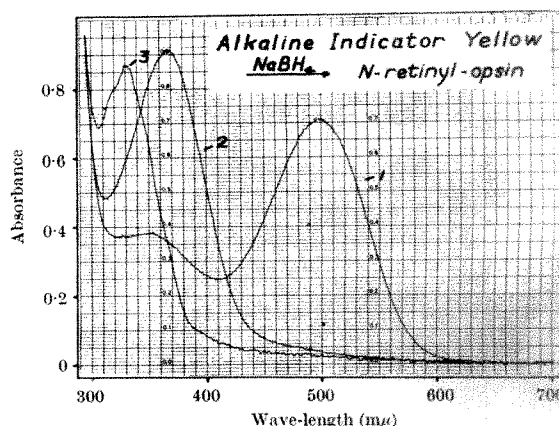


Fig. 3. The reduction of alkaline indicator yellow with sodium borohydride. Rhodopsin (curve 1) is irradiated at pH 9 to produce alkaline indicator yellow (curve 2,  $\lambda_{\max}$  367 m $\mu$ ). Sodium borohydride is then added to reduce the multiple Schiff base linkages between retinal and amino-groups on opsin (curve 3,  $\lambda_{\max}$  330 m $\mu$ ).



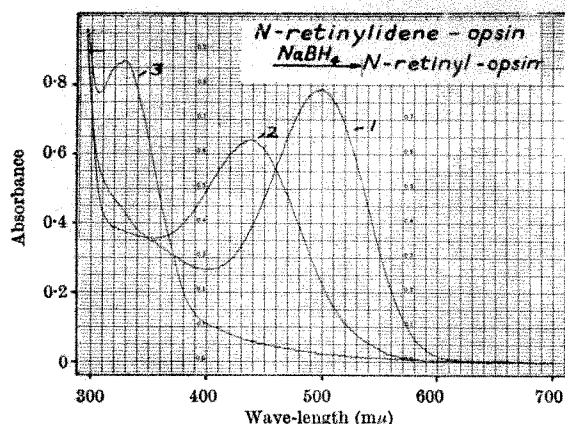


Fig. 4. The reduction of N-retinylidene-opsin with sodium borohydride. A rhodopsin solution (curve 1) is brought to pH 1 in the dark, acid-denaturing the rhodopsin to yield the conjugate acid of N-retinylidene-opsin ( $\lambda_{\max}$  440 m $\mu$ ). After raising the pH to 4 (curve 2), sodium borohydride is added. This reduces the N-retinylidene-opsin to (denatured) N-retinyl-opsin (curve 3).

its original site in native rhodopsin. Rhodopsin is brought to pH 1 and then to pH 4 (curve 2) before adding sodium borohydride. This is done because at pH 4 sodium borohydride is decomposed by hydrolysis less rapidly than at pH 1, yet the Schiff base remains protonated. The spectrum of the reduced derivative appears immediately after the addition of sodium borohydride and is similar in shape and absorption maximum to that obtained when rhodopsin is bleached in the presence of sodium borohydride (curve 3).

Protonated and non-protonated Schiff bases between retinal and a number of aliphatic and aromatic amino-acids were also reduced, and spectral changes observed which were almost identical with those obtained with the reduction of N-retinylidene-opsin and alkaline indicator yellow. At room temperature all these reactions were complete within 10 sec after the addition of sodium borohydride.

Since rhodopsin reacts with sodium borohydride during its bleaching by light to yield a product having the spectrum of a reduced Schiff base, one of the intermediates of bleaching before the hydrolysis to free retinal and opsin must expose the Schiff base linkage. To identify this intermediate, we examined the reaction of sodium borohydride with metarhodopsin I and metarhodopsin II.

Irradiation of a rhodopsin solution (Fig. 5, curve 1) in 66 per cent glycerol, pH 6.5, at  $-20^{\circ}\text{C}$  isomerizes the retinaldehyde chromophore to a steady-state mixture of pigments (curve 2): metarhodopsin I (the all-*trans* chromophore), isorhodopsin (9-*cis* chromophore) and rhodopsin (11-*cis* chromophore)<sup>2</sup>. At  $-20^{\circ}\text{C}$  metarhodopsin I is not converted to metarhodopsin II. The spectrum is not significantly altered by the addition of three 0.5-mg portions of sodium borohydride (curves 3, 4 and 5). To show that sodium borohydride could function at  $-20^{\circ}\text{C}$ , the solution was warmed to room temperature for 1 h to allow the metarhodopsin to hydrolyse to free retinal and opsin. (By this time the borohydride previously added had been hydrolysed.) On re-cooling to  $-20^{\circ}\text{C}$ , curve 6 was obtained. It shows the presence of isorhodopsin and rhodopsin, and a peak near 390 m $\mu$  due to retinal. Addition of two portions of sodium borohydride at  $-20^{\circ}\text{C}$  caused the latter band to be replaced by one near 330 m $\mu$  (curves 7 and 8), showing that sodium borohydride can reduce retinal to retinol even at  $-20^{\circ}\text{C}$ . Thus its failure to react with metarhodopsin I at that temperature is significant.

Similar experiments have been performed with metarhodopsin II at  $-20^{\circ}\text{C}$ . The absorption of this pigment, unlike that of metarhodopsin I, does fall slowly when portions of sodium borohydride are added. However, at this temperature it does not react nearly so rapidly with

sodium borohydride as free retinal or the non-protonated Schiff base of alkaline indicator yellow.

The work at  $-20^{\circ}\text{C}$  suggests that metarhodopsin II is more reactive than metarhodopsin I toward sodium borohydride. Fig. 6 shows an experiment at  $2^{\circ}\text{C}$  which demonstrates this point more clearly. Irradiation of a rhodopsin solution at  $2^{\circ}\text{C}$  and pH 6.5 produces an equilibrium mixture of both tautomers (curve 2). When sodium borohydride is added, the metarhodopsin II absorption at 380 m $\mu$  is replaced within seconds by absorption at 330 m $\mu$ , whereas considerable metarhodopsin remains (curve 3). The disappearance of the remaining metarhodopsin I (curves 4 and 5) is associated with further rise in absorption at 330 m $\mu$ , metarhodopsin II apparently being reduced as fast as formed.

It seems clear, therefore, that metarhodopsin II is reduced much more rapidly by sodium borohydride than metarhodopsin I, if the latter is reduced at all. Thus,

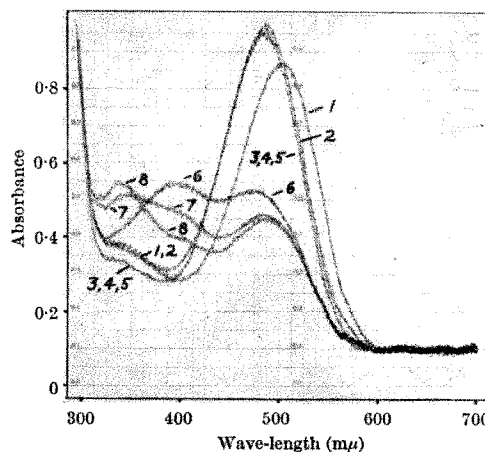


Fig. 5. The failure of metarhodopsin I to react with sodium borohydride at  $-20^{\circ}\text{C}$ . Curve 1 is the spectrum of rhodopsin, pH 6.5, in 66 per cent glycerol at  $-20^{\circ}\text{C}$ . Exhaustive irradiation at this temperature produces a steady-state mixture of rhodopsin, isorhodopsin, and metarhodopsin I (curve 2). Curves 3, 4 and 5, each recorded 30 min after successive additions of sodium borohydride, show no significant changes. Apparently sodium borohydride does not react with any of these pigments. To show that it can act as a reducing agent at  $-20^{\circ}\text{C}$ , the solution is warmed to room temperature for an hour to allow the metarhodopsin I present to hydrolyse to free retinal and opsin. It is re-cooled to  $-20^{\circ}\text{C}$  (curve 6); this displays a peak at about 480 m $\mu$  due to isorhodopsin and rhodopsin, and a peak at about 387 m $\mu$  due to free retinal. The addition of two further portions of sodium borohydride causes the latter band to be replaced by the retinol band at about 330 m $\mu$  (curves 7 and 8, each recorded 30 min after adding portions of sodium borohydride).

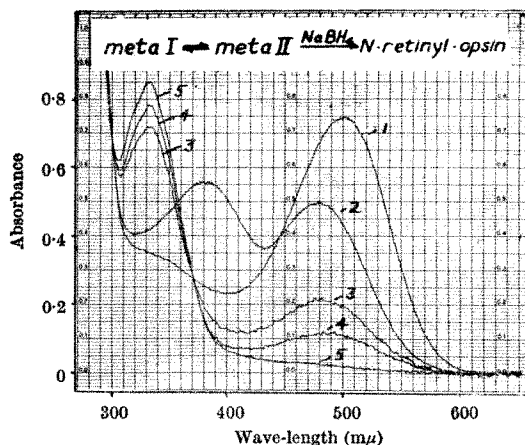


Fig. 6. The reaction of sodium borohydride with an equilibrium mixture of metarhodopsins I and II. Irradiation of rhodopsin (spectrum 1) at  $2^{\circ}\text{C}$ , pH 6.5, yields an equilibrium mixture of metarhodopsin I ( $\lambda_{\max}$  478 m $\mu$ ) and metarhodopsin II ( $\lambda_{\max}$  380 m $\mu$ ) (spectrum 2). Curve 3, recorded within 30 sec after adding sodium borohydride, shows that metarhodopsin II is wiped out, being replaced by N-retinyl-opsin ( $\lambda_{\max}$  333 m $\mu$ ), while considerable metarhodopsin I remains. On warming the solution, the remaining metarhodopsin I was transformed, presumably over metarhodopsin II, to N-retinyl-opsin (curve 4). On re-irradiating in the warm, the remaining rhodopsin and isorhodopsin were bleached and also reduced to N-retinyl-opsin (curve 5).

when rhodopsin is irradiated in the presence of sodium borohydride, metarhodopsin II is probably the first intermediate to be reduced.

### Discussion

Rhodopsin itself is not attacked by sodium borohydride; reduction of the Schiff base linkage of retinal to opsin occurs only after it is irradiated. Nor does potassium borohydride, used earlier in our laboratory to reduce retinal freed by the bleaching of rhodopsin, attack rhodopsin<sup>18</sup>. These observations are not surprising in the light of earlier work which has shown that attachment to opsin protects retinal from a variety of chemical attacks. Lipoxidase, for example, attacks the polyene chain of retinal after rhodopsin is bleached, but has no effect on rhodopsin itself<sup>19</sup>. Similarly, hydroxylamine, during the bleaching of rhodopsin, traps retinal away from opsin by oxime formation, but does not attack rhodopsin<sup>17</sup>. (It is an interesting sidelight that hydroxylamine does take retinal from the cone pigment, iodopsin<sup>18</sup>.)

There are many lines of evidence that configurational changes in opsin accompany the bleaching of cattle rhodopsin<sup>1</sup>. A large increase in entropy occurs in the transition metarhodopsin I  $\rightarrow$  metarhodopsin II<sup>2</sup>; also, as the result of bleaching, new groups on opsin—two sulphhydryl groups and one acid-binding group—are exposed. Further, the optical rotation at 233 m $\mu$  becomes less negative on irradiation<sup>19</sup>. Sodium borohydride may not be able to effect a reduction until such configurational changes have exposed the Schiff base linkage, apparently at the stage of metarhodopsin II.

If one assumes that metarhodopsin I is a protonated, and metarhodopsin II a non-protonated, Schiff base, a further explanation must be considered. Protonation of the Schiff base linkage may block reduction in the earlier intermediates, whereas loss of this proton in the transition from metarhodopsin I to metarhodopsin II may render the latter molecule more susceptible to reduction. This suggestion implies that sodium borohydride may react selectively with the uncharged rather than with the protonated Schiff base, at least in this instance. There is as yet no clear evidence that such selective reduction of the uncharged species occurs generally. On the contrary, model Schiff bases of retinal and various amino-acids, brought to acid pH so that their  $\lambda_{\max}$  is about 440 m $\mu$ , characteristic of the protonated species, react so rapidly with sodium borohydride at room temperature that we are unable to follow the kinetics. The same is true of the reaction of uncharged Schiff bases, however, so that we cannot conclude definitely what species is attacked.

It should be noted that if in rhodopsin the chromophore is bound as a protonated Schiff base, this linkage has

extraordinary properties. The complete stability of the rhodopsin spectrum between pH 4 and 10 means that the Schiff base retains its proton even at the latter pH, implying that it possesses a  $pK$  much higher than 10. We do not know of any model Schiff base that displays such a high  $pK$ . It is possible alternatively that the protonated linkage may be bound into the protein structure in some way that makes it inaccessible to any reagents, even such as might remove a proton. As already said, the conjugate acid of N-retinylidene-opsin, in which retinal is bound to denatured opsin as a protonated Schiff base at the original site, reacts rapidly with sodium borohydride. This indicates that some aspect of the native opsin structure shields the protonated Schiff base linkage from reaction with sodium borohydride.

In summary, sodium borohydride, though it does not react with rhodopsin, reduces an intermediate—apparently metarhodopsin II—in the bleaching of rhodopsin by light. The special vulnerability of metarhodopsin II is probably due to the exposure of the Schiff base linkage between retinal and opsin through unfolding of the protein, as also possibly to the loss of a proton by this linkage. The reduction of the Schiff base linkage anchors retinal firmly to the chromophoric site in opsin as the stable secondary amine, N-retinyl-opsin. Experiments are in progress to identify the amino-acids at the binding site.

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## PROTECTIVE ACTION OF HEPARIN IN EXPERIMENTAL IMMUNE NEPHRITIS

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**H**EPARIN has been found to inhibit various forms of tissue damage in hypersensitivity reactions such as the Shwartzman phenomenon<sup>1</sup> and the Arthus lesion<sup>2</sup>. The results reported here provide evidence that heparin exerts a remarkable protective action on allergic nephritis induced in rabbits by injection of heterologous nephrotoxic serum. Following a description of the results, the possible mechanism of action of heparin will be discussed, taking into account the manifold physiological effects of heparin.

The anti-kidney serum was prepared by immunizing ducks with rabbit kidney homogenate incorporated in complete Freund's adjuvants (Difco). The injections were given once a week during 5 weeks. The animals were bled a week after the last injection and the titre of anti-kidney antibody determined by the complement deviation reaction. In spite of identical techniques of immunization, the antibody titre varied considerably from one bird to another. For these experiments only the most potent sera were used.



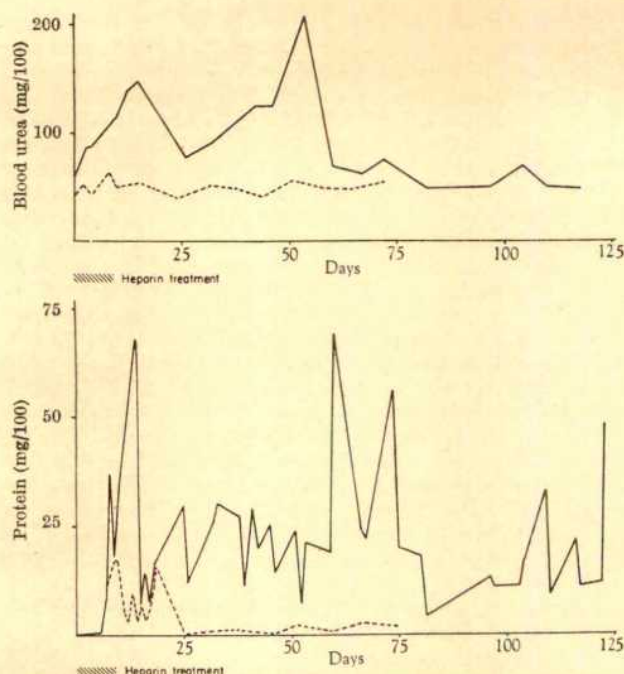


Fig. 1. Protective action of heparin on kidney damage produced by heterologous anti-kidney serum in rabbits. Mean blood urea nitrogen (upper part) and mean daily proteinuria (lower part) in control group as compared with animals treated with heparin. (The dose of antiserum administered was 8 ml.) Each point represents a mean of values obtained in 4 animals. —, 4 rabbits injected with 8 ml. anti-kidney serum; ---, 4 rabbits injected with 8 ml. anti-kidney serum and heparin.

For the induction of glomerulonephritis, adult rabbits weighing 2.5 kg were used. The animals were previously checked for proteinuria, blood urea nitrogen and blood pressure. The anti-kidney serum was administered by a single intravenous injection of 4 ml. in one series, and of 8 ml. in another one. The animals of both series were subdivided into two groups *A* and *B*.

The animals of group *A* received the anti-kidney serum alone.

The animals of group *B* received, with the anti-kidney serum, intravenous injections of heparin in the following manner: 100 mg per animal simultaneously with the injection of the antiserum and 25 mg twice a day during the following 10 days.

A third group of 10 rabbits (group *C*) received an intravenous injection of 8 ml. of normal duck serum.

The animals were placed in individual cages and checked for urine output, proteinuria, haematuria, blood urea nitrogen and blood pressure (measured with the technique described by Grant). Careful histological investigations of the kidney were performed either on biopsy fragments taken between the 10th and 20th day following treatment, or on necropsy preparations from animals which died spontaneously or which were killed deliberately.

Of 19 animals of group *A* receiving the anti-kidney serum alone, all have presented symptoms of severe glomerulonephritis. Ten animals died spontaneously 8–20 days following injection, with symptoms of severe renal failure: proteinuria, haematuria. The blood urea reached, or even exceeded, 150–300 mg/100 ml. (Fig. 1).

The histological examination confirmed the extent and severity of the kidney lesions. There were signs of a generalized endothelial proliferative glomerulonephritis with thrombosis and necrosis of the glomer-

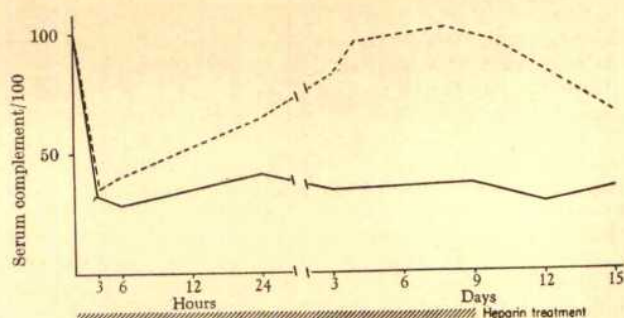


Fig. 2. Daily modifications of serum complement levels after the administration of anti-kidney serum in the control animals and in animals treated with heparin. Each point represents a mean of values obtained in 4 animals. —, 4 rabbits injected with 8 ml. anti-kidney serum; ---, 4 rabbits injected with 8 ml. anti-kidney serum and heparin.

ular capillaries. It was estimated that 60–90 per cent of the glomeruli were involved. These lesions were associated with proliferation of epithelial cells leading to massive crescent-like formations and flocculo-capsular synechiae. In more advanced stages, about 60 per cent of the glomeruli were fibrotic and a great number were completely obliterated. Interstitial fibrosis, fibrinoid degeneration of the arterioles and tubular engorgement with hyaline casts were commonly noted.

The renal lesions were more severe in the animals which received the higher dose of antiserum.

Of the 17 animals forming group *B*, one died with symptoms of renal failure. Haematuria and proteinuria were occasionally observed in the first few days following the treatment, but afterwards they subsided. The blood urea nitrogen remained normal in all animals, except one (Fig. 2).

The histopathological findings confirmed the remarkable protective action of heparin. In animals killed 4 weeks after treatment, the lesions were limited to about 1–5 per cent of glomeruli, all others being apparently normal. The interstitium was normal as well as the arterioles.

Slight and transient proteinuria occurred in some animals of group *C*, and the histological findings here were unimportant.

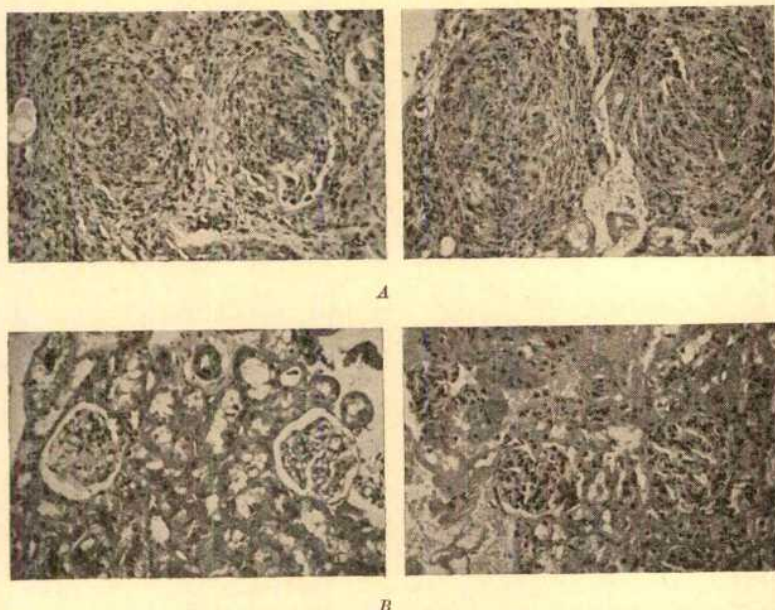


Fig. 3. Photomicrograph of kidney sections obtained from rabbits treated with anti-kidney serum. *A*, No treatment with heparin. Rabbits 249 (left) and 757 (right). Massive glomerular lesions with endocapillary proliferation, producing obliteration of the lumen of the capillaries. Epithelial proliferation with formation of crescents and complete fusion of the flocculum with the capsula. Periglomerular inflammatory reaction. *B*, Treated with heparin. Left (rabbit 738), glomeruli essentially normal. Slight endocapillary proliferation. Right (rabbit 735), signs of moderate endocapillary glomerulonephritis. Slight proliferative reaction and early hyalinosis.



The results presented in Fig. 2 indicate that the injection of nephrotoxic serum causes a dramatic and long-lasting fall of the serum complement. The effect was almost maximal within a few hours and it seemed to persist so long as the inflammatory process continued. In the heparin-treated group the initial fall was almost of the same importance, but during the 2-3 following days the complement titre rose rapidly and reached or even exceeded transiently the normal values.

The administration of heparin to animals receiving heterologous anti-kidney serum, in a dose high enough to cause in 100 per cent of the control animals severe renal failure leading to about 50 per cent of mortality, reduced markedly both the biochemical and the histopathological symptoms of the renal damage. In the treated group the mortality was minimal (1 out of 17 animals), the blood urea nitrogen remained unchanged and the histological lesions were considerably reduced. These results are in agreement with previous observations reported by Silfverskiöld<sup>3</sup> and Kleinerman<sup>4</sup>.

To be effective the treatment with heparin should fulfil several conditions: (1) the heparin should be administered before or immediately after the injection of the antiserum; (2) it should be given in high dosage; (3) it should be continued for at least one week following sensitization.

The mechanism of action of heparin in immune nephritis is not yet clear. Two possibilities can be envisaged: (1) Heparin may act by virtue of its anticoagulant properties and thus prevent the thrombo-embolic disorders commonly observed in hypersensitivity reactions. This mechanism has been evoked to explain the protective action of heparin against cortical necrosis in the generalized Schwartzman reaction<sup>1</sup>. This may also be the explanation of the preventive action of heparin in the Arthus lesion, in which vascular damage is pre-eminent.

(2) Heparin may act also by its anticomplementary properties. Immune nephritis may be similar either to the Arthus type lesions or to the immune cytolytic type reaction. In both types of reaction complement is involved. While in the cytolytic type of reaction the role of complement is essential, in the initiation of the cellular

damage, it is still conjectural in the case of the Arthus reaction.

Heparin has been found, in our experiments, to prevent immune haemolysis *in vitro*. Our findings suggest also that complement is not bound or only transiently bound by antigen-antibody complexes *in vivo* in heparin-treated animals. If complement is an essential factor in inducing tissue damage in immune nephritis, this mechanism cannot be overlooked. It may be emphasized here that Lachmann *et al.*<sup>5</sup> have recently shown by immuno-histochemical investigations the presence of *in vivo* bound complement in the glomeruli in human glomerulonephritis of varying aetiology. In all these cases the distribution of complement was similar to that of bound human  $\gamma$ -globulin, suggesting that binding of complement by the antibody may be related to the tissue damage.

Whatever the mechanism of action of heparin may prove to be, its remarkable protective effects on tissue damage in immune experimental nephritis strongly suggest potential and unexplored applications in similar human pathological conditions. Our first clinical results in acute glomerulonephritis support this impression.

The action of heparin on tissue damage in immune nephritis, induced in rabbits by injection of heterologous anti-kidney serum, has been investigated. The results obtained indicate that, when administered early enough and in sufficient doses, heparin exerted a remarkable protective effect. While the serum complement level was markedly and lastingly depressed in the animals receiving anti-kidney serum, the fall was only transient in the heparin-treated group.

Whether the action of heparin on immune nephritis is related to its anticoagulant properties or to its complement inhibiting effects is discussed.

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## RELATIONSHIP OF APPEARANCE OF ADENOSINE DIPHOSPHATE, FIBRIN FORMATION AND PLATELET AGGREGATION IN THE HAEMOSTATIC PLUG *IN VIVO*

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MANY investigators in the past few years have shown that adenosine diphosphate (ADP) aggregates platelets *in vitro*. This is an important observation, since both haemostasis and thrombus formation are initiated by platelet aggregation and the red blood cells and platelets of whole blood are possible *in-vivo* sources of this nucleotide. Hellem<sup>1</sup> described a constituent of red blood cells which brought about aggregation of platelets and was later identified by Gaarder *et al.*<sup>2</sup>, using the paper chromatographic method for separation of nucleotides, as ADP. Born<sup>3</sup> also had shown earlier that platelets contained high levels of adenosine triphosphate (ATP) which were degraded to ADP as platelet-rich plasma clotted. He then found that ADP added *in-vitro* to platelet-rich plasma brought about platelet aggregation which was inhibited by ATP, by adenosine monophosphate and by adenosine<sup>4</sup>. Although the aggregation of platelets by thrombin has been known for several decades, recent accounts of haemostasis state that thrombin does not appear in shed blood until after platelet aggregation by ADP was completed, so that it has no part in the initiation

of the haemostatic mechanisms. Using ultrastructural techniques *in vivo*, Johnson *et al.*<sup>5</sup> have recently found fibrin, together with large aggregates of platelets, in haemostatic plugs, 30 sec after transection of mesenteric vessels in guinea-pigs. The relationship between the activation of prothrombin to thrombin and the degradation of ATP to ADP, in haemostatic plug formation as well as in the initiation of thrombosis, is seen to be of critical importance.

Born<sup>6</sup> has recently shown that the formation of white bodies or platelet thrombi in the lumen of injured arteries can best be suppressed by infusion of adenosine or 2-chloroadenosine. Haemostatic plug formation was apparently unaffected, since the bleeding time remained unchanged, when the inhibitors were infused, although the formation of thrombi was diminished. Haslam<sup>7</sup> has prevented the aggregation of washed platelets with thrombin by enzymatic conversion of ADP to ATP using pyruvate kinase and 2-phosphoenolpyruvate. This work shows that, at least in this model, ADP must be present for aggregation with thrombin to occur.



We therefore undertook a parallel investigation to document the degradation of ATP to ADP in blood shed after transection of mesenteric vessels in guinea-pigs, correlating it with the appearance of fibrin and aggregation of platelets. The adenosine nucleotides were separated by thin-layer chromatography and the fibrin was identified by ultrastructural techniques. This is the first time the appearance of ADP in haemorrhage, *in vivo*, has been recorded.

The guinea-pigs were anaesthetized with 'Nembutal', the abdomen was opened and the smallest arteriole entering the gut from the inferior mesenteric artery was transected. Samples of blood were collected from the bleeding end of the vessel at designated intervals into capillary tubes, trichloroacetic acid was drawn in and stirring accomplished by moving an iron filing in the capillary tube with a magnet. After 3 min of stirring the extract was separated by centrifugation at 1,500*g* for 10 min. The resulting protein-free extract was applied in 1-ml. volumes in four applications to the cellulose plates for thin-layer chromatographic separation of adenosine nucleotides according to Randerath<sup>8</sup>.

Coagulation of whole blood brings about degradation of ADP in red blood cells and platelets, so the samples were collected with great caution. A siliconized microscope slide was placed under the loop of the gut and tilted so the blood might drain away easily until a few seconds before the sample was to be taken, when a pool of blood was allowed to accumulate so the capillary tube could be rapidly filled.

A 10 per cent solution of trichloroacetic acid was prepared from the crystalline material purchased from Fisher Laboratory Chemical U.S.P. Adenosine and 5' nucleotides (Kit K-2) were purchased from Pabst Laboratories, Division of Pabst Brewing Company, Milwaukee, Wisconsin. Standard curves were obtained using 39.6 mg crystalline ATP ( $\text{Na}_2\text{H}_2\text{ATP} \cdot 4\text{H}_2\text{O}$ , mol. wt. 623) per litre of 0.02 M phosphate buffer, pH 7, and 30.8 mg crystalline ADP ( $\text{NaH}_2\text{ADP} \cdot 2\text{H}_2\text{O}$ , mol. wt. 485) per litre of the same buffer. These curves were based on average molar absorptivity index ( $A_{259}$ ), at 259*m*, on a Beckman spectrophotometer. The standard solutions were mixed with trichloroacetic acid, and applied in 4 applications of 1 ml. each. MN-Cellulose powder 300, Macherey, Nagel and Co., was purchased from Brinkman Instruments, Inc., 115 Cutter Mill Road, Great Neck, New York.

Cellulose plates were prepared by spreading a paste composed of 15 g of cellulose stirred into 90 ml. of distilled water by a Waring blender for 30–45 sec. The plates were then dried in an oven at 105°C for 10 min. Four applications of either protein-free extract of blood to be tested or standard solutions of adenine nucleotides, of 1 ml. each, were placed on the plates. A solvent composed of *n*-butanol/acetone/acetic acid/5 per cent ammonium hydroxide/water (3.5 : 2.5 : 1.5 : 1.5 : 1) was used to develop the test material according to Randerath<sup>8</sup>. After the nucleotides were visualized under an ultra-violet lamp, the separated areas were collected into phosphate buffer, pH 7, and measured spectrophotometrically.

A detailed description of our ultrastructure techniques has been published<sup>5</sup>. The same experiment was conducted, a small mesenteric vessel transected and fixed *in vivo* by dropping cold collidine-buffered<sup>9</sup> 4 per cent osmium tetroxide on the bleeding vessel. The tissue was then dissected out, fixed, dehydrated and embedded in 'Epon' using equal portions of A and B mixture according to Luft *et al.*<sup>10</sup>. The sections were cut on a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate<sup>11</sup> and viewed under a RCA EMU3G electron microscope at 100 kV.

**Adenosine triphosphate degradation to adenosine diphosphate in shed blood.** The results of ATP and ADP assays of blood, shed at successive intervals after transection of small mesenteric arterioles, are shown in Fig. 1. Each point on the graph represents a mean value derived from

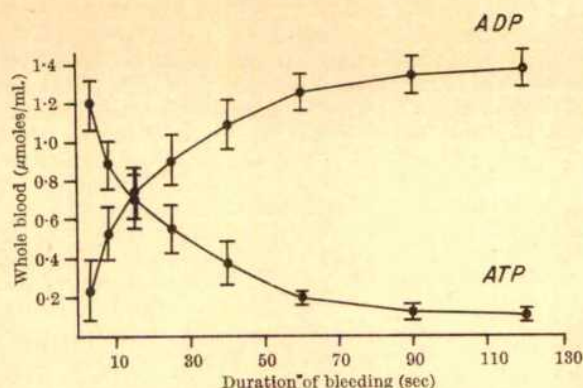


Fig. 1. A graph depicting ATP degradation during bleeding from a transected mesenteric vessel. The points are interpolated values from curves drawn from actual determinations in 12 guinea-pigs. On the ordinate ATP and ADP are expressed as  $\mu\text{moles}$  per ml. of whole blood, and on the abscissa the duration of bleeding is expressed in seconds.

12 guinea-pigs. In most cases ADP appeared 2–3 sec after bleeding began and 50 per cent of the ATP had appeared as ADP within 10–15 sec. A plateau of ATP degradation was reached about 60 sec after the vessel was severed.

Evidence to suggest that the only adenosine nucleotide resulting from adenosine triphosphate is adenosine diphosphate. The total of ATP and ADP remains so constant (Table 1) that there appears to be no other adenosine nucleotide formed. No additional spots appeared on the cellulose plates following application of the protein-free extract of whole shed blood even after 1 min of bleeding. The standard solutions of adenosine monophosphate and adenosine were applied to the plates, however, and they migrated to their appropriate places. We found it necessary to collect the separations of adenosine diphosphate at the initial times even if they were not visible on the plates under the ultra-violet light following developing, since some ADP was found in the cellulose even though its quantity was less than that necessary to give visible separations. The ATP + ADP totals, however, always revealed any loss of material. The data in Table 1 represent a typical experiment.

**Comparison of adenosine nucleotides in circulating and shed blood.** Born has frequently suggested that injury to the vessel wall might result in release of adenosine nucleotides responsible for platelet aggregation. Along with the assay of the shed blood from transection of the mesenteric vessels, we have carried out heart punctures

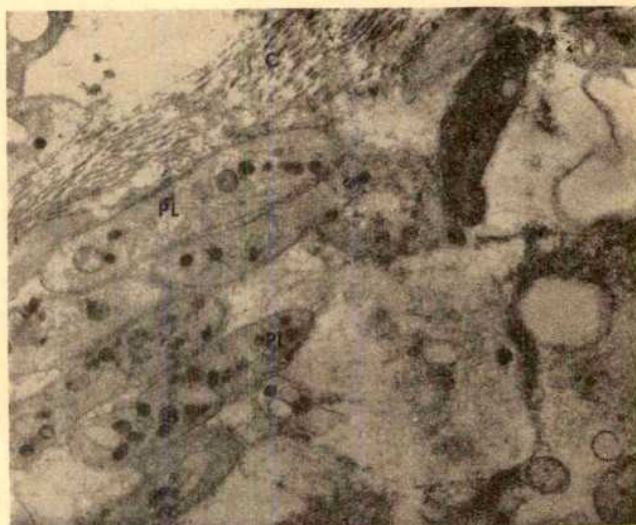


Fig. 2. Platelets (PL) can be seen adhering to collagen (C). This transected mesenteric vessel had been allowed to bleed for 15 sec before *in vivo* fixation with cold collidine-buffered osmium ( $\times c. 10,600$ ).



Table 1. ATP DEGRADATION IN SHED BLOOD IN THE INITIAL STAGES OF HAEMOSTASIS

Time (sec)	ATP $\mu\text{M/ml.}$	ADP $\mu\text{M/ml.}$	Total ATP + ADP $\mu\text{M/ml.}$
3.2	1.052	0.155	1.207
8.4	0.695	0.542	1.237
19.9	0.454	0.762	1.216
32.0	0.359	0.897	1.256

on each guinea-pig to determine the degradation of ATP to ADP in circulating blood. We found ADP in some of the samples secured by heart puncture but not in all. When ADP was detected the amount was subtracted from that measured in shed blood. As can be seen in Table 2, the total ATP + ADP is not significantly different in circulating blood from that found in shed blood. We interpreted this to mean that no detectable amount of ADP was released into shed blood by the cells in the damaged vessel wall.

Table 2. COMPARISON OF ADENOSINE NUCLEOTIDES IN CIRCULATING AND IN SHED BLOOD

	$\mu\text{M per ml. whole blood}$	
	Total ATP and ADP, blood shed at 12 sec	Total ATP and ADP, circulating blood (heart puncture)
1	1.200	1.371
2	1.370	1.497
3	1.380	1.570
4	1.540	1.465
5	1.570	1.532
6	1.410	1.450
7	1.390	1.378
8	1.390	1.475
9	1.445	1.456
Mean	$1.410 \pm 0.032$	$1.466 \pm 0.020$
		$P=0.1$

Whole guinea-pig blood was allowed to clot and ATP degradation was measured. Although this investigation is not yet complete it is apparent that ATP degradation in coagulated blood is radically different from that in shed blood with adenosine monophosphate and perhaps adenosine appearing.

**Ultrastructure of fibrin formation and platelet aggregation after 15 sec bleeding.** (a) A few small platelet aggregates were seen almost immediately after bleeding was initiated. Zucker and Borrelli<sup>12</sup>, using light microscopy, have observed platelet adherence to collagen fibres, while Hovig<sup>13</sup>, using ultrastructural techniques, has shown this phenomenon to be mediated through the formation and release of ADP from platelets. We have also observed the phenomenon of platelet adherence to collagen (Fig. 2). The significance of this type of aggregation is not known, but the foregoing investigators have suggested that collagen aggregation may be important in the initial stages of haemostatic plug formation.

(b) For the first time we have observed fibrin formation in association with red cells rather than with aggregated platelets. Fig. 3 shows fibrin fibres with a periodicity of 230 Å in close proximity to red blood cells. This fibrin, which has been observed in quantity, is not very electron dense and has a feathery appearance.

(c) A newly formed platelet aggregate, judging from the number of granules, both electron opaque and electron lucent, present in most of the platelets and the empty spaces between the aggregated platelets, can be seen in Fig. 4. Two red cells appear in the electron micrograph with fibrin fibres emanating from them into the platelet aggregate. The fibrin fibres are well formed, and the oldest part of the aggregate, judging from the reduced number of granules and the disintegrating platelet membranes, is that nearest the red cells and around the fibrin.

We have reported here that as early as 15 sec after bleeding began, very few platelet aggregates were found, but some formation of fibrin was already present. After 30 sec of haemorrhage many very large platelet aggregates and several easily identifiable fibrin loci<sup>5</sup> were seen. Along with the visualization of the ultrastructure of

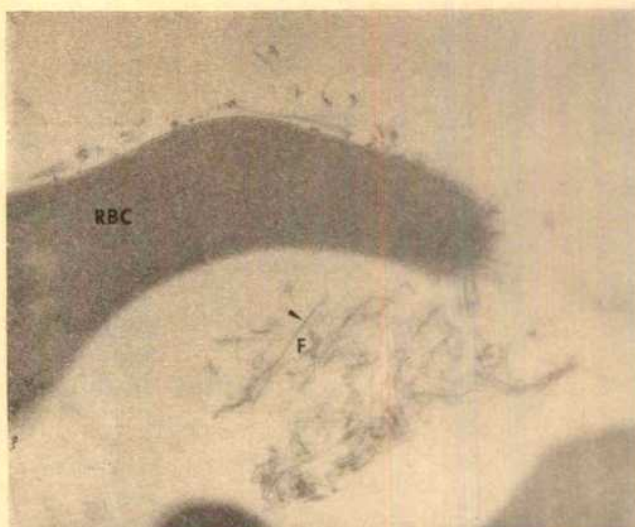


Fig. 3. Fibrin fibres (F) can be seen adjacent to red blood cells (RBC). This transected mesenteric vessel had been allowed to bleed for 15 sec before *in vivo* fixation with cold collidine-buffered osmium ( $\times c. 10,600$ ).



Fig. 4. Fibrin fibres (F) in close proximity to red blood cells (RBC) can be seen radiating into the platelet (PL) aggregate. This transected mesenteric vessel had been allowed to bleed for 15 sec before *in vivo* fixation with cold buffered osmium ( $\times c. 6,850$ ).

platelet aggregates we now know how much ADP is present in whole blood at the time the platelets are aggregating and the fibrin is appearing.

Although both thrombin and ADP induce platelet aggregation, little consideration has been given to the possibility of the interaction of these two stimuli. Because small amounts of thrombin could not be detected and no fibrin was observed by light microscopy in the initial stages of haemostatic plug formation, many investigators have concluded that aggregation by ADP took place well before thrombin became available, and that no interrelationship could have existed. Since we have shown that fibrin is present at the time of appearance of ADP, an interrelationship between activation of prothrombin and ATP degradation seems very likely. Haslam's recent report<sup>7</sup> of inhibition of thrombin aggregation of platelets accomplished by removal of traces of ADP certainly bespeaks an association between these two separate mechanisms.

The ADP measured in these experiments probably comes in large measure from the red blood cells. We have no data, however, to indicate that it has left the red cells and diffused into the serum where it would be available to bring about platelet aggregation. When ATP



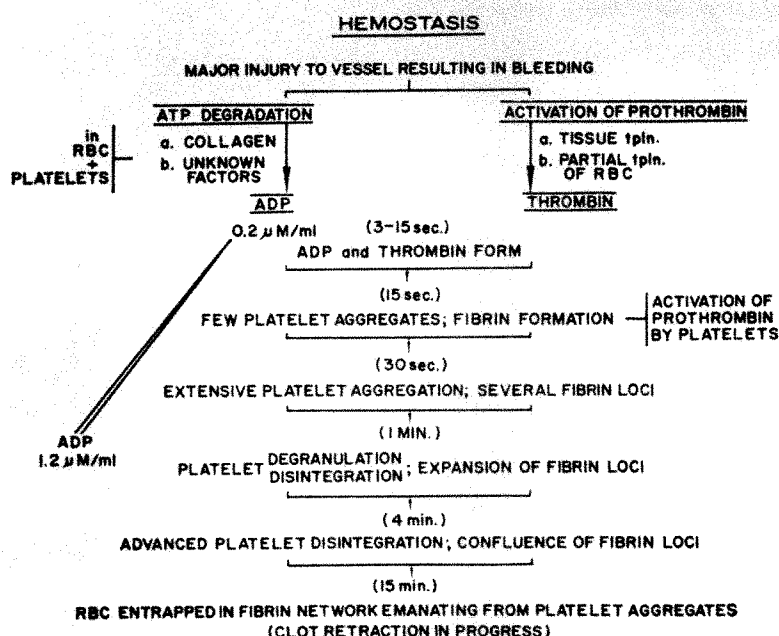


Fig. 5. A schema of the mechanisms of haemostasis after major injury to vessel wall resulting in bleeding

degradation is measured in clotting whole blood, a very complicated pattern emerges, involving formation of adenosine monophosphate and perhaps adenosine after certain critical levels of ADP are reached. The amount of residual coagulation components in shed blood is not known, but the appearance of fibrin does prove that the coagulation mechanisms are initiated.

The stimulus for degradation of ATP in shed blood must certainly come from the damaged vessel wall, even though detectable amounts of adenosine nucleotides are not released into the blood as it passes the injured cells (Table 2). Hellem<sup>1</sup> has stated that ADP release from red blood cells is brought about by contact with a foreign surface. The stimulus may be from the contact with the air, with collagen, or tissue thromboplastin from the injured vessel wall or some as yet undescribed component. The injured vessel wall must change very rapidly, for aggregated platelets building up to form a haemostatic plug must alter the characteristics of the orifice through which the blood is escaping. The con-

centrations of ADP formed in whole shed blood are much higher than those necessary to initiate platelet aggregation in platelet-rich plasma in either rabbit or human material.

The role of red blood cells in the arrest of bleeding should not be disregarded. Since few platelets were seen in our electron micrographs after 15 sec of bleeding and 20 times as many red blood cells are present in circulating blood as platelets, the ADP we measured probably comes in large part from the red blood cells. The close proximity of fibrin to red blood cells suggests that the partial thromboplastin of red blood cells described as thromboplastic cell component by Shinowara<sup>14</sup> may account for the activation of prothrombin to thrombin, thus converting fibrinogen to fibrin.

Fig. 5 summarizes our data on haemostatic plug formation when major injury to a vessel wall resulting in bleeding has occurred.

This work was supported in part by U.S. Public Health Services grant HE-06033-04.

These results show that thrombin and ADP form a few seconds after blood is shed, and both are available to bring about platelet aggregation before the necessary platelets are available. Our schema of the mechanisms

of haemostasis (Fig. 5) attempts to incorporate both activation of prothrombin and degradation of ATP. The interrelation of these two mechanisms remains to be elucidated.

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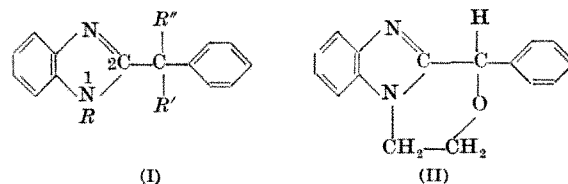
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## PROTECTION OFFERED TO POLIOVIRUS-INFECTED TISSUE-CULTURE CELLS BY METHOXY- AND HYDROXY-METHYL COMPOUNDS RELATED TO 2-BENZYL-BENZIMIDAZOLE

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THE high degree of protection conferred by 1-alkyl-2-( $\alpha$ -hydroxybenzyl)-benzimidazoles (I;  $R$  = alkyl,  $R'$  = H,  $R''$  = OH) (ref. 1) on rabbit-embryo kidney cells, infected with types 1, 2 and 3 poliovirus, makes derivatives with modified alkyl substituents at position 1 of particular interest. We have found 1-( $\beta$ -methoxyethyl)-2-( $\alpha$ -hydroxybenzyl)-benzimidazole (I;  $R$  =  $-\text{CH}_2\text{CH}_2\text{OMe}$ ,  $R' = \text{H}$ ,  $R'' = \text{OH}$ ) (ref. 2) (m.p.  $135^\circ\text{--}136^\circ$ ) to be less toxic than the simple 1-alkyl derivatives towards rabbit-embryo kidney cells, the maximum tolerated concentration of the ether being  $300 \mu\text{moles/l.} (\mu\text{M})$ .



This compound, at  $150 \mu\text{M}$  concentration, affords rabbit-embryo kidney cells considerable protection against the cytopathic consequences of infection with types 1, 2

Table 1. PROTECTION GIVEN BY 1-BUTYL-, 1- $\beta$ -METHOXYETHYL- AND 1-PENTYL-HBB TO CELLS INFECTED WITH TYPES 1, 2 AND 3 POLIOVIRUS

Virus		Mean delay (days) between infection with virus and degeneration of half the cell population						
Type	Conc.	Control*	0.25 M.T.C.		Control*	0.5 M.T.C.		
			1-Bu (15 $\mu$ M)	1-MeOCH <sub>2</sub> CH <sub>2</sub> - (75 $\mu$ M)		1-Bu (30 $\mu$ M)	1-MeOCH <sub>2</sub> CH <sub>2</sub> - (150 $\mu$ M)	1-Pentyl (50 $\mu$ M)
1	10 <sup>-1</sup>	1.0	1.5	1.25	1.0	4.75	1.5	1.25
	10 <sup>-2</sup>	1.75	3.75	2.5	1.75	> 5.0	> 5.0	2.25
	10 <sup>-3</sup>	2.0	6.0	4.5	2.25	> 5.0	> 5.0	3.5
	10 <sup>-4</sup>	0.75	1.25	1.0	0.75	1.5	1.25	1.0
3	10 <sup>-1</sup>	1.25	2.0	1.5	1.0	2.75	2.0	1.75
	10 <sup>-2</sup>	1.75	2.75	2.25	1.5	3.75	3.0	2.25
	10 <sup>-3</sup>				1-Pentyl (25 $\mu$ M)			
					2.25			
2	10 <sup>-1</sup>	1.25	4.25	2.75	4.0			
	10 <sup>-2</sup>	1.75	6.25	4.25	> 5.0			
	10 <sup>-3</sup>	2.25	7.5	> 5.0				

Times are quoted to the nearest quarter of a day.

Approximate initial virus concentration (before dilution): 10<sup>7.2</sup> TCD<sub>50</sub> units per ml. (types 1 and 2 virus) and 10<sup>7.4</sup> TCD<sub>50</sub> units per ml. (type 3 virus).

\* Infected control containing no protective agent. Uninfected and untreated controls always survived more than 5 days. Examination of culture tubes was usually stopped on the fifth day.

M.T.C., maximum tolerated concentration.

and 3 poliovirus. The length and shape of the  $\beta$ -methoxyethyl- are similar to those of the butyl-substituent. Table 1 shows that the protection given by the 1- $\beta$ -methoxyethyl derivative (I;  $R = -CH_2CH_2OMe$ ,  $R' = H$ ,  $R'' = OH$ ) lies between that given by the 1-butyl (ref. 1) and 1-pentyl (ref. 3) derivatives (all tested in tissue-culture by the method previously outlined<sup>1,4</sup>).

An attempt to prepare 1-( $\beta$ -hydroxyethyl)-2-( $\alpha$ -hydroxybenzyl)-benzimidazole by demethylation of the 1- $\beta$ -methoxyethyl compound gave instead the cyclic ether (II) (ref. 2) (m.p. 161°–162°). This ether (maximum tolerated concentration 600  $\mu$ M) is much less toxic to rabbit-embryo kidney cells than other benzimidazoles we have tested and, at 300  $\mu$ M, has selective protective action (activity/toxicity ratio) against the 3 poliovirus types which, although high, is less than that of the 1- $\beta$ -methoxyethyl derivative (Tables 1 and 2). It is slightly less effective than the 1-pentyl compound against the type 2 virus, but more effective than the 1-pentyl compound against the types 1 and 3 viruses. The two ethers, (I;  $R = -CH_2CH_2OMe$ ,  $R' = H$ ,  $R'' = OH$ ) and (II), offer particularly good protection against the cytopathic effects of the types 1 and 2 viruses (Tables 1 and 2). The activity of the cyclic ether (II) makes it necessary to reconsider the role of the  $\alpha$ -hydroxy group.

Table 2. PROTECTION GIVEN BY CYCLIC ETHER (II) AND BY 2-( $\alpha$ -METHOXYBENZYL)-BENZIMIDAZOLE TO CELLS INFECTED WITH TYPES 1, 2 AND 3 POLIOVIRUS

Virus		Mean delay (days) between infection with virus and degeneration of half the cell population			
Type	Conc.	Control	Ether (II) (300 $\mu$ M)	$\alpha$ -Methoxy-HBB (150 $\mu$ M)	HBB (100 $\mu$ M)
1	10 <sup>-1</sup>	1.0	3.5	2.0	2.0
	10 <sup>-2</sup>	1.5	4.75	3.0	3.0
	10 <sup>-3</sup>	2.0	6.75	3.75	3.5
	10 <sup>-4</sup>	0.75	1.5	1.0	1.0
3	10 <sup>-1</sup>	1.0	2.0	1.75	1.5
	10 <sup>-2</sup>	1.75	3.0	2.0	2.0
	10 <sup>-3</sup>		(150 $\mu$ M)*		
2	10 <sup>-1</sup>	1.0	2.0	1.75	2.0
	10 <sup>-2</sup>	1.5	3.0	3.0	3.25
	10 <sup>-3</sup>	2.0	4.5	4.5	5.0

\* Half M.T.C. was used except when the cyclic ether was tested with the type 2 virus. (Strictly the value for HBB itself should be 105  $\mu$ M), but 100  $\mu$ M HBB has been taken as the standard. Untreated-uninfected control cells began to die from over-crowding during the seventh day. Otherwise the footnotes to Table 1 apply.

2-Benzylbenzimidazole (I;  $R = R' = R'' = H$ ) has very low selective activity against type 2 poliovirus, while its  $\alpha$ -hydroxy derivative HBB (I;  $R = R' = H$ ,  $R'' = OH$ ) has high selective activity<sup>5</sup>. Replacement of the  $\alpha$ -hydroxy group by an  $o$ -hydroxy group, similarly sited in space but substituted in the side-chain phenyl group giving structure (III;  $R = OH$ ), slightly reduces the protective activity<sup>6</sup>. In contrast, 2-( $p$ -hydroxybenzyl)-benzimidazole has low activity<sup>6</sup>. We have now examined 2-[ $\alpha$ -(hydroxymethyl)-benzyl]-benzimidazole (I;  $R = R' = H$ ,  $R'' = -CH_2OH$ ) (ref. 2) (m.p. 150°–151°; maximum tolerated concentration 200  $\mu$ M) and find that at 80  $\mu$ M it gives protection to cells infected with type 2 poliovirus similar to that given by 50  $\mu$ M HBB (Table

3). Against the cytopathogenicities of types 1 and 3 poliovirus, the hydroxymethyl compound at 80  $\mu$ M is inferior to 50  $\mu$ M HBB and it provides just detectable protection, of doubtful significance, under the condition of our assays.

Table 3. PROTECTION GIVEN BY 2-[ $\alpha$ -(HYDROXYMETHYL)-BENZYL]-BENZIMIDAZOLE, THE HYDROCHLORIDE OF GLYCERIC ACID (IV) AND HBB TO CELLS INFECTED WITH TYPE 2 POLIOVIRUS

Mean delay (days) before onset of cytopathic effects				
Virus conc.	Control	$\alpha$ -Hydroxymethyl compound (80 $\mu$ M)	Glyceric acid (IV) hydrochloride (60 $\mu$ M)	HBB (50 $\mu$ M)
10 <sup>-1</sup>	1.5	1.75	1.5	1.75
10 <sup>-2</sup>	2.0	2.75	2.25	2.75
10 <sup>-3</sup>	3.0	4.0	3.25	3.75

Initial virus concentration (before dilution)  $\sim 10^{6.8}$  TCD<sub>50</sub> units per ml. Otherwise the footnotes to Table 1 apply.

In order to determine whether an alkyl group at position 1 would increase the activities, as is the case with HBB (ref. 1), 1-ethyl-2-[ $\alpha$ -(hydroxymethyl)-benzyl]-benzimidazole (I;  $R = Et$ ,  $R' = H$ ,  $R'' = -CH_2OH$ ) was prepared by condensing *N*-ethyl-*o*-phenylenediamine with tropic acid by the method described previously<sup>1</sup>. White needles were obtained from aqueous methanol (yield 7 per cent from tropic acid), m.p. 158°–159° (found: C, 77.1; H, 7.0; N, 10.6; C<sub>17</sub>H<sub>18</sub>N<sub>2</sub>O requires C, 76.7; H, 6.8; N, 10.5 per cent). Introduction of the ethyl group results in considerable increase in protective action. At half maximum tolerated concentration, the 1-ethyl derivative (I;  $R = Et$ ,  $R' = H$ ,  $R'' = -CH_2OH$ ) is superior to HBB (100  $\mu$ M) against type 2 poliovirus and is similar to HBB (100  $\mu$ M) against the type 1 and type 3 viruses (Table 4).

Table 4. PROTECTION GIVEN BY 1-ETHYL-2-[ $\alpha$ -(HYDROXYMETHYL)-BENZYL]-BENZIMIDAZOLE TO CELLS INFECTED WITH POLIOVIRUS

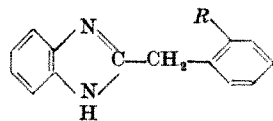
Virus		Mean delay (days) before onset of cytopathic effects		
Type	Conc.	Control	1-Ethyl compound 0.5 M.T.C. (100 $\mu$ M)	HEB (100 $\mu$ M)
1	10 <sup>-1</sup>	0.75	1.0	1.0
	10 <sup>-2</sup>	1.0	1.25	1.5
	10 <sup>-3</sup>	1.5	2.0	2.0
3	10 <sup>-1</sup>	0.75	1.25	1.0
	10 <sup>-2</sup>	1.5	1.75	1.75
	10 <sup>-3</sup>	1.75	2.0	2.0
2	10 <sup>-1</sup>	0.75	1.75	1.25
	10 <sup>-2</sup>	1.25	3.25	2.75
	10 <sup>-3</sup>	1.75	4.75	3.75

Initial virus concentrations (before dilution)  $\sim 10^{7.2}$  TCD<sub>50</sub> units per ml. Otherwise footnotes to Table 1 apply.

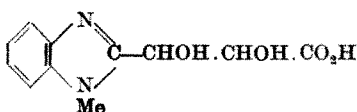
Each assay (Tables 1 to 4) has been carried out on two separate occasions.

Thus it is not necessary for a hydroxy group to be attached directly to the  $\alpha$ -carbon atom in order to preserve reasonable activity against type 2 poliovirus. The activity of the cyclic ether (II) indicates that an alkoxy group might be as effective (Table 2). 2-( $\alpha$ -Methoxybenzyl)-benzimidazole (I;  $R = R' = H$ ,  $R'' = OMe$ ) (ref. 2) (m.p. 160°–161°) was examined in order to discover the effect of replacing the  $\alpha$ -hydroxy by an  $\alpha$ -methoxy group. It is less toxic to the cells than HBB and shows similar protective action at half maximum tolerated concentration. Table 2 shows that the selective protective action against the type 2 virus is slightly less than that of HBB, while it is slightly more effective than HBB with respect

to the types 1 and 3 viruses. Thus the  $\alpha$ -hydroxy group is not a necessary feature for high activity against the type 2 virus. However, it is just conceivable that some dealkylation might occur in the tissue-culture system. Investigations of other  $\alpha$ -substituted derivatives of 2-benzylbenzimidazole and of their D- and L-isomers are in progress.



(III)



(IV)

2-(*o*-Carboxybenzyl)-benzimidazole (III;  $R = \text{CO}_2\text{H}$ ) was tested because of the activity of the *o*-hydroxy compound<sup>6</sup>. However, at 30  $\mu\text{M}$  (half maximum tolerated concentration) it shows no protective action against the type 1 or type 2 virus and only a small effect, of doubtful significance, against the type 3 virus. Another carboxylic acid (IV) was tested as the hydrochloride at 60  $\mu\text{M}$  (half maximum tolerated concentration) and was found to have no activity with respect to the types 1 and 3 viruses but to offer slight protection against the type 2 virus (Table 3). Mandelic acid at 90  $\mu\text{M}$  (half maximum tolerated concentration) provides no protection against any of the 3 virus types.

2-(*o*-Carboxybenzyl)-benzimidazole (III;  $R = \text{CO}_2\text{H}$ ) was obtained by heating under reflux for 7 h, *o*-phenylenediamine (2.7 g, 0.025 mole) and homophthalic acid (4.5 g, 0.025 mole) in 2M hydrochloric acid (37 ml.). Cooling, filtering and drying the crystals gave a greenish product (5.9 g, yield 94 per cent). The solid, suspended in water, was dissolved in a slight excess of M sodium hydroxide, filtered, and treated with carbon dioxide until no more precipitation occurred. On crystallization from methanol, it gave the *o*-carboxy derivative (III;  $R = \text{CO}_2\text{H}$ ) as white needles, m.p. 320° (dec.) (found: C, 71.2; H, 4.5; N, 10.7;  $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_2$  requires C, 71.5; H, 4.8; N, 11.1 per cent).

The glyceric acid derivative (IV) was prepared at the same time as the *bis*-derivative<sup>1</sup> by heating under reflux for 7 h, *N*-methyl-*o*-phenylenediamine<sup>1</sup> (2.44 g, 0.02 mole), tartaric acid (1.5 g, 0.01 mole) and 4M hydrochloric acid (20 ml.). The dihydrochloride of 1,2-*bis*-[2'-(1'-methylbenzimidazolyl)]-ethylene glycol<sup>1</sup> (1.65 g) separated on cooling. On standing the filtrate at 0°, a further crop of crystals (1.4 g) separated. Crystallization from ethanol-

ether after charcoal treatment gave  $\beta$ -[2-(1-methylbenzimidazolyl)]-glyceric acid hydrochloride as white prisms, soluble in saturated sodium bicarbonate, m.p. 212°–213.5° (yield 51 per cent) (found: C, 47.1; H, 5.0; N, 10.1;  $\text{C}_{11}\text{H}_{13}\text{ClN}_2\text{O}_4$  requires C, 47.5; H, 4.8; N, 10.2 per cent).

Table 5 summarizes the approximate relative activities of compounds, all at half maximum tolerated concentration, in protecting rabbit-embryo kidney (ERK) cells against the cytopathic effects of poliovirus type 1 (*L Sc*, 2 ab), type 2 (*P* 712, *Ch*, 2 ab) and type 3 (*Leon* 12 ab) (the virus strains used in this present work). The information is based on our most up-to-date work. Activities are given on an arbitrary scale from  $\alpha$  (very high protection) to  $\epsilon$  (slight protection), as described previously<sup>1</sup>. Until we have completed our investigation of percentage inhibitions of virus growth, Table 5 provides a rough guide to the relative effectiveness of the most interesting compounds we have examined<sup>1,3,4</sup>.

Table 5. APPROXIMATE RELATIVE EFFECTIVENESS IN PROTECTING ERK CELLS INFECTED WITH POLIOVIRUS

Compound	M.T.C. ( $\mu\text{M}$ )	Protective influence*		
		Type 1	Type 2	Type 3
DL-HBB	210	$\epsilon$	$\gamma$	$\epsilon$
D-HBB	210	$\delta$	$\beta$	$\delta$
1-Methyl-HBB†	210	$\delta$	$\beta$	$\delta$
1-Ethyl-HBB†	180	$\gamma(-)$	$\beta$	$\gamma$
1-Propyl-HBB†	80	$\alpha$	$\alpha$	$\beta$
1-Butyl-HBB†	60	$\beta$	$\alpha$	$\gamma$
1-Pentyl-HBB	100	$\gamma$	$\beta$	$\delta$
1-Isopropyl-HBB	100	$\epsilon$	$\gamma$	$\epsilon$
1-Benzyl-HBB	100	$\alpha$	$\alpha$	$\beta$
1- $\beta$ -Methoxyethyl-HBB	300	$\beta$	$\beta$	$\gamma$
1,3-Diethyl-2-( $\alpha$ -hydroxybenzyl)-benzimidazolium iodide	70	$\epsilon$	$\delta$	$\epsilon$
2-( $\alpha$ -(Hydroxymethyl)-benzyl)-benzimidazole	200	0	$\gamma$	0
1-Ethyl-2-( $\alpha$ -(hydroxymethyl)-benzyl)-benzimidazole	200	$\epsilon$	$\gamma$	$\epsilon$
2-( $\alpha$ -Methoxybenzyl)-benzimidazole	300	$\epsilon$	$\gamma$	$\epsilon$
Cyclic ether (II)	600	$\beta$	$\beta$	$\gamma$

\* Ranges are given from  $\alpha$  (very high) to  $\epsilon$  (low); 0 means no activity.

† Same data apply to the DL- and D-isomers.

Experiments on the highly active compounds show that they do not inactivate the virus particles in the absence of cells and that they possess little or no retarding effects on the passage of virus into the cells. Presumably the compounds exert their actions within the cells. The DL- and D-1-propyl-, DL- and D-1-butyl- and DL-1-benzyl-2-( $\alpha$ -hydroxybenzyl)-benzimidazoles are the most effective compounds discovered so far in our investigations<sup>1,3,4</sup>.

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## AMINO-ACID COMPOSITION OF HUMAN DERMAL COLLAGEN

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THE amino-acid composition of collagens from various mammalian and other<sup>1</sup> species reveals a uniform pattern characterized by high concentrations of glycine, proline and hydroxyproline, low concentrations of aromatic and sulphur-containing amino-acids, and significant amounts of hydroxylysine. The amino-acid composition of human collagens from bone, tendon<sup>2</sup>, dura mater and uterus<sup>3</sup> has been reported. This investigation

reports the isolation and amino-acid composition of the citrate-soluble, insoluble and urea-extractable collagens from human skin.

Specimens of normal adult skin were obtained from the abdominal wall of fresh autopsy material. The epidermis was removed from the stretched skin by scraping with a scalpel and the skin was lyophilized. The dry weights of the samples ranged from 1.5 to 3 g. They were passed through a micro Wiley mill, resulting in a fine powder which greatly facilitated the subsequent isolation of the collagens. The lipids were removed by extracting<sup>4</sup> with

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20 ml. of ether for 2 h. This operation was repeated twice. After drying, each sample was extracted with 20 ml. of distilled water for 45 min in a Vir-tis homogenizer at 5° C to remove soluble proteins. The water extraction was repeated five times. The last extract contained insignificant amounts of protein as determined by the method of Lowry<sup>4</sup>. The residue was extracted five times with 0.1 M disodium phosphate, pH 8.9, in a manner similar to the water extraction. This step removed other connective tissue proteins, acid and neutral polysaccharides and a small amount of collagen. After washing, the acid-soluble collagen was extracted from the residue by five extractions with 20 ml. of 0.1 M citrate buffer, pH 3.8, in a way similar to that described for the water and phosphate extractions. The combined citrate extracts were dialysed against 0.01 M disodium phosphate and the precipitated acid-soluble collagen was collected by centrifugation, washed with cold water and lyophilized. The residue, after citrate extraction, was washed twice with 0.4 M NaCl, distilled water and lyophilized. This was the insoluble collagen.

Two skin specimens were subjected to an additional extraction. The extraction proceeded as described here up to and including the citrate extraction. After removal of the citrate buffer by dialysis the residue was extracted with 6 M urea for 45 min at 4° C in a Vir-tis homogenizer. The urea-insoluble residue was removed by centrifugation at 15,000 r.p.m. for 30 min, the supernatant was filtered through a fine sintered glass filter, dialysed against distilled water for 3 days to remove the urea, and lyophilized. This was designated urea-soluble collagen.

Reducing hexoses were estimated by the anthrone method without previous hydrolysis<sup>5</sup>. Hexosamines were determined by the method of Boas<sup>6</sup>. Two samples of urea-soluble collagen were examined with wide-angle X-ray diffraction and compared with rat tail tendon collagen. The samples were placed in a micro-beam X-ray with a 100 $\mu$  glass capillary to collimate the X-ray beam. The exposure with nickel-filtered copper radiation at 35 kV and 20 m.amp lasted 10–12 h.

Our insoluble collagen contained elastic tissue. This was removed by autoclaving for 6 h at 25 lb./in.<sup>2</sup> pressure in distilled water. The insoluble elastin and other impurities were removed by high-speed centrifugation and the gelatin supernatant was filtered through a 'Micropore' filter (German Instrument Co.) and lyophilized.

One mg samples of the various collagens were hydrolysed in constant boiling (6 M) HCl for 22 h in evacuated sealed tubes at 110° C. The hydrolysates were dried in vacuum desiccators over NaOH. Amino-acid analyses were performed using a Technicon Autoanalyser which uses the system of Piez and Morris<sup>7</sup>.

Three satisfactory extractions of human skin were used to examine the composition of citrate-soluble and insoluble collagens. Additional samples were extracted with 6 M urea. The insoluble collagen was found to contain 0.2 per cent hexosamine and 0.45 per cent reducing hexose by the anthrone method<sup>5</sup>.

The amino-acid compositions of the citrate-soluble and insoluble collagens of human skin are shown in Table 1. These represent the average of hydrolysates from three different specimens. The analysis shows the characteristic distribution of amino-acids found in collagens from most sources and is not significantly different from human Achilles tendon, dura mater and uterus<sup>2,3</sup>. No corrections were made for destruction of amino-acids during acid hydrolysis.

The investigation of the characteristics and composition of the portion of the insoluble collagen that is made soluble by 6 M urea is in the nature of a preliminary investigation. In each extraction only 4–5 per cent of the initial collagen was dissolved in the 6 M urea. A second extraction yielded no additional material, nor did any subsequent extractions. Following the removal of the urea by dialysis the lyophilized material was quite

Table 1. AMINO-ACID COMPOSITION OF HUMAN SKIN COLLAGENS

Amino-acid	Citrate soluble collagen*	Insoluble collagen*	Urea soluble collagen†	Human Achilles tendon (Eastoe)
Residues/1,000 residues				
Hydroxyproline	84.5	90.9	91.3	92.1
Aspartic acid	47.0	47.2	50.6	48.4
Threonine	18.0	18.3	19.0	18.5
Serine	27.1	36.9	33.8	36.0
Glutamic acid	72.8	77.7	83.6	72.3
Proline	123.8	125.1	124.8	126.4
Glycine	344.9	324.4	339.9	324.0
Alanine	107.3	114.5	120.3	110.7
Cystic acid	Trace	Trace	Trace	Trace
Valine	25.9	24.5	23.0	25.4
Methionine	6.2	7.0	6.6	5.7
Isoleucine	11.7	10.4	12.1	11.1
Leucine	26.5	24.8	25.2	26.0
Tyrosine	4.4	3.5	3.2	3.6
Phenylalanine	12.9	12.6	14.1	14.2
Hydroxylysine	5.1	5.9	8.5	8.9
Lysine	27.0	26.6	29.8	21.0
Histidine	5.9	5.4	6.4	5.4
Arginine	47.1	49.0	51.4	49.0

\* Average of hydrolysates from 3 dermis specimens.

† Average of hydrolysates from 2 dermis specimens.

soluble in cold water, indicating that degradation had occurred. However, a single determination of the molecular weight by light scattering gave a value of about 300,000, which is compatible with tropocollagen. X-ray diffraction examinations of the precipitated urea-soluble collagen show patterns consistent with non-oriented collagen. The ring corresponding to the 2.86 Å nodal repeat of collagen was distinct, but a diffuse ring close to the primary beam suggested that part of the collagen molecules may have been packed in fibrous regions.

The urea-soluble collagen contained reducing hexose in amounts equal to skin collagens. The amino-acid composition is shown in Table 1 and represents the average of two skin specimens. The pattern is generally typical of collagen, but glutamic acid, isoleucine, phenylalanine, hydroxylysine and lysine seem to be present in significantly higher concentrations than in the insoluble collagen of human skin from which it was extracted. Further investigation is required before the significance of the urea soluble material can be clarified.

Although no investigator will claim to have isolated homogeneous collagen, a good index of purity is the carbohydrate content. The value of 0.45 per cent reducing hexose found in the insoluble collagen of human skin is in good agreement with the careful investigation of Blumenfeld *et al.*<sup>8</sup> on the reducing hexoses of swim bladder ichthyocoll. Since the glucose and galactose found in their investigation were not polymerized and were linked to the collagen by L-glycosidic bonds, this represents the minimum of hexose in collagen. However, Blumenfeld and Gallop found no hexosamine in ichthyocoll, and this finding was confirmed in our laboratory. The insoluble collagen, however, contained 0.2 per cent hexosamine.

The portion of insoluble collagen that is extracted by 6 M urea is of great interest. There have been reports that urea alters or denatures collagen<sup>9,10</sup>. It is most likely a degraded collagen as indicated by the ready solubility of the lyophilized material in cold distilled water.

No previous reports of the amino-acid composition of urea-extractable collagen have been found. As mentioned earlier, the amino-acid analysis reported here is typical of collagen but contained excesses of glutamic acid, isoleucine, phenylalanine, hydroxylysine and lysine when compared with insoluble skin collagen. These deviations could not be explained on the basis of preferential solution of one of the collagen sub-units since, when compared to the sub-units of rat skin collagen reported by Piez, Eigner and Lewis<sup>11</sup>, the urea-soluble collagen bore no resemblance to the pattern of any sub-unit with respect to the deviant amino-acids. It is hoped that ultra-centrifuge examinations and sub-unit fractionation will clarify the relationship of the urea-soluble material to intact collagen.

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## ALPHA-KERATIN

### Molecular and Fine Structure of $\alpha$ -Keratin (IV)

THE elusive nature of the structure of  $\alpha$ -keratin has recently become the subject of renewed speculations stimulated by the impact of electron microscope evidence<sup>1-3</sup>. It is therefore relevant to repeat our earlier warning<sup>2</sup> that the much favoured concept of a '9+2' arrangement of the aggregates of  $\alpha$ -helices within the microfibrils\* was advanced, following interpretation of image details of dimensions comparable with the resolving power of the electron microscope, in terms of the theory of image formation applicable to extended objects.

Consequently, until the analysis of the high-resolution electron microscope images of transverse sections of mammalian hairs and quills has been successfully completed<sup>4,5</sup>, it would not be prudent to insist on the correctness of the foregoing model (which falls also on other grounds<sup>2,6</sup>), let alone to express views about the measure of agreement between the electron microscope and X-ray evidence<sup>7</sup>; for there appears to be equally strong divergence of opinion about what should be considered as the theory applicable to the case of equatorial scattering of X-rays by a system of the aggregates of long chain molecules<sup>7-11</sup>. It is, therefore, reassuring that more recent publications give exclusive attention to this latter problem<sup>10,11</sup>, thus acknowledging the complexity of electron microscope evidence.

We would like to recall that only in what are essentially under-focused conditions, using as a criterion Fresnel diffraction at the edge of the section, do the electron microscope images of microfibrils show a peripheral ring of a variable number of intensity maxima surrounding a central region of one or more intensity maxima<sup>2</sup>. This type of ultrafine structure has been observed in the images of transverse sections of keratin from many sources (porcupine quill, Lincoln, Cotswold and Australian Merino wools and human hair) after treatment with solutions of reducing reagents, followed by specific or non-specific reactions involving metal compounds<sup>12-14</sup>.

It should, of course, be quite clear (and has been amply substantiated by the results of our preliminary tilting experiments<sup>4</sup>) that the high-resolution image of a microfibril in metal-treated keratin cannot be regarded as a simple orthogonal projection from specimen plane to image plane<sup>2</sup>. However, in view of a singular reluctance to accept the wave-optical approach to the problem of image formation, it seems necessary to repeat the outline of our earlier argument. Thus, single metal atoms or their small aggregates deposited along the loci of  $\alpha$  helices perturb the electron wave to give in the image plane an intensity distribution which is subject to modification after small changes of focus, as a consequence of phase changes between the scattered and the background waves. It was, therefore, not unexpected to find that when the coherence of the illuminating beam was altered, by variation of the semi-angular aperture of the condenser lens, or the distribution of the scattered wave in the image plane affected, by variation of the semi-angular aperture of the

objective lens, changes were observed in the distribution of the intensity maxima in the images of microfibrils<sup>2</sup>, as in the case of small changes of focus<sup>2</sup>.

In view of the adverse effect on the image detail of (i) the electron noise, especially where fast photographic emulsions are involved<sup>15</sup>, and (ii) the contamination under the influence of the electron beam, it should not be surprising that the information available in the images of microfibrils could not be easily interpreted in terms of the ultrastructure of metal-treated keratin. Consequently, the direct solution of the problem must be sought using, *inter alia*, information theory.

Any method used to resolve difficulties of this kind should not rely on an *a priori* assumption of the existence of a unique symmetry in the individual images of microfibrils. Moreover, there should be no ambiguity (pace ref. 1) about the procedure adopted, during repetitive photographic printing, for the mutual angular orientation of the successively superimposed images of microfibrils.

Two useful techniques free from any bias of this kind and applicable to any system with cylindrical symmetry have been developed by Markham, Frey and Hills<sup>16</sup> and can be conveniently adapted to an analysis of the peripheral regions of the microfibrillar images in keratin. Following the first method, the exposure time ( $t$ ) of a highly magnified image (in our case  $\times 10^7$ ) is determined, and  $n$  exposures, each of  $t/n$  duration, are made, successive exposures being taken after rotation of the photographic paper by an angle  $2\pi/n$  about the centre of the image,  $n$  being an integer. If the image has  $m$ -fold symmetry, and  $n=m$  or is an integral fraction of the symmetry order, then  $m$  intensity maxima are observed, instead of the general blur of the original photograph, cf. Figs. 1b and 1a, respectively.

Twelve images selected at random were examined using this method and revealed the symmetries indicated in row (i) of Table 1. Entries in the last column, under the 'number of peripheral intensity maxima— $m$ ', indicate the number of microfibrils with no symmetry or symmetries other than those indicated in the preceding columns. Examples of  $m$ -fold symmetry ( $m=5, 6, 7, 8$  and  $9$ ) are given in Fig. 1b, together with the corresponding unrotated images, Fig. 1a.

In the second method, the enlarged image is rotated about its centre while the system is illuminated by a stroboscopic flash with a variable frequency. Thus, when the latter is  $n$ -times the frequency of rotation of the image

Table 1. FREQUENCY OF IMAGES OF METAL-TREATED MICROFIBRILS OF KERATIN SHOWING: (i)-(iv),  $m$  PERIPHERAL INTENSITY MAXIMA; (v) POLYGONAL CROSS-SECTION

Material		Reducing treatment	Metal deposited	No. of peripheral intensity maxima— <i>m</i>						Total No. of images
				5	6	7	8	9	None	
(i)	Porcupine quill-tip	Thioglycollic acid	Os	1	2	1	2	2	4	12
(ii)	Lincoln wool fibres	Tetrakis (hydroxymethyl) phosphonium chloride	Ag	2	7	4	3	2	4	22
(iii)	Lincoln wool fibres	Thioglycollic acid	Ag	4	3	5	2	0	8	22
(iv)	Total:			7	12	10	7	4	16	56
				Polygonal form						
				5	6	7	8	9	None	
(v)	As above			14	15	11	2	1	27	

\* It is to be regretted that by proposing a new term 'filament', to replace the well-tried 'microfibril', the systematization of nomenclature for fine structural components of keratin, Mercer *et al.* (*Nature*, **201**, 367, 1964) disregarded the long-established connotation of the former term (for example, *Textile Terms and Definitions*, Textile Institute, 1954, *Filament—A Fibre of Indefinite Length*).



Table 2. EFFECT OF INSTRUMENTAL PARAMETERS ON SYMMETRY IN IMAGES OF MICROFIBRILS OF PORCUPINE QUILL-TIP, REDUCED WITH THIOGLYCOLLIC ACID SOLUTION AND TREATED WITH OSMIUM TETROXIDE

I			II			III					
$\theta_c = 4 \times 10^{-4}$ rad			$\theta_c = 8 \times 10^{-4}$ rad		$\theta_c = 4 \times 10^{-4}$ rad	$\theta_c = 4 \times 10^{-4}$ rad					
$\theta_a = 9.1 \times 10^{-3}$ rad			$\theta_a = 9.1 \times 10^{-3}$ rad			$\theta_a = 5.5 \times 10^{-3}$ rad	$\theta_a = 9.1 \times 10^{-3}$ rad	$\theta_a = 4.5 \times 10^{-3}$ rad	$\theta_a = 9.1 \times 10^{-3}$ rad		
Arbitrary focus			$\Delta f = -0.2 \mu$			$\Delta f = 0$			$\Delta f = 0$		
<i>a</i>	6	6	<i>g</i>	None	None	<i>m</i>	None	8	<i>s</i>	None	None
<i>b</i>	5	6	<i>h</i>	6	8	<i>n</i>	7	9	<i>t</i>	None	9
<i>c</i>	6	8	<i>i</i>	None	7	<i>o</i>	6	None	<i>u</i>	7	7
<i>d</i>	8	None	<i>j</i>	7	None	<i>p</i>	6	9	<i>v</i>	7	6
<i>e</i>	None	None	<i>k</i>	8	9	<i>q</i>	5	7	<i>w</i>	None	None
<i>f</i>	9	6	<i>l</i>	7	6	<i>r</i>	6	7	<i>x</i>	6	5

$\theta_c$ , condenser semi-angular aperture;  $\theta_a$ , objective semi-angular aperture.

possessing an inherent  $m$ -fold symmetry, then—as before—for  $n=m$  or  $n$  equal to an integral fraction of such a symmetry order,  $m$  stationary intensity maxima are seen. The same twelve images, row (i) of Table 1, were investigated by the stroboscopic method and the results (see, for example, five images in Fig. 1c) confirmed the symmetries obtained by the first method. The stroboscopic technique was used in all subsequent work, as being the more convenient of the two.

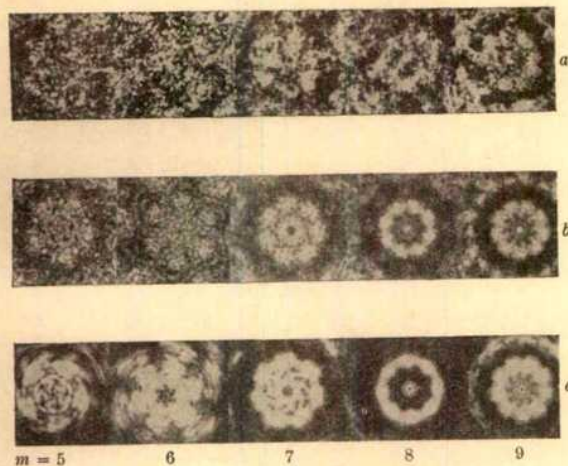


Fig. 1

It was of some importance to attempt a quantitative analysis of the available images of microfibrils. This was carried out using observations from two samples of Lincoln wool fibres, rows (ii) and (iii) of Table 1. If it is assumed that the six orders of peripheral symmetry are equally likely to occur, then the  $\chi^2$  test applied to all microfibrils (data in row (iv)) reveals that these results could have arisen by chance and, therefore, no unique symmetry order could be suggested. Thus it is evident that the ultrafine detail of images of microfibrils is not inherently constant.

We have applied similar tests to six images of microfibrils of porcupine quill-tip, comparing the symmetries derived from images recorded at an arbitrary position of focus (judged as 'best' in a through-focus series) with those from images under-focused by  $\Delta f = -0.2 \mu$ , from the original position. It is evident, from Table 2 (column I), that the symmetry changed in four cases (*b*, *c*, *d* and *e*) but remained unaltered in the other two. When the effect of the coherence of illumination was investigated (that is, the size of condenser aperture varied), images *g* to *l* in Table 2 (column II), the symmetry of only one image remained unchanged. Nine of the twelve images (*m-x*, in Table 2, column III) investigated, after a change of objective aperture, revealed changes in symmetry. In an independent investigation Dobb (ref. 17) and private communications, using the first of the above techniques, has

established that no unique symmetry exists in the images of the microfibrils of metal-treated keratin.

In some instances the shape of the rotated images appeared, at certain values of  $n$ , as a 'straight-sided' polygon. Fifty-six images of microfibrils in the transverse sections of Lincoln wool fibres and porcupine quill were investigated, see row (v) in Table 1. Twenty-seven images showed no particular external shape on rotation. Moreover, out of twenty-nine images which revealed positive results in this respect, fourteen images yielded two different but distinct forms. As a particular example, the microfibril on the extreme right-hand side of Fig. 1c (shown to possess nine-fold symmetry of peripheral maxima) exhibited pentagonal and heptagonal external forms. These latter observations are of some importance since Kassenbeck recently reported that the microfibrils in keratin possess a unique pentagonal cross-section<sup>2</sup>.

The results presented here add considerable weight to our earlier doubt regarding the validity of models for the structure of microfibrils based on the evidence obtained from electron micrographs taken at a random focal position. Moreover, the inconclusive nature of the electron microscope evidence available at present and the only partial success of the quantitative analysis of the X-ray diffraction data, stress the need for broadening the attack on the whole problem following, for example, the lines indicated by Lundgren and Ward<sup>18</sup>. In the forthcoming publication we hope to show that a simple consideration of the dimensions and an idealized packing of the microfibrils (without the refinement of Fraser *et al.*<sup>19</sup>), the densities and mean residue weights of the relevant fractions can lead directly to the evaluation of the possible number of the  $\alpha$ -helices in the microfibrils<sup>6</sup>.

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### Ultrafine Structure of $\alpha$ -Keratin

THE equivocal evidence for the structure of the  $\alpha$ -keratin microfibril as revealed in transverse section has been discussed<sup>1,2</sup>, and it is clear that other methods of approach to the important problem of the arrangement of protein molecules within the microfibril must be used together with sectioning techniques. Again, there is little experimental evidence about the length of the individual protein molecules in the microfibrils and their possible mode of longitudinal polymerization. Thus, in this communication we present a technique for dispersing the microfibrils, prior to negative staining, which is proving useful in the examination of lateral and longitudinal arrangement of protein molecules in keratin.

Fractionation methods applied to fibrous keratin give rise to two main fractions<sup>3</sup>. One fraction is rich in sulphur compared with the original fibre, has a molecular weight of 22,000 and exists in buffer solutions as a random coil<sup>4</sup>. The other fraction is low in sulphur compared with the original fibre, may have a molecular weight of 100,000, and can be regenerated as oriented films which give an X-ray diffraction photograph largely of the  $\alpha$  type but with traces of the cross and parallel  $\beta$  configurations<sup>5</sup>. Harrap and Gillespie<sup>6</sup> have examined extensively the fractionation of wool keratin after alkaline reduction and have shown that treatment by sodium thioglycollate at 4° C for 18 h at pH 10.5 extracts a protein enriched in the high sulphur fraction and leaves a fibrous residue enriched in the low sulphur protein. This residue has been shown to give a disoriented X-ray diffraction pattern of the  $\alpha$  type<sup>7</sup>. As high sulphur content proteins have been tentatively identified with the matrix which surrounds the microfibrils, it was thought that this type of extraction might give a relatively clean preparation of microfibrils which, because of the reduction of disulphide bonds, might be induced to break up into smaller units by the application of ultrasonic irradiation.

The technique finally adopted was to reduce Merino 64's wool, which had been purified by washing with ether, alcohol, and distilled water, with 0.1 M thioglycolic acid in 0.1 M disodium hydrogen phosphate adjusted to pH 10.0 or 10.5 with sodium hydroxide. The fibrous residue was then transferred to an intracellular physiological buffer<sup>8</sup> containing potassium, magnesium, chloride, and phosphate ions at pH 7.0, and irradiated with ultrasonics at 15 c/s and 20 W cm<sup>-2</sup> for 5 h. The temperature in the water-cooled cell remained at 12° C throughout the irradiation. The dispersed material was centrifuged at 500g for 5 min, the supernatant liquid discarded, the sediment washed with buffer and recentrifuged and, finally, resuspended in 1 per cent sodium phosphotungstate at pH 5.6 and irradiated with ultrasonics for a further period of about 1 h. Drops of the dispersed material were then placed on 'holey' carbon film on specimen supporting grids and examined in a Siemens Elmiskop I electron microscope after desiccation.

It may be noted that, as in the case of transverse sections<sup>1</sup>, optimum contrast in the specimen is found at a slightly under-focused setting of the objective lens with respect to true focus as defined by the absence of Fresnel diffraction at the edge of a hole. Because of this the structure of the carbon film is out of focus and tends to distort the appearance of the specimen. Fig. 1 shows a typical area of the preparation and it is evident that the microfibrils have been dispersed into long filaments or protofibrils; indeed there was no evidence of intact microfibrils remaining in the preparation. Microdensito-

meter traces with a long slit have been made on the negatively stained filaments and reveal widths between 12 Å and 40 Å; however, a width of approximately 20 Å predominates. In the region indicated by the arrows there are three filaments about 12 Å, 18 Å and 40 Å in width, but their identities are not maintained for any great length. In Fig. 1 and other micrographs, some of the 20 Å wide filaments appear to be composed of still smaller filaments twisted together, but it is not yet possible to say how many of these smaller filaments are present because of the background noise.



Fig. 1. Merino wool reduced, dispersed by ultrasonic irradiation, and stained with phosphotungstic acid ( $\times 275,000$ )

It may be tentatively suggested that the smallest filaments seen here are single protein molecules, and that the predominant 20 Å wide filaments are two- or three-strand ropes. Nevertheless, other arrangements of the molecules cannot be excluded. From these results it is difficult to say whether or not any one arrangement is maintained for a great distance along the microfibril, and there is no indication of a unique structure for the microfibril. Further work is being carried out.

Finally, it should be noted that, in an independent investigation, Dobb<sup>9</sup> has dispersed the microfibrils using a different technique and again finds predominant evidence of 20 Å wide protofibrils.

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## LETTERS TO THE EDITOR

## GEOPHYSICS

## Magnetic Variation Measurements in Iceland

IN certain parts of the Earth, notably Japan<sup>1</sup>, Germany<sup>2,3</sup> and near Alert and Mould Bay in arctic Canada<sup>4</sup>, the pattern of time-variations in the geomagnetic field exhibits considerable local irregularities. These have been attributed to the effects of electric currents induced in highly conducting portions of the Earth, and the suggestion has been made that this high conductivity is evidence of anomalously high temperatures beneath the regions concerned.

There are differences in the pattern of magnetic behaviour between the locations mentioned, presumably because of differences in the geometrical distribution of conducting material. Thus, in Germany and at Alert, the vertical component of magnetic bay-type disturbances changes sign between stations 100 or 200 km apart, while at Mould Bay the vertical component for a range of periods is highly attenuated over a considerable area.

There is no direct proof that temperature, rather than composition or other parameter, is responsible for the increased electrical conductivity. It therefore appeared desirable to examine the pattern of magnetic variation across a region where high temperatures in the crust and upper mantle were to be expected. Iceland, which lies on the Mid-Atlantic Ridge, provides such a region. The central zone of the Island (Fig. 1) includes virtually all the post-Pleistocene volcanic activity as well as the highest-temperature hot springs. This zone is in line with the central rift of the Mid-Atlantic Ridge, along which high heat flow has been measured farther south.

We have recently operated, for a period of several days, recording three-component magnetometers at three stations, shown on the map. Records are also available, for the same period, from the University of Iceland

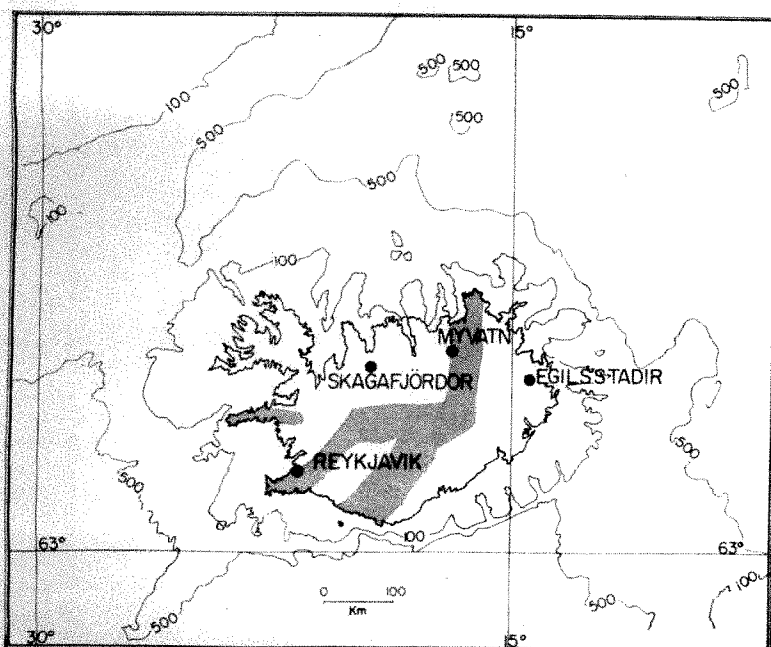


Fig. 1. The shading indicates areas of post-Pleistocene volcanic activity. Depth contours are in fathoms

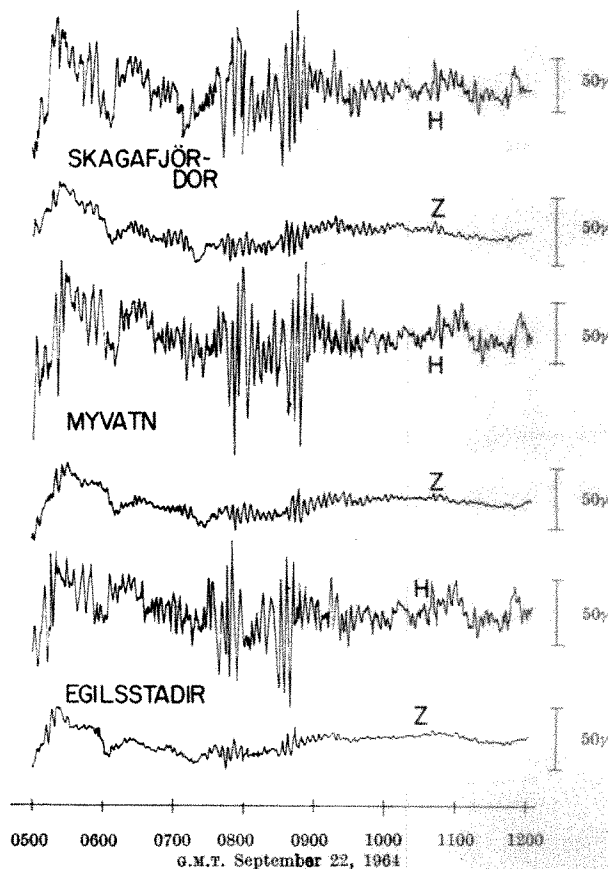


Fig. 2

magnetic observatory at Reykjavik. Of the temporary stations, Myvatn is on the central zone, while Skagafjörður and Egilsstaðir are located on Tertiary basalts, respectively west and east of the zone. The distance between the latter two is 225 km; this is at least as great as the distance across which reversals in the sign of the vertical component were found in Germany and at Alert.

Records for two components, Z and H, for a portion of a moderately disturbed day are shown in Fig. 2. Considering that Iceland is located in the auroral zone, the coherence between the stations, particularly in H, is remarkably high. No reversals in Z are observed. There is, however, definite attenuation of certain periods in Z, not at Myvatn, but at Egilsstaðir. A preliminary examination of all records suggests that this attenuation is greatest for periods lying between 300 and 900 sec. Comparison with theoretical power spectra for different models<sup>5</sup> suggests that a horizontal layer of high conductivity near the base of the crust could be responsible.

The puzzling feature is the fact that the anomalous effect is not associated with station Myvatn. It will require further observations to confirm and outline the

area of high conductivity, but one possibility is suggested by the submarine contours on Fig. 1. The axis of the Mid-Atlantic Ridge, which enters Iceland from the south-west, is not well defined north of the Island. At depth, the axis of thermal or magmatic activity may continue easterly beneath Egilsstadir, then trend northerly beyond the north-east corner of Fig. 1, toward Jan Mayen Island. On this interpretation, the north-south line of recent volcanic activity which passes through Myvatn owes its location to tectonic control in the crust. Near-surface, linear bodies of hot rock near Myvatn may distort the electromagnetic field, but only at periods too short to be detected on these records.

It is well known that magnetic variation measurements on oceanic islands are affected by the boundary of the highly conducting sea-water. To minimize this influence, the three stations were chosen to be approximately equal distances from the coast. The possibility remains that the different orientation of the coastline nearest to the different stations is responsible for the anomalous behaviour of the vertical component at Egilsstadir. However, the azimuth of the horizontal disturbance vector has been examined for different events, and the phenomenon appears to be independent of it. This would support the suggestion that internal conductivity differences rather than the coastline are chiefly responsible.

More detailed examination of the records, including spectral analysis, is in process, and plans are being made to extend the observations. The work was supported by the National Research Council of Canada, and was carried out with the assistance of the staff and students of the Department of Physics, University of Iceland, and geophysicists of the State Electrical Authority of Iceland.

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## RADIOPHYSICS

### Polarization of Jovian Emission at Decametre Wave-lengths

DURING the period September 1–December 31, 1963, observations of the polarization of radio-wave emission from Jupiter were made at 15.2, 16.2, 18.2, 22.2, and 24.2 Mc/s. The Southwest Research Institute two-helix polarimeter<sup>1</sup>, utilizing a twin channel receiver, was expanded so that determination of the cross-correlation of the right and left circular components, in addition to the two opposite circular components, was made separately for each Jupiter burst. This type of radio polarimeter scheme has been described by Cohen<sup>2</sup>. The calibrated polarimeter thus provided determination of the polarization fraction ( $m$ ), axial ratio ( $r$ ) and sense of the Jupiter bursts observed.

One of the predictions regarding the decametre polarization made by Ellis and McCulloch<sup>3</sup>, assuming a cyclotron radiation mechanism, may be stated as follows:

$$\Sigma|r| \propto N^x, \quad x = 1.05$$

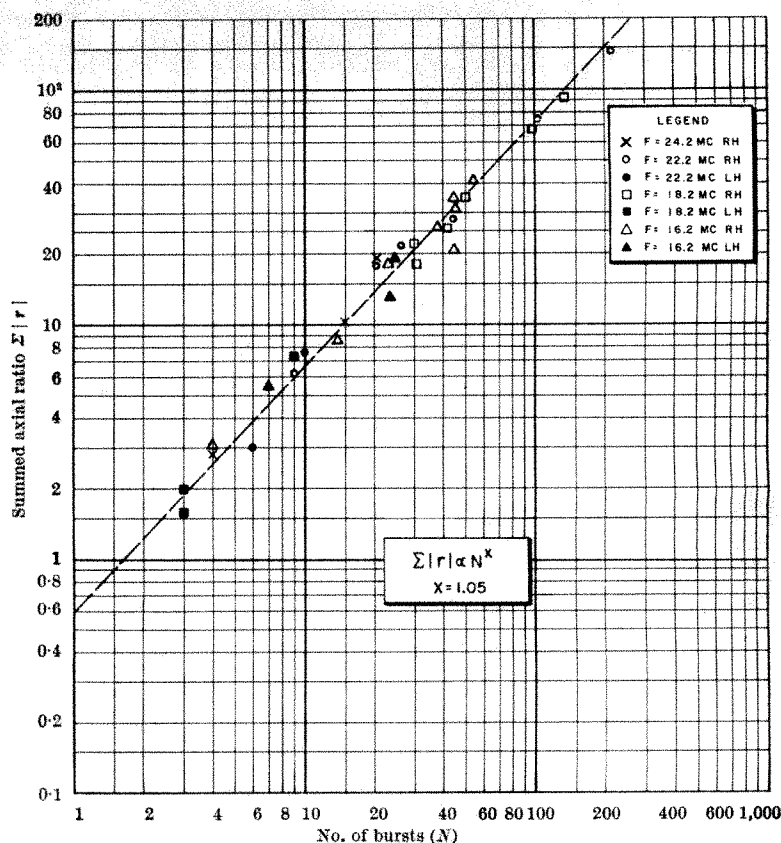


Fig. 1. Jupiter burst axial ratio data obtained during September–December, 1963.

where  $N$  = number of Jupiter bursts,  $\Sigma|r|$  = sum of the  $r$ 's for the corresponding  $N$  bursts taken without regard to the sign of  $r$ .

The points shown in Fig. 1 were obtained by calculating  $r$  and the corresponding System III<sub>(1957-0)</sub> longitudes for the best Jupiter bursts observed. The bursts were divided into six 60° longitude ranges and  $\Sigma|r|$  and  $N$  obtained in each range for the left-hand and right-hand bursts separately. The results are plotted in Fig. 1 for the 16, 18, 22, 24 Mc/s frequencies. The solid line has been drawn through the points arbitrarily with the slope 1.05. A summary of the  $x$  value, and the total number of left- and right-hand bursts obtained for each frequency are given in Table 1.

Table 1. VALUES OF  $x$ , ASSUMING  $\Sigma|r| \propto N^x$ , AND POLARIZATION SENSE DISTRIBUTION FOR EACH FREQUENCY

$F$ (Mc/s)	$x$	Per cent deviation	Total number of bursts		RL
			RH	LH	
24	1.14	10.5	40	0	0
22	1.08	2.8	412	10	2
18	0.916	-12.4	386	7	1
16	1.01	-3.8	267	56	0
Totals	—	—	1,105	73	3

The deviation from the predicted slope is within  $\pm 13$  per cent for all frequencies reported and is roughly equal to the experimental error for  $|r|$ . This agreement is similar to that reported by Dowden<sup>4</sup> for polarization measurements at 10.1 Mc/s. It should be noted that for the September observations  $m$  was assumed to be unity (as was also assumed by Dowden), while the  $r$  data for October–December include the effects of the  $m$  determination. No distinction has been made in the data reported here because there is no apparent effect on the results.

The results indicate consistent values of burst axial ratios independent of System III longitude. This and the observed dependence of  $\Sigma|r|$  on  $N$  are both consonant with the cyclotron source model within experimental error. It should be noted, however, that differing



average values of  $r$  associated with the three primary sources have been obtained by Carr *et al.*<sup>5</sup> and from the data obtained in 1962 with the earlier two-helix polarimeter<sup>1</sup>. The question of  $r$  versus longitude remains to be settled by a greater quantity of precise  $r$  determinations.

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## PHYSICS

### Measurements of Cavity Loss in a Pulsed Ruby Laser

In a cavity laser system, oscillation by stimulated emission occurs when the gain is sufficient to overcome the total loss in the cavity. Cavity loss not only determines the threshold condition of laser emission, it is also related to various properties of the laser output, such as the mode structure and the mode pulling effects. It is therefore important to measure accurately the cavity loss in a laser system. Previous workers have used the threshold electric input energy of the xenon flashtube as a means of evaluating the cavity loss for a pulsed ruby laser<sup>1-3</sup>. The non-linear behaviour of the xenon flashtube can cause measurement error, especially when the measurement was made over a wide range of temperature. There are two parts to this non-linear property: the total and peak radiant outputs in the pumping band are not linearly proportional to the electric input to the flashtube<sup>4</sup>, and the dependence on time of the radiant output varies with the electric input. However, if the time-varying flashtube radiant output power in the pumping band of the laser is directly measured, the total threshold radiant energy can be obtained by time integration of this pumping power up to the onset of the laser emission, assuming that the fluorescence decay time is long. This results in a loss measurement independent of the non-linearity of the flashtube.

For a pulsed ruby laser, we may assume a four-level system<sup>5</sup> to include both the  $\bar{E}^2(E)$  and  $2\bar{A}^2(E)$  metastable levels. The other upper states besides the broad pumping band are neglected in this analysis<sup>5</sup>. The rate equation for the ground state population density is

$$\frac{dn_1}{dt} = -P(t,e)n_1 + (n_2 + n_3)\frac{1}{\tau} \quad (1)$$

where  $n_1$ ,  $n_2$ , and  $n_3$  are the population density of the ground-state, the  $\bar{E}^2(E)$  and the  $2\bar{A}^2(E)$  levels, respectively,  $\tau$  is the fluorescence decay time of the metastable levels, and  $P(t,e)$  is the probability of excitation within the entire pumping band in per atom per sec. It depends on the electrical input  $e$  to the flashtube and is a function which varies with time.

In the measurement procedure, the pumping input was maintained at a sufficiently high level such that the laser threshold condition was reached within 200  $\mu$ sec from the beginning of the pumping. Since  $\tau$  is 3.2 msec<sup>6</sup>, the second term on the right-hand side of equation (1) may be neglected. We then obtain, at threshold:

$$n_1 = n_0 e^{-E_T} \quad (2)$$

where  $n_0 = n_1 + n_2 + n_3$  and  $E_T = \int_0^{t_0} P(t,e)dt$ .  $t_0$  is the time at the onset of the laser emission since pumping started at  $t=0$ .  $E_T$  is therefore proportional to the total threshold radiant pumping input energy which is a function of the temperature  $T$  for a given laser.

We further assume that the two metastable states, separated by 29 cm<sup>-1</sup>, are in thermal equilibrium and are related to each other by:

$$n_3/n_2 = e^{-42/T} \quad (3)$$

The normalized population inversion is then given by:

$$\delta = \frac{1}{n_0} \left[ n_2 - \frac{g_2}{g_1} n_1 \right] = \frac{1}{(1 + e^{-42/T})} - \frac{n_1}{n_0} \left[ \frac{1}{(1 + e^{-42/T})} + \frac{g_2}{g_1} \right] \quad (4)$$

where  $g_1 = 4$  and  $g_2 = 2$  are the statistical weights of the ground state and the  $\bar{E}^2(E)$  level, respectively.  $\delta$  is related to  $\alpha$ , the gain per unit length along the amplifying medium, by<sup>4</sup>:

$$\delta = \frac{g_2}{g_1} \frac{\Delta\nu_T}{(\Delta\nu_r \cdot \alpha_r)} \cdot \alpha \quad (5)$$

where  $\Delta\nu_T$  and  $\Delta\nu_r$  are the  $R_1$  line-width at temperature  $T$  and at room temperature, respectively, and  $\alpha_r$  is the absorption coefficient in cm<sup>-1</sup> at room temperature.  $\alpha$  multiplied by the length of the rod must be equal to the loss per pass of the laser cavity at threshold.

Combining equations (2), (4), and (5), we obtain:

$$\frac{E_T}{E_R} = \frac{\ln \left[ 1 - \frac{g_2}{g_1} \frac{\Delta\nu_T \alpha}{(\Delta\nu_r \alpha_r)} (1 + e^{-42/T}) \right] - \ln \left[ 1 + \frac{g_2}{g_1} (1 + e^{-42/T}) \right]}{\ln \left[ 1 - \frac{g_2}{g_1} \frac{\alpha}{\alpha_r} (1 + e^{-42/T}) \right] - \ln \left[ 1 + \frac{g_2}{g_1} (1 + e^{-42/T}) \right]} \quad (6)$$

where  $E_R$  is the value of  $E_T$  at room temperature. Using the temperature dependence of  $R_1$  line-width measured by Aagard<sup>7</sup> and by McCumber and Sturge<sup>8</sup>, and also taking<sup>4</sup>  $\alpha_r = 0.272$  cm<sup>-1</sup>,  $E_R/E_T$  was calculated as a function of  $\Delta\nu_T$  for  $\alpha = 0, 0.10, 0.135$ , and  $0.2$ . The results are plotted in Fig. 1.

Cavity loss measurement was performed on a 1.25 in. long sapphire-clad ruby rod with a 0.080-in. diameter ruby core of 0° orientation and of 0.05 per cent Cr<sup>3+</sup> concentration. This rod was purchased from Linde Company in 1962. The ends are coated with 89 per cent reflectivity aluminium mirrors. An F/T-524 flashtube was used for the pump. Two photomultipliers were used. One monitored the flashtube output after passing through a green filter with a pass band similar to the green absorption band of the ruby. The output was proportional to  $P(t,e)$ . The other photomultiplier monitored the laser output, thereby yielding the value of  $t_0$ . The time integration of the output of the first photomultiplier up to  $t_0$

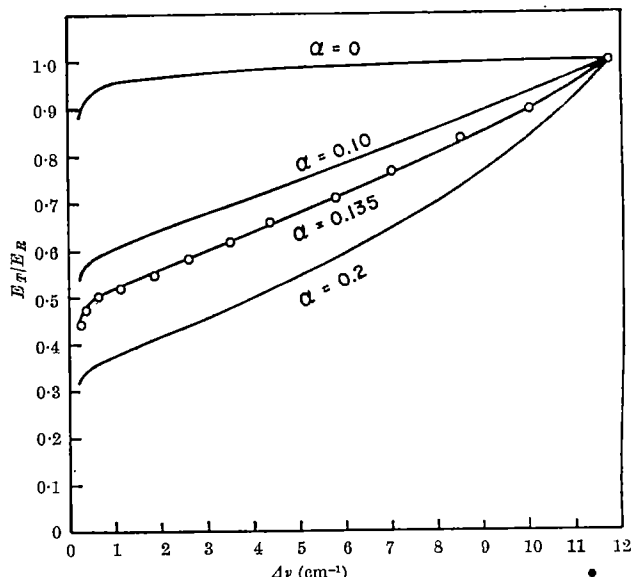


Fig. 1. Normalized threshold pumping input energy  $E_T/E_R$  as a function of  $R_1$  line-width, for four different values of loss per cm  $\alpha$ . The circles are the experimental data measured on a 1.25 in. sapphire-clad ruby rod.

then yielded a quantity proportional to  $E_T$ . Since only the value of  $E_T$  normalized to that at room temperature was required, the proportionality constant was cancelled out. The measurement was carried out in the range from 77° K to 293° K and the results plotted in Fig. 1. The agreement between the experimental data and the theoretical calculation for  $\alpha = 0.135$  is nearly perfect.

This yields a total cavity loss of 43 per cent per pass in the ruby rod studied. The end reflectivity loss contributes 11 per cent per pass. Assuming the diffraction loss and other end losses of 25 per cent, the scattering loss is 7 per cent per pass. These results are very comparable to those measured at reduced temperature on other Linde ruby laser rods grown during the same period of time<sup>2</sup>.

The method described here eliminates the inaccuracy and difficulty due to the non-linear property of flashtube in the conventional laser cavity loss measurements. Since measurements are made at relatively high pumping input, there is no need to search tediously for the threshold pumping energy. The measurement results obtained indicate that the cavity loss is essentially constant over the temperature-range investigated, and the assumed four-level system is an adequate description for the pulsed ruby laser.

I thank R. L. Aagard and J. F. Ready for their advice, and G. N. Otto for making the experimental measurements.

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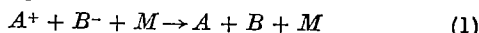
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### Three-body Ionic Recombination

THOMSON'S theory<sup>1</sup> of three-body recombination of positive and negative ions:



invokes the artifice of a fictitious trapping distance such that recombination may be supposed to occur if either ion collides with a neutral molecule when within this distance from the other. In recent years the theory has been elaborated by several investigators<sup>2</sup> and the predictions regarding the recombination coefficient have consequently been considerably modified. However, the basic artifice is retained though it does not form an altogether satisfactory foundation on which to build a precise theory. We here outline an entirely different approach exploiting the fact that a quasi-equilibrium is quickly established in which the rate at which bound ion-pairs are formed and destroyed is very much greater than the rate at which their number density changes<sup>3</sup>. In so far as the contribution from charge transfer is merely additive, our approach is effectively exact in the region where the three-body recombination coefficient  $\alpha$  is directly proportional to the number density,  $N(M)$ , of neutral atoms.

Let the number density of ion-pairs having internal energy between  $x$  and  $x+dx$  be  $n(x)dx$  in the quasi-equilibrium state and be  $n_0(x)dx$  in thermodynamic equilibrium. Write:

$$\rho(x) = \frac{n(x)}{n_0(x)}, \quad x < 0 \\ = 1, \quad x > 0 \quad (2)$$

Considering a gas of neutral atoms and ion-pairs of internal energy  $x$  denote the rate coefficient describing those collisions which change the internal energy to between  $y$  and  $y+dy$  by  $K(x,y)dy$ .

In the quasi-equilibrium state we have that:

$$n(x) \int_{-I}^{\infty} K(x,y)dy = \int_{-I}^{\infty} n(y)K(y,x)dy \quad (3)$$

where  $-I$  is the most negative internal energy reached. With the aid of (2) and the principle of detailed balancing it may be shown that (3) is equivalent to:

$$\rho(x) \int_{-I}^{\infty} K(x,y)dy = \int_{-I}^{\infty} \rho(y)K(x,y)dy \quad (4)$$

If  $K(x,y)$  is known (and in any particular case it may be calculated) this integral equation for  $\rho(x)$  may be solved to any desired accuracy. The rate recombination equals the net rate at which ion-pairs flow downwards in  $x$ -space past any fixed level  $-J$  so that:

$$\alpha N(A^+)N(B^-) = N(M) \int_{y=-J}^{\infty} \int_{x=-I}^{\infty} \{n(y)K(y,x) - n(x)K(x,y)\} dx dy \quad (5)$$

where  $N(A^+)$  and  $N(B^-)$  are the number densities indicated. Hence  $\alpha$  may be found.

We have as yet carried out detailed computations only on ions recombining in their parent gas (for which case  $K$  is determined by resonance charge transfer). The preliminary value which we obtained for  $\alpha$  in this special case does not differ appreciably from that given by the original simple formula of Thomson (as corrected by Loeb<sup>1</sup>) and the temperature dependence is the same. A fuller account of the work will be published elsewhere.

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## GEOLOGY

### Late Quaternary Terraces on Mount Carmel (Haifa, Israel)

REMNANTS of two marine quaternary terraces, corresponding to a sea-level of 5 m and 15 m above sea-level respectively, have been revealed by boreholes and excavations during housing projects on the N.N.E. piedmont of Mt. Carmel in Haifa, Israel.

The area of approximately two square kilometres, known as the Kyriat Eliahu suburb of Haifa, is covered mainly with alluvial soils and some mountain talus. Numerous middle palaeolithic artefacts<sup>1</sup> which are obviously *non in situ* have been found scattered over the surface. In several places the bore holes and excavations encountered marine calcareous sandstone, resting on a platform cut in the mountain formations (dolomite and marly chalk of Cenomanian age) (Fig. 1).

The younger (lower) terrace has been traced on a stretch of 1.5 km up to the present shore (Fig. 2). A conglomerate, 0.5 m thick, cemented by calcareous sandstone and typical of beach environment, was found resting on a chalky bedrock at 4 m, thus determining the level of the terrace-forming sea as approximately 5 m above the present sea. The calcareous sandstone is light in colour, is virtually unweathered and is mainly of an oolitic type<sup>2</sup>. Its maximum thickness known so far is 1 m. The calcareous sandstone is

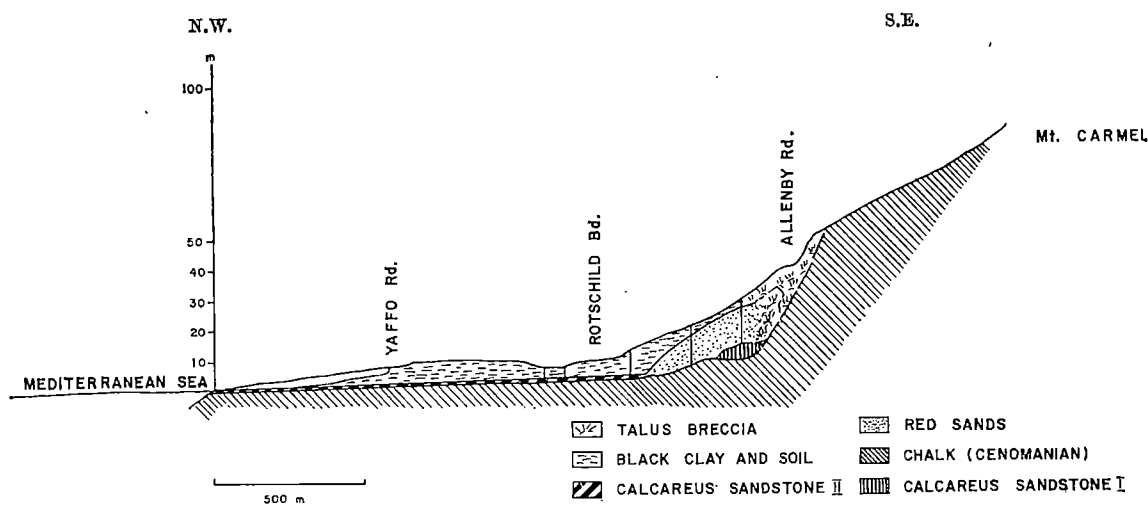


Fig. 1

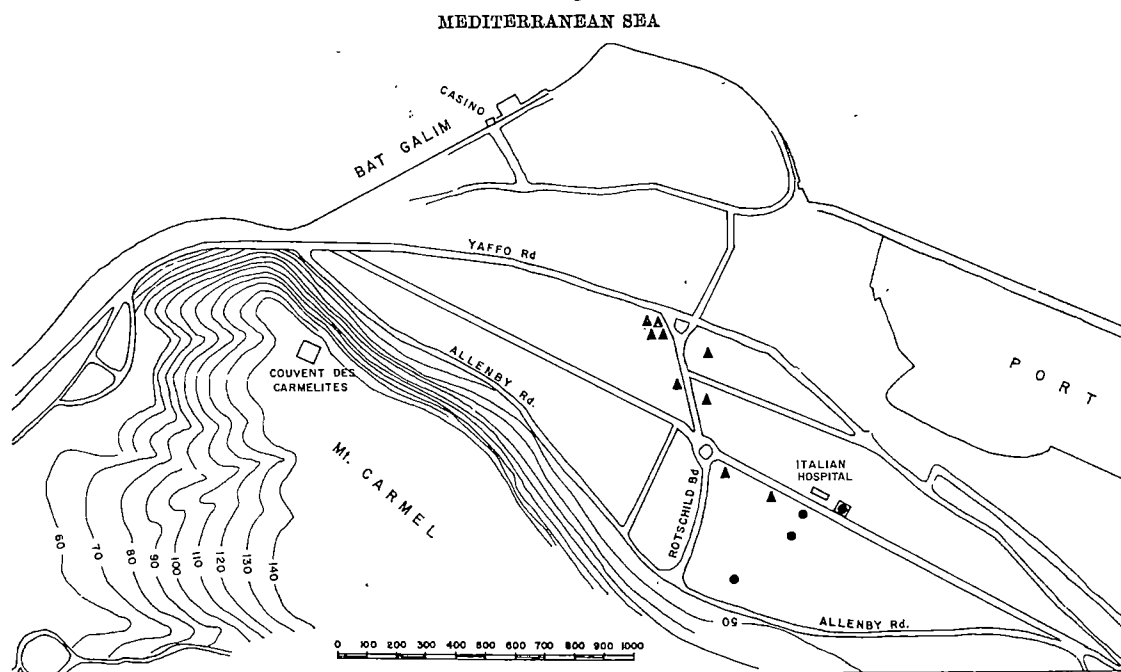


Fig. 2. ●, Upper terrace; ▲, lower terrace

covered by a black plastic clay of swampy character containing flint pebbles.

The marine layer of the upper (older) terrace is up to now known only in three localities, an excavation and two boreholes. This is a coarse, reddish, very fossiliferous calcareous sandstone, not oolitic, passing downwards and upwards to a strongly recrystallized hard coquina. Only *Loripes lacteus* in enormous quantities has been determined until now with certainty, together with some *Miliolidae* and *Streblus* spp.

The patchy character of the layer shows itself clearly in a large recent excavation. Its thickness varies there from 0 to 1 m. The discontinuity of the layer and the red colour of the sand and clayey sand overlying it point to a strong and prolonged weathering. The calcareous sandstone rests directly on the bed-rock, which is dolomitic in one of the localities and chalky in others, or on a sporadically distributed conglomerate, 20 cm thick, cemented partially by calcareous sandstone. The marine layer is covered by red-brown clayey sands containing flint pebbles and gradually passing upwards to a dark grey clay. The level of the marine sandstone in a borehole near the mountain is at 14 m, thus giving 15–16 m as a most probable level of the terrace-forming sea.

On general geological and petrographical considerations the younger terrace is regarded as having been formed by the Late Monastirian sea<sup>1</sup>. The older terrace corresponds, according to its level and by comparison with the Lebanon coast<sup>2</sup>, to the Main Monastirian sea<sup>3</sup>, although no *Strombus bubonius* has as yet been found in it. To our best knowledge only one specimen of *S. bubonius* has been found so far in Israel (several years ago<sup>5</sup> in the vicinity of the area under discussion) and no evidence exists that it was *in situ*.

We thank the Soil Mechanics Laboratory of the Technion, Haifa, which kindly provided the samples and data from the boreholes.

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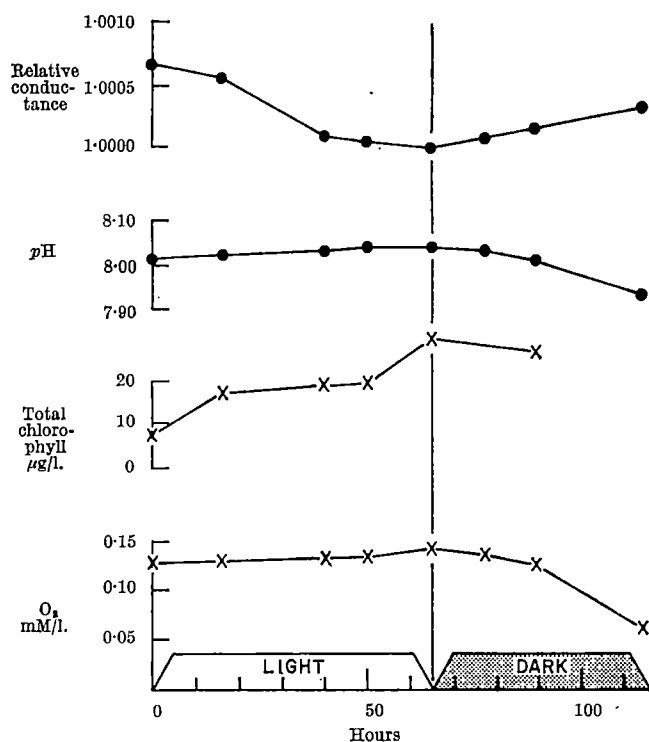


Fig. 1. Concurrent changes in electrical conductance, pH, chlorophyll and dissolved oxygen during the successive periods of illumination and darkness

During the following 49-h dark period, pH dropped from 8.03 to 7.93, and oxygen from 0.14 mM/l. to 0.06 mM/l. Chlorophyll content showed decreasing tendency. The conductivity ratio increased by 0.03 per cent.

Park *et al.*<sup>1</sup> explained the change in conductance due to transformation of bicarbonate ion to carbonate ion and vice versa. In our experiment the observed change in the conductance can be explained by the same mechanism; photosynthesis transforms bicarbonate ion to carbonate ion and respiration does the opposite.

When we know more about the electrochemical properties of sea-water, it may be feasible to estimate the magnitude of net photosynthesis and respiration by the measurement of electrolytic conductance.

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### Uptake of Chromium(III) by Particles in Sea-water

ATOMIC reactors at Hanford Laboratories, Washington, introduce easily measurable quantities of chromium-51 into the Columbia River. Although in hexavalent form when produced, some of the radioactive chromium is probably reduced to the trivalent state while passing downstream. Chemical species likely to exist are  $\text{CrO}_4^{2-}$ ,  $\text{Cr}^{+3}$  (aq.) and  $\text{Cr}_2\text{O}_3 \cdot n\text{H}_2\text{O}$ . Observations of Osterberg and Cutshall (unpublished) confirm the presence of several species in the river. They found that  $^{51}\text{Cr}$  could

be removed from river water using membrane filters, chelating resins and both cation and anion exchange resins. Membrane filters (0.65 µ pore size) retained about 20 per cent of the  $^{51}\text{Cr}$  from river water collected near Bonneville Dam. Seymour<sup>2</sup> noted earlier that filters with a 0.45 µ pore size removed about 25 per cent of  $^{51}\text{Cr}$  from river water taken near the mouth of the Columbia River. Measurements made during a recent cruise in the lower 210 km of the river showed that the percentage of  $^{51}\text{Cr}$  attached to particles gradually increased downstream<sup>3</sup>.

The presence of  $^{51}\text{Cr}$  off the mouth of the Columbia River appears closely related to the position of the river effluent.  $^{51}\text{Cr}$  was consistently found on membrane filters, with glass fibre prefilters, to 72 km offshore. In April 1962, 32 pc./l. was found 'as particles' at the mouth of the river. This value decreased to 16 pc./l. at 24 km, 2 pc./l. at 104 km, and was not appreciably above background 136 km–264 km from shore<sup>4</sup>. In June 1962, comparable levels of 'particulate'  $^{51}\text{Cr}$  were found out to 40 km. In April 1963,  $^{51}\text{Cr}$  was detected 40 km from the mouth in water with a salinity of 30 parts per thousand (nominally considered to be the Columbia River effluent), then decreased to background 72 km offshore.

Krauskopf<sup>5</sup> used various substances in an attempt to remove  $\text{CrO}_4^{2-}$  from artificial sea-water. Addition of  $\text{MnO}_2 \cdot n\text{H}_2\text{O}$  rapidly removed most of the chromate, whereas  $\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$ , 'clay', apatite, and 'plankton' were not effective over exposure periods of two to fourteen days. On the other hand, Yamagata and Iwashima<sup>6</sup> found  $\text{MnO}_2$  to be inefficient (14.5 per cent) in removing chromium (valence state not given) from sea-water. Chromate ions do not appear to be actively adsorbed on most common particles in the sea, but may be subject to scavenging by some hydrocolloids. There is no evidence that marine pelagic organisms naturally contain much chromium. Generally less than 1 p.p.m. has been reported in ashed samples<sup>7</sup>. Thus, the particulate  $^{51}\text{Cr}$  from filtered sea-water off the mouth of the Columbia must have been either scavenged from solution by hydrocolloids, or present in some form other than chromate (that is,  $\text{Cr(III)}$ ).

At the commonly assumed pH value of 8.2 and an oxidation potential of 0.40 V for open ocean water, chromium should exist predominantly as  $\text{Cr(VI)}$  (ref. 8) and should not be associated with particles. However, a small decrease in oxidation potential of pH would favour the formation of hydrous chromic oxide. This should not be possible in well-oxygenated surface water, but might occur in river water mixed with upwelled water, since the latter is very low in oxygen. The pH of surface water along the Oregon coast has been observed to drop to 7.9 as a result of either river run-off or upwelling<sup>2</sup>. Assuming that  $\text{Cr(III)}$  could exist in nearshore, surface waters of the ocean, it was decided to test the physical behaviour of this species in sea-water.

Chromium, as  $^{51}\text{CrCl}_3$ , was added to plain and nutrient-enriched filtered sea-water (pH 8.0) in the amount of 14 µc./100 ml. ( $6 \times 10^{-3}$  µg Cr including carrier). Enrichment consisted of phosphate, nitrate, silicate, trace metals and vitamins.

The appearance of  $^{51}\text{Cr}$  on membrane filters used to determine radionuclides in suspended matter from the ocean off Oregon indicated that absorption might be important. To investigate this possibility, filters (2.5 cm, 0.45 µ pore size) were soaked in plain sea-water containing  $^{51}\text{Cr(III)}$ . After varying periods, the radioactivity on the unrinsed filters was counted. Adsorption was rapid at first. The amount on the filters at 1 h was three times the amount after 10 min, and after 24 h was 23–30 times the amount after 10 min. Filters which soaked for 24 h recovered 33–43 per cent of the total radioactivity.

Filtered sea-water was adjusted to pH values of 6.0, 7.0 and 8.5 and re-filtered at time intervals ranging from 1 min to 24 h. At pH 6.0, 1.7 per cent of the activity was

recovered on filters; at pH 7.0, 4.0 per cent, and at pH 8.0, 1.5 per cent. There was no significant difference in recovery as a function of time.

Glass beads with an average diameter of 0.2 mm were washed successively in 3 N HCl and distilled water and were then exposed to  $^{51}\text{Cr(III)}$  in filtered sea-water for 24 h. The beads were washed into a weighing tube, rinsed with sea-water, and the supernatant removed. A linear correlation of activity recovered with weight of beads (and, therefore, surface area) was observed.

Uptake of  $^{51}\text{Cr(III)}$  by ten species of phytoplankton algae was measured, but no appreciable differences among species were noted. However, small cells appeared to accumulate somewhat more isotope per cell per unit time than did large cells. Experiments used both living cells in the light and in the dark, and dead cells killed either by heat (100° C for 10 min) or by the addition of formaldehyde. In some experiments various amounts of  $^{51}\text{CrCl}_3$  were added. In others, various numbers of cells were used. When variable amounts of isotope were added to aliquots of rich culture (200 cells/ml.) of *Skeletonema costatum*, the percentage adsorbed was inversely related to the quantity added. For example, after 1.5 h 50 per cent of  $110 \times 10^3$  c.p.m. was recovered, while 70 per cent of  $0.1 \times 10^3$  c.p.m. was recovered.

When a constant amount of isotope was added to cultures of *S. costatum* with cell concentrations ranging from 8 to 80/mm<sup>3</sup>, a doubling of cell concentration resulted in a 1.5 to 3-fold increase in isotope accumulation. Above 80 cells/mm<sup>3</sup>, a doubling of cell concentration produced a linear increase of 22 per cent in isotope accumulation. This relationship prevailed up to 320 cells/mm<sup>3</sup>.

In general, uptake was rapid. Equilibrium was always reached within 24 h. Rates of uptake and final equilibrium values were the same for equal cell numbers of living phytoplankton both in the light and in the dark and for cells killed either with heat or by the addition of formaldehyde.

There is no doubt that Cr(III) is actively adsorbed on living and dead particles at all pH values normal for sea-water. The existence of chromium in this state in sea-water is still problematic, since it is not thermodynamically favoured. However, recent evidence (Johnson and Cutshall, unpublished) suggested that Cr (probably III) attached to particles in the Columbia River may remain bound to particles in sea-water. It will be necessary to demonstrate the actual oxidation state of chromium in sea-water. In addition it would be very desirable to establish the rate of uptake of anionic chromium by phytoplankton and other suspended matter.

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## CHEMISTRY

### ( $\text{Re}_3\text{Br}_{11}$ )<sup>2-</sup> Anion in $\text{Cs}_2\text{Re}_3\text{Br}_{11}$

THE detailed structure of the ( $\text{Re}_3\text{Br}_{11}$ )<sup>2-</sup> anion, the formation of which was recently reported by Ferguson and Robinson<sup>1</sup>, has been determined by means of X-ray crystal structure analysis of its caesium salt. It shows the same general configuration as the ( $\text{Re}_3\text{Cl}_{11}$ )<sup>2-</sup> ion (ref. 2). De-

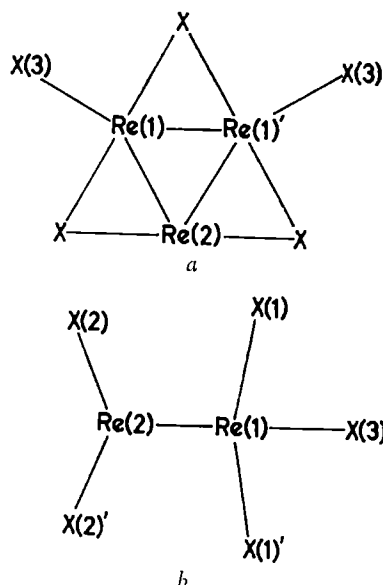


Fig. 1. The ( $\text{Re}_3\text{X}_{11}$ )<sup>2-</sup> ion showing (a) atoms in the plane of the rhenium triangle and (b) atoms out of this plane

tailed analysis of bond lengths and angles reveals some interesting differences between the ( $\text{Re}_3\text{Cl}_{12}$ )<sup>3-</sup> ion (refs. 3 and 4) and the two ( $\text{Re}_3\text{X}_{11}$ )<sup>2-</sup> ions, which raise questions as to the nature of the chemical bonds involved. Further, there is an important difference between ( $\text{Re}_3\text{Cl}_{11}$ )<sup>2-</sup> and ( $\text{Re}_3\text{Br}_{11}$ )<sup>2-</sup>.

Crystals of  $\text{Cs}_2\text{Re}_3\text{Br}_{11}$  are small black needles, orthorhombic, with space group *Pnma*. There are four formula units in a unit cell of dimensions  $a=9.53$ ,  $b=16.08$ ,  $c=13.72$  Å. The calculated density is  $5.38 \text{ g cm}^{-3}$ . A trial structure was proposed on the basis of projections of the Patterson function down each crystallographic axis and was confirmed by three-dimensional electron density maps. All atomic co-ordinates, including individual isotropic thermal parameters, have been refined by three-dimensional least squares using 1412 observed reflexions in the levels ( $0 \dots 6kl$ ) for which the *R* index is 0.167.

Like ( $\text{Re}_3\text{Cl}_{11}$ )<sup>2-</sup>, the ( $\text{Re}_3\text{Br}_{11}$ )<sup>2-</sup> ion has the ( $\text{Re}_3\text{Cl}_{12}$ )<sup>3-</sup> general configuration but with one terminal bromine atom missing from the plane of the rhenium triangle (see Fig. 1). It possesses a crystallographic mirror plane which passes through Re(2) and its two attached terminal bromine atoms (2) and (2'), and also through the bromine atom which bridges Re(1) and Re(1'). In fact it possesses symmetry  $C_{2v}$  within limits of error. Estimated standard errors for Re—Re and Re—Br bonds are  $\leq 0.01$  Å, and for angles,  $\leq 0.5^\circ$ . All bond distances and some angles are listed in Table 1 together with the corresponding mean dimensions for the ( $\text{Re}_3\text{Cl}_{11}$ )<sup>2-</sup> ion (refs. 2 and 5) which has no crystallographic symmetry. These figures show that differences within the chloro-anion which were of only possible significance have been confirmed by quite significant differences in the bromo-anion. On the other hand, there remains one highly significant difference between the two ( $\text{Re}_3\text{X}_{11}$ )<sup>2-</sup> ions.

The Re—Re lengths in ( $\text{Re}_3\text{X}_{11}$ )<sup>2-</sup> do not change on replacing Cl by Br. Furthermore, the Re—Re bonds involving the halogen-deficient rhenium atom are significantly shorter than the third bond, which has the same

Table 1. BOND LENGTHS AND ANGLES

	( $\text{Re}_3\text{Br}_{11}$ ) <sup>2-</sup>	( $\text{Re}_3\text{Cl}_{11}$ ) <sup>2-</sup>
Re(1)—Re(1')	2.48 Å	2.48 Å
Re(1)—Re(2)	2.43	2.43
Re—X(1)	2.49, 2.46	2.30
Re—X(2)	2.39, 2.37	2.29
Re—X(3)	2.72	2.57
Re—X(bridge)	2.52, 2.55, 2.56	2.35
Re—X(bridge)—Re	59°, 57°	61°
X(1)—Re—X(1')	159°	159°
X(2)—Re—X(2')	134°	153°



length as those in  $(\text{Re}_3\text{Cl}_{12})^{3-}$ . This suggests a difference of bond order among the Re—Re bonds.

On replacement of Cl by Br, there is a reduction of  $19^\circ$  in the angle subtended by the out-of-plane atoms  $X(2)$  and  $X(2)'$  at the deficient Re(2), and the Re—Br(2) bonds themselves are significantly shorter than any other in the bromo-anion. These observations suggest that the Re—Br bonds to the non-deficient Re(1) are under considerable strain which is relieved on removal of one halogen atom by bond bending and also by bond shortening to what is presumably a closer approximation to a true single bond length. The first quantitative treatment of the bonding in these trinuclear anions, given by Cotton and Haas<sup>4</sup>, assumes that four orbitals from each rhenium atom are directed to the corners of a square and that all Re—Re bonds are equivalent. For  $(\text{Re}_3\text{Cl}_{12})^{3-}$ , the only structure then known, the first assumption leads to bonds with the out-of-plane chlorine atoms which are bent by about  $10^\circ$  only. However, relative to this scheme, the bending of these bonds in  $(\text{Re}_3\text{Br}_{11})^{2-}$  is increased markedly to  $23^\circ$ . The observations, first, of a preference for highly bent Re—X bonds and, secondly, of the non-equivalence of Re—Re bonds, do indicate that some modification or extension of the Cotton and Haas scheme is required for  $(\text{Re}_3\text{X}_{11})^{2-}$  ions.

The unit cell dimensions and space group of  $\text{Cs}_2\text{Re}_3\text{Br}_{11}$  were previously attributed by us (see ref. 1) to the compound  $\text{CsRe}_3\text{Br}_{10}$  on the basis of bulk analyses. This was because crystals of more than one composition appear to separate under the conditions of preparation and bulk analyses are therefore misleading. We have since characterized a second crystal form from these preparations. The small, black, roughly spherical crystals are orthorhombic with cell dimensions  $a = 11.1$ ,  $b = 14.6$ ,  $c = 14.6$  Å and probable space group  $Ama2$ . They thus have very similar cell dimensions and the same probable space group as  $\text{Cs}_3\text{Re}_3\text{Cl}_{12}$  and are assumed to be the isostructural  $\text{Cs}_3\text{Re}_3\text{Br}_{12}$ . The existence of this  $(\text{Re}_3\text{Br}_{12})^{3-}$  ion is of interest in view of the very considerable steric repulsions which must exist between bromine atoms.

We thank Dr. J. E. Fergusson and Mr. B. H. Robinson for sample preparation, and for advice. This project was supported financially by the New Zealand Universities Research Committee.

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<sup>1</sup> Fergusson, J. E., and Robinson, B. H., *Proc. Chem. Soc.*, 189 (1964).

<sup>2</sup> Fergusson, J. E., Penfold, B. R., and Robinson, W. T., *Nature*, 201, 181 (1964).

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### Distribution of Benzcarbazole Isomers in Petroleum as Evidence for their Biogenic Origin

A RECENT paper<sup>1</sup> has discussed the distribution of different types of nitrogen compounds in the  $200^\circ$ – $500^\circ$  C fraction from petroleum of varying geological age. Carbazoles and benzcarbazoles were found to be predominant nitrogen types in all crudes, accounting for 25–70 per cent of total nitrogen in this boiling range, and as much as 2 per cent by weight of the  $200^\circ$ – $500^\circ$  C fraction. Carbazoles and their higher benz analogues also appear to account for a major part of the nitrogen in the  $500^\circ$  C plus residues from these crudes. Among the benzcarbazoles the 1,2-isomers predominate, the 3,4-benzcarbazoles are

present in minor amounts, and the 2,3-benzcarbazoles are altogether absent. A similar distribution has been noted in catalytically cracked gas oils<sup>2</sup>.

An examination of the possible origin of these important nitrogen compound types is worth while because it might provide indirect evidence on the origin of petroleum itself. The indole nucleus is found in Nature in the amino-acid tryptophan and many of the alkaloids<sup>3,4</sup>, while the hexahydrocarbazole nucleus exists intact in the alkaloids strychnine and aspidospermine. Similarly, as indicated in Fig. 1, certain natural alkaloids appear structurally related to the 1,2-benzcarbazoles, and, in a less direct fashion, to the 3,4-benzcarbazoles. Interestingly, no alkaloids known to the author possess the potential 2,3-benzcarbazole nucleus. It is striking in this connexion that the relative probability of producing a benzcarbazole from the naturally occurring alkaloids (that is, 1,2-benzcarba- most likely, 3,4-benzcarbazoles less likely, and 2,3-benzcarbazoles unlikely) is precisely reflected in the actual occurrence of the various benzcarbazole isomers in petroleum. In the absence of alternative explanations for the relative occurrence of the benzcarbazole isomers in petroleum, this suggests that these petroleum nitrogen compounds were formed from ancient plant deposits. The preference for the angular 1,2- and 3,4-benzcarbazole structures in petroleum also parallels the general preference in Nature for angular as opposed to linear structures (for example, the steroids).

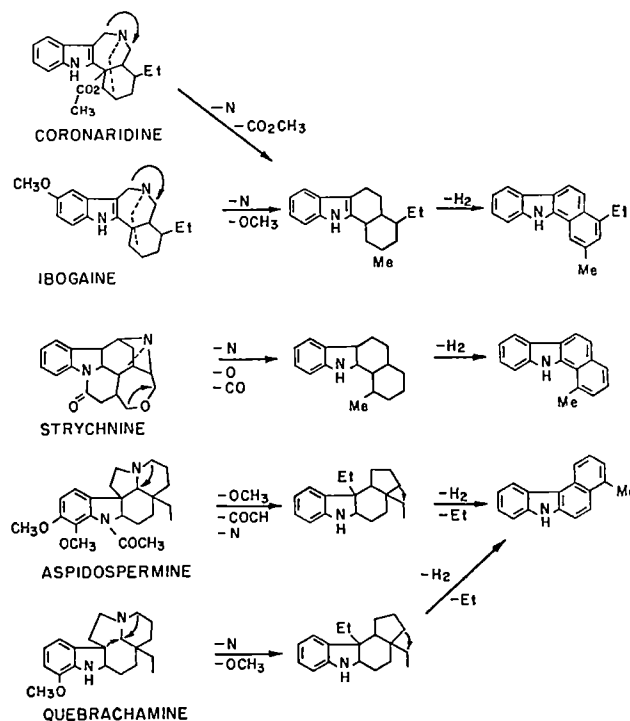


Fig. 1

The relative absence of the indoles in petroleum<sup>1</sup> can be rationalized in terms of the much greater reactivity of this class of compounds toward acid and other environments (for example, ref. 2).

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### Influence of Gases on the Rate of Evaporation of Water

Two communications on this subject have been published recently. Sechrist<sup>1</sup> found that water containing dissolved carbon dioxide, or surrounded by an atmosphere of this gas, evaporated 15–50 per cent more rapidly than water in the presence of air. Kingdon<sup>2</sup> measured the rates of evaporation of water in the presence of 11 different gases, including hydrogen, oxygen, argon, carbon dioxide and butane. Rates varied over a range of 15 per cent in all gases except hydrogen and helium, which produced rates about 40 per cent less. Kingdon defined a corrected rate of evaporation as the measured rate divided by the diffusion coefficient of water vapour in the surrounding gas. The corrected rate in butane was double that found in oxygen or nitrogen. Other gases also augmented the corrected rate relative to oxygen or nitrogen, except hydrogen or helium, which reduced it five-fold.

Both workers attributed their results to the influence of the gases on the physicochemical state of the water surface. Sechrist suggested that carbon dioxide catalyses the conversion of polymerized liquid water to single molecules, and Kingdon postulated that some gases weaken hydrogen bonding at the water surface. Both these hypotheses imply that certain gases may raise the condensation coefficient of water vapour molecules at a water surface. The existing evidence suggests, however, that this coefficient may be unity<sup>3</sup>. Accordingly a further experiment seemed advisable.

In both the previous experiments the water was held within a shallow pan, so that water vapour migrated upwards from the surface. I have found that reversal of this direction reverses the trend of the observations.

A drop of water, volume 0.0070 ml., was generated at the tip of a thin glass tube attached to a micrometer syringe. The delivery tube was mounted in the rubber stopper of a flask which could be filled with a known dry gas. Rates of evaporation were readily measured by determining residual volumes after known intervals of time. With the flask standing on the bench so that water vapour migrated mainly downwards, drops evaporated 70 per cent more rapidly in hydrogen than in oxygen. In argon the rate was less than in oxygen by about 5 per cent, barely significant in this work. These relative rates follow a trend opposite to that found by Kingdon. With the flask inverted, however, water evaporated 10 per cent more rapidly in argon and 50 per cent more slowly in hydrogen. Thus with water vapour migrating mainly upward results agreeing with Kingdon were obtained.

It is concluded that in all the reported experiments the relative effects observed are influenced more by buoyancy than by any other factor. Whether the underlying mode of diffusion is viscous or turbulent, water vapour departs from a water surface less rapidly if opposed gravitationally and more rapidly otherwise. Thus examination of Kingdon's measurements reveals a strong positive correlation between the density of the surrounding gas and the corrected rate of evaporation.

Kingdon also observed that evaporation proceeded spasmodically except when hindered by hydrogen or helium, and suggested that the phenomenon arose from localized weakening of hydrogen bonds in the water surface. Spasmodic flow, however, is a common consequence of an unstable density gradient, and indeed has been observed within liquid water itself while evaporating<sup>4</sup>.

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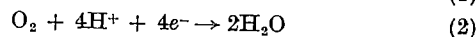
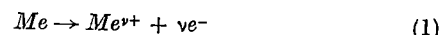
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### Kinetics of Oxygen Reduction on the Amalgam – Acid Solution Interface

In earlier work on the kinetics of electrochemical corrosion on the amalgam–acid solution interface, I found on the basis of experiments carried out with the radioactive tracer method that the oxygen dissolved in the acid solution has a considerable effect on the electrochemical corrosion rate<sup>1,2</sup>. In the presence of dissolved oxygen the following part-processes occur during the corrosion of the amalgamated metal:



As a result of these electrochemical part-processes a so-called mixed potential ( $E^M$ ) develops on the amalgam–acid solution interface<sup>3</sup>, where the dissolution velocity of the amalgamated metal equals the velocity of the oxygen reduction:

$$\left(\frac{dn}{dt}\right)_1^{E^M} = \left(\frac{dn}{dt}\right)_2^{E^M} \quad (3)$$

and the time profile of corrosion can be described by the following differential equation which is characteristic of a reaction of zero order:

$$\left(\frac{dn}{dt}\right)_1^{E^M} = k_1 \quad (4)$$

The following correlation exists between the specific rate constant and the half time of the reaction:

$$k_1 = \frac{C_0}{2t_{1/2}} \quad (4a)$$

where  $C_0$  is the initial concentration of the amalgam.

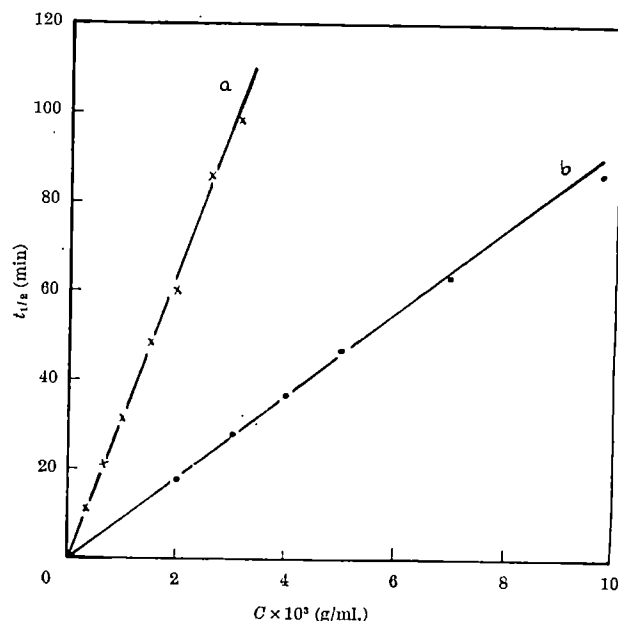


Fig. 1. Half-time of the corrosion process as a function of the initial concentration of the amalgam. Temperature: 18° C. Volume of the amalgam: 2 ml. (a) Zinc amalgam; (b) thallium amalgam

Fig. 1 shows the half times of the corrosion of zinc amalgams tagged with <sup>65</sup>Zn, and of thallium amalgam tagged with <sup>204</sup>Tl of different concentrations, in a 0.01 N H<sub>2</sub>SO<sub>4</sub> solution saturated with air, as a function of the initial concentration of the amalgam. The value of the rate constants was determined from the slope of the straight lines. These experiments support the assumption that the diffusion of the dissolved oxygen is a rate determining factor in the corrosion of amalgams. The quantity of oxygen which reaches the unit surface of amalgam in unit time can be given by Fick's 1st law:

$$\left(\frac{dn}{dt}\right)_2^{E^M} = \frac{D}{\delta}(c_a - c_s) \quad (5)$$

where  $D$  is the diffusion constant of the dissolved oxygen,  $\delta$  the thickness of the Nernst adhesion layer,  $c_a$  the concentration of the dissolved oxygen in the solution, and  $c_s$  that in the immediate vicinity of the amalgam surface. Due to oxygen reduction on the amalgam surface  $c_s = 0$ , thus from equations (3) and (5) the value of  $k_2$  can be given as:

$$k_2 = \frac{D}{\delta} c_a \quad (6)$$

$c_a$  is constant, because the same quantity of oxygen is reduced on the amalgam-acid solution interface as is dissolved in the acid solution-air interface. If the appropriate values ( $D = 1.98 \times 10^{-5}$  cm<sup>2</sup>/sec,  $c_a = 25 \times 10^{-3}$  g-mole O<sub>2</sub>/ml. and  $\delta = 5 \times 10^{-3}$  cm) are substituted into equation (6) then the value of  $k_2$  will be:

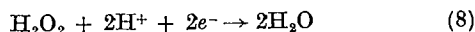
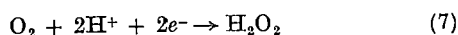
$$k_2 = 9.88 \times 10^{-10} \text{ g-mole O}_2/\text{cm}^2/\text{sec}$$

From the experimental data of Fig. 1a the value of the specific rate for the corrosion of zinc amalgam is:

$$k_1 = 34.6 \times 10^{-10} \text{ g-equiv. zinc/cm}^2/\text{sec}$$

This means that the reduction of 1 g-mole of oxygen results in the corrosion of 4 g-equiv. of zinc, thus in the corrosion of zinc amalgam the cathodic part process can indeed be described by equation (2).

It is known from the work of Kolthoff *et al.*<sup>4</sup> and, more particularly, by that of Bagotzky *et al.*<sup>5</sup>, that the reduction of O<sub>2</sub> on the mercury cathode takes place in two steps according to the following gross equations:



The intermediate product, hydrogen peroxide, formed during the reaction is thermodynamically unstable, as the oxidation-reduction normal potential of the O<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system is  $E_h^0 = +0.682$  V, and of the H<sub>2</sub>O<sub>2</sub>/H<sub>2</sub>O system  $E_h^0 = +1.77$  V. Thus the presence of H<sub>2</sub>O<sub>2</sub> can be attributed to reaction kinetical reasons: it can be identified only in oxygen reductions where the cathodic reduction on the electrodes requires high activation energies. According to the experimental data of Fig. 1b, oxygen reduction in the corrosion of thallium amalgam proceeds according to equation (7). The value of the experimentally determined rate constant is:

$$k_1 = 17.4 \times 10^{-10} \text{ g-equiv. thallium/cm}^2/\text{sec}$$

thus the reduction of 1 g-mole of oxygen results in the corrosion of 2 g-equiv. thallium.

In order to throw light on the reduction kinetics of amalgams, the time profile of the electrochemical corrosion of amalgamated metals in solutions saturated with inert gas has been examined in the presence of a number of oxidizing agents (IO<sub>3</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, methylene blue, etc.). The results of these experiments will be reported in another communication<sup>6</sup>.

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### Solvent Extraction of Xenon

SOLVENT extraction of xenon has never been reported. Two years ago, one of us (R. K.) noticed that caesium-138 (half-life 32.2 min) was extracted in di-isobutylketon (DIBK) when he was carrying out a rapid solvent extraction separation of short-lived selenium activities from uranium fission products<sup>1</sup>. The caesium-138 activity did not appear in the DIBK phase, if the hydrobromic acid solution of uranium fission products was heated or air-bubbled prior to the extraction of selenium activities into the organic solvent. This phenomenon suggests to us that xenon-138 (half-life 17 min) is extracted into the DIBK phase.

We designed the following experiment to obtain more clear and direct evidence about the possibility of organic solvent extraction of the fission xenon. Direct DIBK extraction of xenon activities from hydrobromic acid solution of uranium fission products causes a greater difficulty, because iodine activities extracted into DIBK phase seriously disturb the  $\gamma$ -ray spectra of xenon nuclides. In this investigation, carbon tetrachloride was used for the extraction of <sup>133</sup>Xe and <sup>135</sup>Xe activities from <sup>133</sup>I and <sup>135</sup>I nuclides which were separated and purified from the fission products in an iodide form.

Fifty mg of UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O was irradiated in the *Triga Mark II* reactor of St. Paul's University for 5 h at a neutron flux of  $5 \times 10^{11}$  n/cm<sup>2</sup>/sec. After cooling for one day, <sup>133</sup>I and <sup>135</sup>I were separated along with 20 mg of iodide carrier from the uranium fission products using a standard separation method for iodine<sup>2</sup>. Activities of the final solution, that is, 10 ml. of water containing a few drops of 2 M NaHSO<sub>3</sub>, consists primarily of <sup>133</sup>I, <sup>135</sup>I and their daughters (<sup>133</sup>Xe, <sup>133m</sup>Xe, <sup>135</sup>Xe and <sup>135m</sup>Xe). After standing for several hours, the aqueous phase was shaken for 1 min with 10 ml. of carbon tetrachloride by a mechanical device. The organic phase was then washed twice by shaking vigorously with a 10-ml. portion of water containing a few drops of 2 M NaHSO<sub>3</sub> for 30 sec. Carbon tetrachloride thus obtained was transferred into a polyethylene vial and its  $\gamma$ -ray spectrum was taken on the Technical Measurement Corporation multichannel analyser coupled with 3-in.  $\times$  3-in. thallium activated sodium iodide crystal.

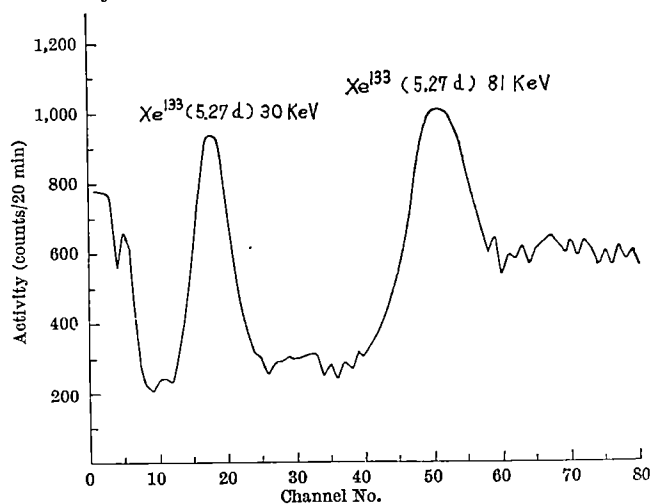


Fig. 1.  $\gamma$ -Ray spectrum of <sup>133</sup>Xe

The spectra obtained are illustrated in Figs. 1 and 2. The photopeaks were found to consist primarily of <sup>133</sup>Xe, <sup>133m</sup>Xe, <sup>135</sup>Xe and <sup>135m</sup>Xe by the  $\gamma$ -ray energies and decay rate measurements.

When carbon tetrachloride containing xenon activities was evaporated off under an infra-red lamp, the residue showed no sign of activities other than the background over the energy range tested. The result also gives additional evidence for the organic solvent extraction of



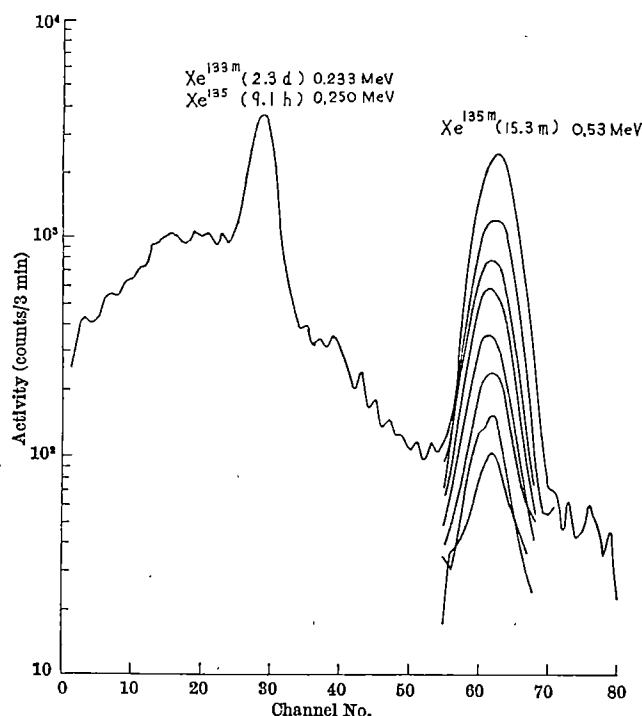


Fig. 2.  $\gamma$ -Ray spectra of  $^{135}\text{Xe}$  and  $^{135\text{m}}\text{Xe}$ . Successive spectra of  $^{135\text{m}}\text{Xe}$  were taken with an interval of approximately 10 min

xenon. This method enables us to obtain xenon tracers repeatedly from their parent iodine nuclides. Because the carbon tetrachloride extraction system provides a highly specific separation procedure for tracer quantities of xenon, it will apply directly to the separation of short-lived xenon activities from fresh fission products. Thus, the method recorded here would be of particular value for the decay scheme investigations of short-lived xenon isotopes.

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### Mechanism of Double-bond Migration catalysed by Group VIII Metal Complexes

RECENT evidence<sup>1</sup> on the palladium (II) catalysed isomerization of olefins has been interpreted as conflicting with earlier mechanisms based on the transfer of hydride<sup>2-5</sup>. Since we have invoked such a mechanism<sup>6</sup>, we should like to point out an alternative interpretation of this evidence, consistent with a hydride transfer mechanism. Our interpretation requires that an abnormally large isotope effect operates in the isomerization of deuterated olefins and that this possibility was overlooked by Davies<sup>1</sup>. Evidence is presented in support of this contention.

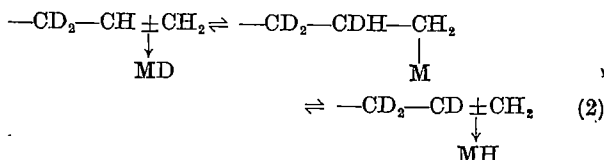
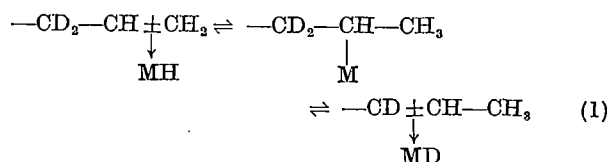
Davies isomerized 3-*d*<sub>2</sub>-octene-1 and interpreted his results as requiring a migration of deuterium or hydrogen atoms from C<sub>3</sub> to C<sub>2</sub> and from C<sub>2</sub> to C<sub>1</sub>. He then concluded

that this ruled out both a  $\pi$ -allyl mechanism and a reversible hydride-alkyl equilibrium. Instead, a mechanism involving a carbene intermediate was tentatively proposed.

It should be mentioned that another recent communication<sup>7</sup> proposes a  $\pi$ -allyl mechanism, but we have found it difficult to reconcile such a mechanism with our unpublished observation that isomerization cannot be effected with simple palladium  $\pi$ -allyl complexes under conditions where olefine complexes were effective.

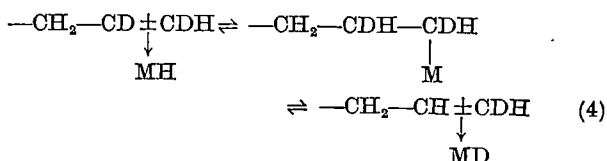
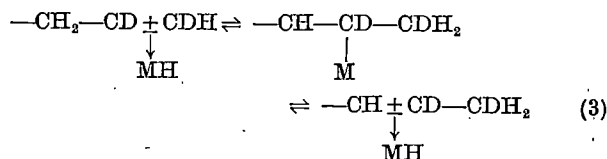
Before excluding the reversible alkyl-hydride mechanism, however, Davies's results must be considered together with the possibility of a large deuterium isotope effect, since reactions involving hydride shifts sometimes show  $k_H/k_D$  values as high as 7 (refs. 8-10).

For an olefine deuterated at the third carbon atom, as used by Davies, the reversible hydride addition mechanism may be represented thus:



Equations (1) and (2) give only a few of the total possible equilibria. Equation (1) is the isomerization step and must give rise to a metal deuteride. Equation (2) represents equilibria which do not give rise to isomerization, but which can result in exchange between metal deuteride and metal hydride. Given the assumption that terminal metalation, as represented in equation (2), is a more probable event than non-terminal metalation, which is a pre-requisite for isomerization, a large  $k_H/k_D$  for breaking carbon-hydrogen bonds will have two effects. First, deuteration at C<sub>3</sub> will lead to a reduction in the rate of isomerization and, secondly, the double-bond will appear to shift by deuterium moving from C<sub>3</sub> to C<sub>2</sub> and hydrogen from C<sub>2</sub> to C<sub>1</sub>, to an extent related to the isotope effect. The first result has not been tested, the second is in agreement with Davies's observations.

In order to obtain further evidence in support of the alkyl-hydride mechanism, we have sought to reverse the effect due to isotopic substitution found by Davies. This can be done by isomerizing hexene-1 deuterated at C<sub>1</sub> and C<sub>2</sub> only. Reactions (1) and (2) then become:



If the breaking of a CD bond is slower than that of a CH, the isotope effect would be expected to reduce the rate of equation (4) relative to equation (2) and of equation (1) relative to equation (3). Thus, in this case the rate of isomerization should be increased and the reaction should

proceed via a  $C_2$  to  $C_1$  hydride shift. Both these results were found.

The deuterated hexene-1 was prepared by the deuteration of hexyne-1 (ref. 11) and was shown by nuclear magnetic resonance to be a mixture of  $C_6H_9CD = CDH$  (64 per cent) and  $C_6H_9CD = CD_2$  (36 per cent). With both  $PdCl_2$  in glacial acetic acid and  $RhCl_3 \cdot 3H_2O$  in isopropyl alcohol the rate of isomerization of the deuterated olefine was about three times faster than that of the protonated olefine (followed by gas phase chromatography and infrared). Also, analysis of products by nuclear magnetic resonance demonstrated that no detectable movement of deuterium occurred during isomerization.

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<sup>1</sup> Davies, N. R., *Nature*, 201, 490, 1964; *Austral. J. Chem.*, 17, 221 (1964).

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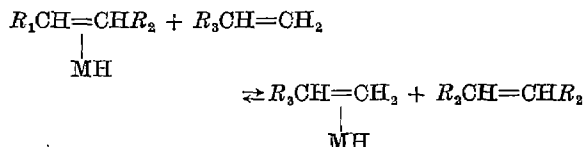
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THE mechanism proposed by Harrod and Chalk, for the palladium II chloride catalysed isomerization of olefines, involves as the effective catalyst an olefine-hydride complex of palladium of such stability that the hydride bond is preserved while the co-ordinated olefine is exchanged with free olefine:



In the earlier publication to which these authors refer<sup>1</sup>, the mechanism which was considered to have been disproved was one in which the hydride complex had a transient existence only. While, therefore, the experimental results can be explained in terms of a large isotope effect, such an explanation is based on the existence of such a relatively stable hydride. My approach differed from that of Chalk and Harrod essentially in that, despite the analogue of the cobalt carbonyl hydride, I rejected the possibility of a stable hydride complex, or, in fact, one in which the hydride originated other than from the isomerizing olefine. An isotope effect would, of course, be expected to alter the reaction rate and this was in fact observed, the rate of isomerization of the  $C_2$  deuterated olefine being markedly slower. However, by such a mechanism, even a large isotope effect would not have prevented deuterium from appearing ultimately at the terminal carbon atom.

Although the assumption that the catalyst is not converted into a stable hydride may possibly prove to be incorrect, it is a conclusion which seems reasonable in view of the evidence available. Thus the hydride complexes of palladium have been reported to be extremely unstable.  $(PdHCl)(PEt_3)_2$ , in which the hydrogen is under the stabilizing influence of two groups of high ligand field strength, could nevertheless not be prepared by methods analogous to those which led to the platinum analogue<sup>2</sup>. Furthermore, a recent study of the kinetics of isomerization of allyl benzene with a palladium II chloride catalyst has led to the conclusion that if the effective catalyst is in fact a hydride complex, then all, and not just a small

fraction, of the catalyst will be present in this form. So far, attempts to detect such hydrogen in the nuclear magnetic resonance spectrum, during the course of the reaction, have proved fruitless<sup>3</sup>. In view of the very large and unique chemical shifts which are characteristic of such hydrogen<sup>4</sup>, this would appear to be surprising if the catalyst were present in any quantity as a hydride complex.

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### Chelation of Ferrous Sulphate Solutions by Desferrioxamine B

DESFERRIOXAMINE B is a specific iron complexing agent originally obtained from an iron-bearing metabolite of *Actinomyces* (*Streptomyces pilosus*)<sup>1,2</sup>. It is available for clinical use as 'Desferal'® (desferrioxamine B methane-sulphonate) and has undergone extensive evaluation in the treatment of chronic iron overload states<sup>3</sup>. The compound, a trihydroxamic acid derivative, has a strong affinity for ferric ions (stability constant  $\log K = 30.6$ ) with which it reacts to form a complex known as ferrioxamine, but it is reported to form relatively little complex with ferrous ions<sup>4</sup>. During the course of investigations involving the use of desferrioxamine B in the treatment of acute iron poisoning in dogs<sup>5</sup>, it was necessary to determine the extent to which ferrous ions form a complex with the chelate. In this communication we wish to present preliminary results relative to the ability of desferrioxamine B to complex with ferrous iron.

The complex formed when ferrous ions are reacted with desferrioxamine B appears to be the same as when ferric ions are reacted. The evidence is as follows: (1) Ferrous ions react with desferrioxamine B in a 1 : 1 molar ratio as estimated by the continuous variation method of Job<sup>6</sup> as modified by Vosburgh<sup>7</sup>. Ferric ions react in a similar manner. (2) The spectral absorption curve (220–700 mμ) of the complex formed when ferrous sulphate and desferrioxamine B are reacted (in the presence of ascorbic acid) is identical to that obtained when ferric perchlorate and desferrioxamine B are reacted (Fig. 1). (3) The molecular extinction coefficient of the iron–desferrioxamine complex formed by reacting either ferrous or ferric ions with desferrioxamine B was 2,480. These measurements were made using a 0.05 M *tris* buffer (pH 7 at 37° C) with a Beckman model DB spectrophotometer. (4) The complex formed by reacting either ferrous sulphate ( $2 \times 10^{-4}$  M in 1 per cent ascorbic acid) with desferrioxamine B ( $2 \times 10^{-4}$  M) or ferric chloride ( $2 \times 10^{-4}$  M) with desferrioxamine B ( $2 \times 10^{-4}$  M) yielded ferric sulphate when reacted with sulphuric acid at a ratio of 6 : 1 (Fig. 2).

The following evidence suggests that the reaction between ferrous ions and desferrioxamine B involves an oxidation to the ferric state prior to complexation: (1) No spectrophotometric evidence (220–760 mμ) for the formation of a complex occurred when ferrous sulphate and desferrioxamine B were reacted under oxygen-free conditions. An oxygen-free medium was accomplished by bubbling nitrogen which had been previously passed through a saturated pyrogallol solution through the ferrous sulphate (in 1 per cent ascorbic acid) and the desferrioxamine B solutions for 10 min prior to, and after, mixing them. Subsequent exposure of this mixture to air or oxygen led to the formation of the ferric complex.

\* Trade name: CIBA Pharmaceutical Co., Summit, New Jersey.

Table 1. EFFECT OF pH ON THE REACTION OF FERROUS SULPHATE WITH DESFERRIOXAMINE *B* METHANE SULPHONATE IN THE PRESENCE OF ATMOSPHERIC OXYGEN AT 25° C

pH	4.28	5.30	5.80	6.70	7.20
Velocity constant ( $K_1$ )	0.0158	0.226	2.585	4.665	6.860
$t^{1/2}$ (min)	43.73	3.07	0.268	0.149	0.010

The calculations were based on equations for first-order kinetics.

(2) The reaction of either ferrous or ferric ions with desferrioxamine *B* was not affected by the presence of the reducing agents, ascorbic acid, sodium sulphite, and hydroxylamine hydrochloride. However, mercapto-acetic acid, sodium thiosulphate and sodium dithionite prevented the reaction of ferrous and ferric ions with desferrioxamine *B* in the presence of oxygen at pH 7. Normally, all six reductants are capable of reducing and maintaining iron in the ferrous state. However, thiosulphate, dithionite and mercapto-acetate are the more powerful reductants.

Further investigations indicate that the reaction between equimolar concentrations of ferrous ions and desferrioxamine is pH dependent as is the reaction between ferric ions and desferrioxamine (Fig. 3). An acetic acetate buffer was used to establish pH values below 7. A *tris* buffer was used to establish pH values of 7 and above. The reaction mixtures were incubated at 37° C for 20 min prior to the estimation of the ferrioxamine concentration. As shown, the reaction between ferrous ions and desferrioxamine goes to completion at pH values above 5 ( $I = 0.05$ ), whereas the reaction between ferric ions and desferrioxamine goes to completion below a pH of 5. Possible explanation for the higher degree of complexation of ferrous salts at pH values above 5 is that at high pH values the oxidation of ferrous ions to the ferric form is accelerated. Less than maximum complexation of ferric ions and desferrioxamine *B* occurs at high pH values because fewer ferric ions are free to react with desferrioxamine because of hydration and the subsequent strong tendency towards the formation of insoluble hydroxides.

Maximum formation of ferrioxamine was achieved at a low pH when the molar ratio of ferrous sulphate to desferrioxamine was 8:1 or the molar ratio of desferrioxamine to ferrous sulphate was 7:1. The reaction rate of

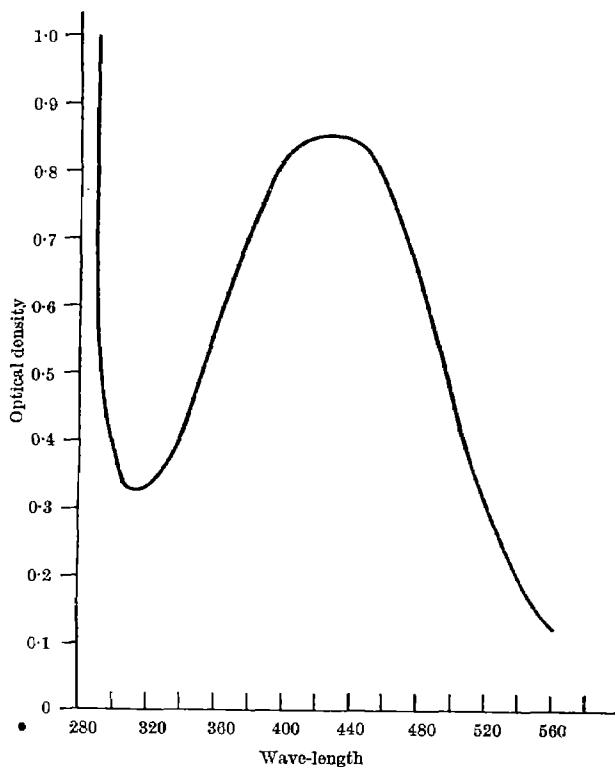


Fig. 1. Spectral absorbance curve for ferrioxamine ( $2 \times 10^{-4}$  M) formed by reaction of ferric perchlorate with desferrioxamine

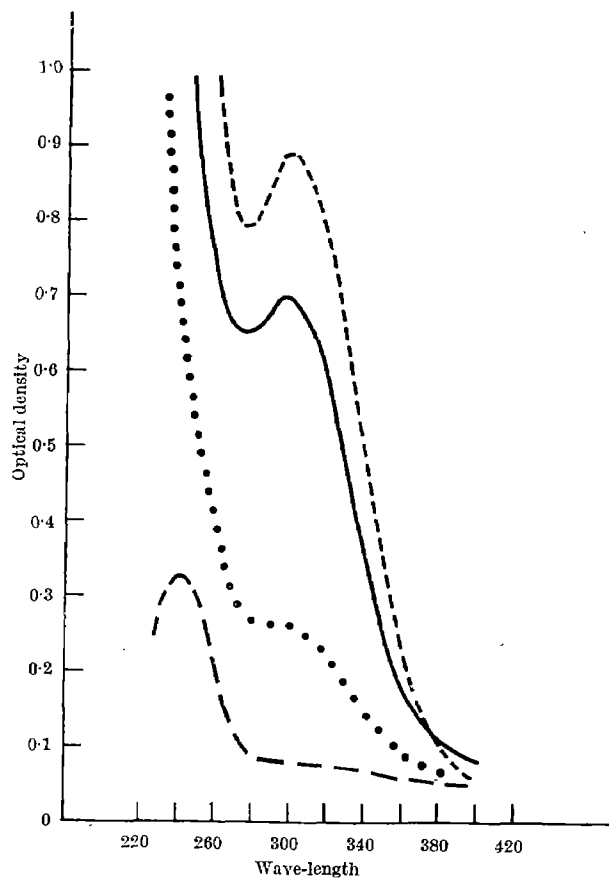


Fig. 2. Spectral absorbance curves of products formed by the reaction of ferrioxamine and iron salts with  $H_2SO_4$ . ...,  $Fe^{++}$  formed complex +  $H_2SO_4$ ; ---,  $Fe^{+++}$  +  $H_2SO_4$ ; —,  $Fe^{+++}$  formed complex +  $H_2SO_4$ ; — — —,  $Fe^{++}$  +  $H_2SO_4$ .

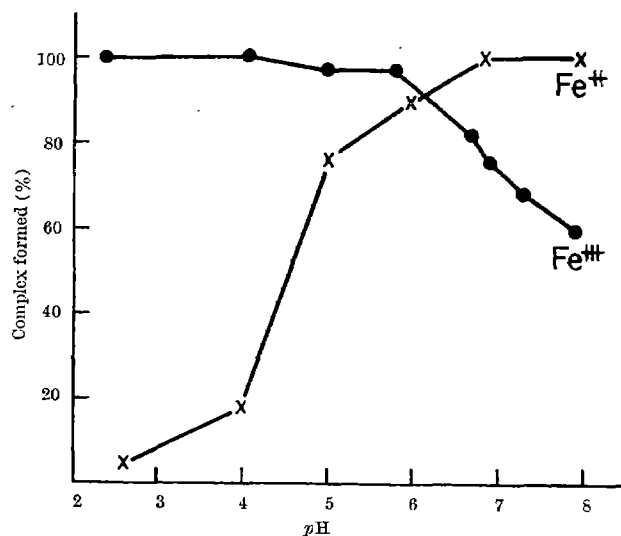


Fig. 3. Effect of pH on the formation of ferrioxamine from  $Fe^{++}$  and  $Fe^{+++}$  salts

ferrous sulphate with desferrioxamine *B* is increased over 4,000-fold with an increase in pH from 4.28 to 7.20 (Table 1). (Equations were used for first-order kinetics.)

The foregoing results strongly suggest that desferrioxamine *B* is highly specific for ferric ions and that it reacts with ferrous ions normally under conditions which permit or facilitate prior oxidation to the ferric form. However, the possibility does exist that a colourless unstable ferrous complex is formed between ferrous ions and



desferrioxamine B and that in the presence of oxygen the ferrous complex is rapidly oxidized to the ferric complex (ferrioxamine). In either case, it appears that this oxidation is catalysed by desferrioxamine B and the catalysis is inhibited by only the more powerful reducing agents.

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## BIOPHYSICS

### Small-angle X-ray Diffraction on Cross-striated Muscle

As an object for testing circular aperture systems for small-angle cameras<sup>1</sup> we used dried bovine skeletal muscle. The main features of the test photographs seem to us worth reporting as, so far as we are aware, some of them have not been reported before.

In Fig. 1 the small-angle diagram obtained from bovine skeletal muscle is shown schematically (fibre axis vertical, perpendicular to direction of the primary beam, copper-K $\alpha$ -radiation, 50 kV, 20 m.amp, 7  $\mu$ m nickel foil, about 50 h exposure time).

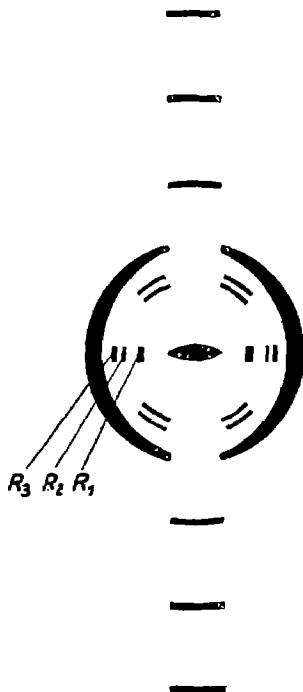


Fig. 1. Small-angle diagram of the dried skeletal muscle of ox (schematic)

There are three fairly sharp reflexions  $R_1$ ,  $R_2$ ,  $R_3$  with intensities strong, weak, medium corresponding to spacings  $d_1 = 60.0 \pm 0.2$  Å;  $d_2 = 51.4 \pm 0.2$  Å;  $d_3 = 49.2 \pm 0.2$  Å respectively. The fact that these reflexions do not spread out perpendicular to the equator indicates that the objects responsible for them must be almost perfectly aligned with respect to the fibre axis.

At a somewhat bigger angle there is a sickle-shaped reflexion the maximum of intensity of which is on the equator and comparable with that of  $R_1$ . Its position corresponds to a spacing of about 40 Å, but varies from one sample to the other by about  $\pm 2$  Å. The value 40 Å is that most frequently obtained. The considerable length of the sickle indicates that the objects giving rise to it are aligned rather badly about the fibre axis as the preferred orientation.

On the meridian 10 orders corresponding to a period of 54 Å show up. The first order is, however, split into four points, each of which is split further into a pair of closely neighbouring reflexions. This is in agreement with a diagram obtained by Cohen and Hanson<sup>2</sup> from dried fibres of F-actin. According to Bear<sup>3</sup> the existence of an essentially larger period (of 350–420 Å) may be concluded from variations of the value of the 54 Å-spacing as deduced from the different orders. From precise measurements of the positions of the 10 registered orders we obtained a value of  $406 \pm 5$  Å for this large period.

In diagrams of dried muscle a rather intense anisotropic continuous small-angle scattering occurs close to the primary beam (Fig. 1). This extends over a considerably larger range perpendicular to the fibre direction than parallel to it. As the small-angle scattering gives a reciprocal image of the scattering particles, it may be concluded that in dried muscle small-angle scattering is due to oblong particles with their main extension essentially parallel to the fibre axis. A double logarithmic plot of the scattering curve—as suggested by Porod<sup>4</sup>—is in agreement with this assumption. This has in its central portion a straight part with slope  $-1.4$ , indicating that the scattering particles have the shape of long rodlets. A quantitative discussion of the scattering curve would, however, not be justified.

A search of reflexions corresponding to the various orders of a 420 Å period as observed by Huxley on living muscle was without result, presumably because they become swamped by the intense continuous small-angle scattering.

Finally, we wish to put on record some differences of the X-ray diagrams obtained from muscle allowed to swell in distilled water after drying as compared with the dry muscle.

Small-angle scattering recorded with a camera with linear slit system has a peak corresponding to a spacing of 180 Å superimposed on the continuous scattering. The diffuse reflexion which for the dried muscle corresponds to a spacing of 4.6 Å is shifted in the soaked muscle to 3.35 Å. The meridional 5.14 Å and 1.49 Å reflexions of the wide angle diagram of dried muscle appear not to be influenced by swelling, whereas the equatorial 9.8 Å reflexion of dried muscle is shifted to 11 Å after swelling.

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## BIOCHEMISTRY

## Measurement of Chenodeoxycholic Acid and Deoxycholic Acid or their Derivatives in a Mixture

THE measurement of the dihydroxycholic acids found in human bile (chenodeoxycholic acid,  $3\alpha,7\alpha$ -dihydroxycholic acid and deoxycholic acid,  $3\alpha,12\alpha$ -dihydroxycholic acid) or of their ester derivatives or conjugates with glycine or taurine has always presented difficulties where the original material to be analysed contains both acids or derivatives of them. Before 1953, the only method available depended on the repeated precipitation of the barium salt of chenodeoxycholic acid. Its value may be gauged from the fact that until this time chenodeoxycholic acid was considered to be a very minor constituent of human bile, a belief shown to be erroneous by Wootton<sup>1</sup> in 1953 when he separated methyl cholate from the two dihydroxy methyl esters by a column chromatographic procedure which utilized silica gel as the supporting medium and pentane/hexane and petroleum ether as the developing solvents. The proportions of methyl chenodeoxycholate and methyl deoxycholate in a mixture could be readily estimated by an investigation of the infra-red spectra of solutions in carbon disulphide. By these means, Wootton demonstrated that chenodeoxycholic acid was, in fact, a major constituent of human bile. The method is relatively insensitive compared with later methods of bile acid measurement which have been devised, requiring about a milligram of the mixed dihydroxy compounds to ensure reasonable accuracy of estimation of the proportion of the two esters.

Later, Sjövall<sup>2</sup> found that by using *iso*-propyl ether:heptane (20:80) as the mobile phase and 70 per cent acetic acid as the stationary phase, separation of the two dihydroxy acids on paper was obtained after the system had operated for 18 h. Pure heptane was also found to be suitable as the mobile phase, effective separation being obtained after 72 h. Such a system was found to be capable of handling quantities of the order of several micrograms. The glycine conjugates of chenodeoxycholic and of deoxycholic acids were also reported by Sjövall to have been separated by paper chromatography using *iso*-propyl ether:heptane (80:20 or 85:15) as the mobile phase and 70 per cent acetic acid as the stationary phase. However, no separation could be

achieved between the taurine conjugates of the two dihydroxy bile acids. In the work reported by Sjövall, estimation of the different bile acids was carried out by measurement of the ultra-violet adsorption in 65 per cent aqueous  $H_2SO_4$  of the acids eluted from the paper.

The fluorescence of the bile acids and their derivatives in concentrated  $H_2SO_4$  has been known for many years, the fluorescence of cholic acid in  $H_2SO_4$  being reported by von Hammarsten<sup>3</sup> in 1922. However, it was not until many years later that Turner *et al.*<sup>4</sup>, Wootton and Osborn<sup>5</sup> and Osborn<sup>6</sup> reported in detail the fluorescent properties of the major human bile acids and their derivatives in  $H_2SO_4$  under various conditions of development of fluorescence with respect to temperature and time.

A method based on the fluorescence properties of methyl chenodeoxycholate and methyl deoxycholate, involving the measurement of the fluorescence of a mixture in  $H_2SO_4$  at 4° C and 100° C, enables the proportions of each in a mixture to be accurately determined at concentrations as low as 0.5  $\mu g/ml$ .  $H_2SO_4$ . The factor increase in fluorescence from 4° C, after standing at this temperature for 1 h, to that obtained after subsequent heating at 100° C for 20 min, is about 9 for methyl chenodeoxycholate, whereas with methyl deoxycholate the factor increase is about 33. Intermediate rises in fluorescence are shown by mixtures of the two compounds in varying proportions, when similarly treated (Fig. 1). When this method is used for analysing a mixture of the two dihydroxy compounds, it is unnecessary to determine the factor increase in fluorescence for other than the pure substances, since the necessary intermediate points on the graph may be obtained by assuming the fluorescences are additive.

It is necessary when working over such a wide range of fluorescence to use two quinine sulphate reference standards. The fluorescence developed at 4° C, 1 h, is measured against the lower standard and that at 100° C, 20 min, against the second standard which is ten times as concentrated as the lower one. Once the relative proportions of chenodeoxycholate and deoxycholate in a mixture have been determined, the absolute amounts of each may be estimated by preparing a mixed standard of the two in the same ratio, fluorescence being induced by heating an  $H_2SO_4$  solution at 100° for 20 min.

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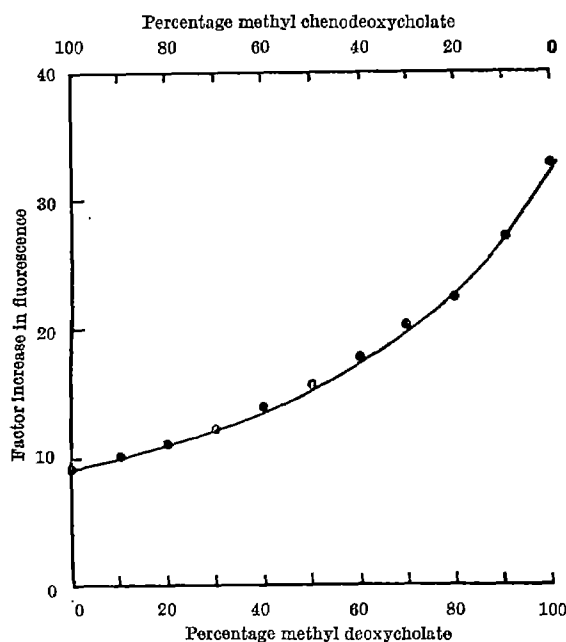


Fig. 1

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## Amino-acids on Hands

RECENT methods for the determination of amino-acids by ion-exchange chromatography at the  $10^{-8}$  and  $10^{-9}$  mole levels<sup>1,2</sup> are now able to give a quantitative measure of observations that have hitherto been of a qualitative nature<sup>3,4</sup>. For example, it is well known that finger-marks on paper chromatography are to be avoided because they are deeply coloured on treatment with ninhydrin reagent. It is probably less well appreciated that amino-acids might be introduced, some considerably in excess of the  $10^{-8}$  mole level, at any or all steps of preparative manipulation prior to chromatography, and these contaminants would then be unwittingly measured along with the material of analytical interest.

Work that necessitated very rigid exclusion of accidental contamination led to the following experiments. (1) A

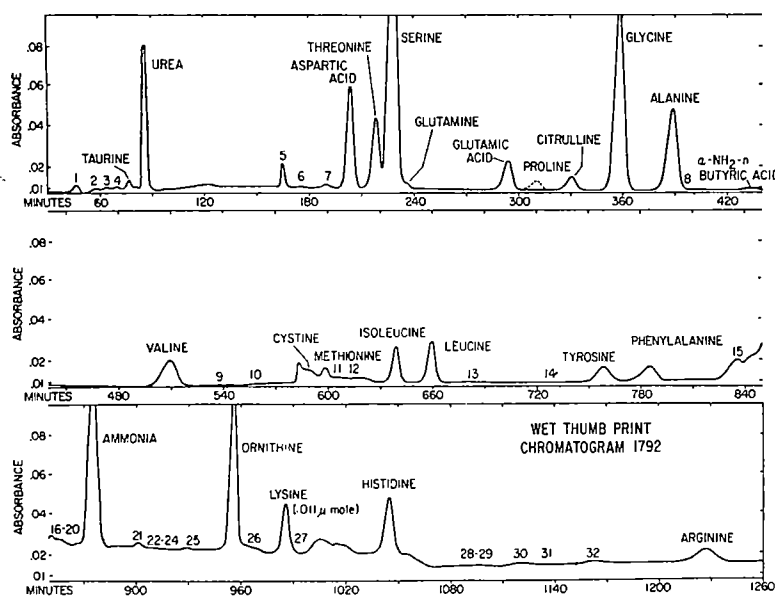


Fig. 1. Amino-acids found by ion exchange chromatography in a single thumb print made on a wet glass surface. For visual comparison of peaks, the amount of lysine (0.011  $\mu$ mole) is indicated on the figure. On the abscissa, 1 min is equivalent to 0.5 ml. of column effluent volume; column diameter, 0.636 cm; volumetric input to column, 30 ml. per h

dry beaker (25 ml.) received a single print from a dry thumb, the hands having been washed 2 h previously; average casual laboratory conditions were thus approximated. The print was then washed into a flask with water, the water was evaporated and the dried residue was transferred with 0.1 N HCl to an ion exchange column. Analysis was carried out by means of the fully automatic procedure of Hamilton<sup>1</sup>, which uses a single column of high resolving power, packed with narrowly classified spherical particles of 17.5  $\mu$  diameter of Dowex 50  $\times$  8 (refs. 5-7). In this chromatogram (not shown) 17 constituents were revealed of the order of  $5 \times 10^{-9}$  to  $2 \times 10^{-10}$  moles; proline, citrulline,  $\alpha$ -amino-*n*-butyric acid, cystine and methionine were missing. (2) A beaker rinsed with water to wet the walls received a single thumb print. This print was analysed as described. The chromatogram obtained is shown in Fig. 1, and the amounts of amino-acids that were found are shown in Table 1.

Table 1. AMINO-ACIDS OF A SINGLE WET THUMB PRINT

Amino-acid	$\mu$ moles	Amino-acid	$\mu$ moles
Taurine	0.001	Valine	0.013
Urea	0.470	Cystine	<0.0001
Aspartic acid	0.023	Methionine	0.002
Threonine	0.018	Isoleucine	0.008
Serine	0.106	Leucine	0.011
Glutamic acid	0.009	Tyrosine	0.006
Proline	0.011	Phenylalanine	0.007
Citrulline	0.004	Ammonia	Off scale
Glycine	0.071	Ornithine	0.034
Alanine	0.029	Lysine	0.011
$\alpha$ -amino- <i>n</i> -butyric acid	<0.0001	Histidine	0.018
		Arginine	0.005

Several observations can be made. Of the amino-acids, the serine peak was the largest; asparagine is not resolved from serine in this system of analysis and its presence or absence was not established. It is interesting to note that Embden and Tachau<sup>8</sup> isolated and identified serine from sweat in 1910. A very small shoulder on the rear of the serine peak was probably due to glutamine; its identity was not, however, established. Glycine was the next most abundant amino-acid. Aspartic acid was present in much greater relative amounts than in normal plasma. Citrulline and ornithine were also present in relative amounts somewhat greater than that usually encountered in plasma. Since these two amino-acids, as demonstrated here on skin surface, are not found in ordinary proteins,

their presence together in materials (other than body fluids) may suggest, but certainly does not prove, possible finger contamination. Cystine was virtually absent; it was detected with certainty only in very concentrated finger washings. Methionine was present in very low concentration; beta-alanine was absent. In general, these findings were similar for the finger washings of three other subjects, one of non-laboratory personnel.

In the final experiment, the fingers and palms of both hands were washed in 200 ml. of water and the washings were concentrated to 1 ml. volume; 0.5 ml. of the concentrate was analysed. The common amino-acids were very abundant and many other ninhydrin positive unknown substances became apparent. The largest of these unknown peaks is numbered 1 to 32 on the chromatogram; in addition to these, 10 other lesser peaks were noted but are not shown. No. 10 plus the small precystine peak and 15 and 16 denote base line changes associated with solvent front phenomenon due to buffer changes.

After hydrolysis for 24 h in boiling 6 N HCl under reflux in a stream of nitrogen, the amounts of aspartic acid, serine, threonine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine were increased. This result was to be expected as the washings were not filtered to remove minute scales that might have been shed from the skin, or bacteria. The method is capable of detecting the hydrolytic products from 1  $\mu$ g of protein. Hydroxyproline was absent from the hydrolysate. After hydrolysis the amounts of taurine,  $\alpha$ -NH<sub>2</sub>-butyric acid, and ornithine were unchanged, while urea and citrulline were somewhat reduced. Of the numbered peaks, 1, 20, 21 and 26 were essentially unchanged by hydrolysis; 14 was increased; 5, 7 and 30 were greatly diminished; the rest disappeared. Presumably, many of these are low molecular weight peptides or other bound forms of amino-acids.

These results show that hand contamination of specimens could lead to erroneous findings. From a medical or clinical point of view, they suggest a further enquiry into the excretion or secretion of amino-acids by the skin, in health and disease; some similarity between skin and kidney, both ectodermal in origin, might be expected. The possibility that internal metabolic amino-acid abnormalities might be reflected in amino-acid distribution on the skin has not apparently been explored. The methods usually used to collect urine from very young infants may be subject to error due to skin contamination, as would blood samples from finger, heel or ear pricks.

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## Influence of D<sub>2</sub>O on Resistance of Plant and Animal Cells, Cellular Models and Actomyosin to some Denaturing Agents

ACCORDING to the denaturation theory of injury<sup>1,2</sup> the response of a cell to the effect of various agents is determined by conformational changes induced in macromolecules of protoplasmic proteins. In seeking to define conditions which would increase the resistance of various proteins to denaturing influences and to determine their effect on the stability of living cells, our attention was directed to the influence of D<sub>2</sub>O on proteins. Deuterization increases the resistance to heating in certain proteins, such as ribonuclease<sup>3</sup>, gelatin<sup>4</sup>, collagen<sup>5</sup> and keratin<sup>6</sup>. We investigated the effect of D<sub>2</sub>O on resistance to heating and high hydrostatic pressure in plant (leaf epidermis of *Campanula persicifolia* L.) and animal (ciliated epithelium of the palate in the frog *Rana temporaria*) cells on one hand, and in protein preparations (muscle actomyosin of frogs and glycerin models of frog ciliated cells<sup>7</sup>) prepared according to Hoffmann-Berling<sup>8</sup>, on the other. The following resistance indices of cells and proteins were studied in the media containing D<sub>2</sub>O (test) or H<sub>2</sub>O (control): (a) the temperature at which 5-min heating irreversibly stopped protoplasmic streaming in plant cells; (b) the hydrostatic pressure which, after 5 min, stopped protoplasmic streaming in the same cells; (c) the minimal pressure which, after 5 min, stopped protoplasmic streaming in the plant cells; (d) the temperature at which ciliary movement of the frog cells ceased after 5 min or 1 sec heating; (e) the hydrostatic pressure which stopped ciliary movement after 5 min; (f) the degree of suppression of actomyosin ATPase activity after 15-min heating at 40° C; (g) the degree of suppression of actomyosin ATPase activity after 15 min at 1,300 atm.; (h) the temperature at which, after 5 min, the movement of glycerin models of ciliated epithelium in the presence of ATP is prevented. We also investigated the influence of the duration of the contact between D<sub>2</sub>O and cells, cellular models and actomyosin, on their resistance to heating and high hydrostatic pressure. The results were as follows:

(a) The thermostability of plant cells proved to be considerably higher in D<sub>2</sub>O than in H<sub>2</sub>O: the criteria being met at 52.7°–53.0° C in the controls, and 55.4°–55.7° C in the tests (99 per cent D<sub>2</sub>O). The increase in heat resistance was independent of whether the cells were kept in D<sub>2</sub>O for 4–5 h or for only 5 min before heating.

(b) Deuterization increases the resistance of plant cells to high hydrostatic pressure. In 99 per cent D<sub>2</sub>O protoplasmic streaming was irreversibly stopped only when the cells were subjected to 2,300 atm., compared with 2,000 atm. in controls. The difference of 300 atm. is statistically significant.

(c) In D<sub>2</sub>O a complete but reversible stoppage of protoplasmic streaming in the same cells occurred after 5 min at a pressure of 1,680 atm., and in H<sub>2</sub>O after the action of 1,500 atm. The difference is statistically significant.

(d) Living ciliated cells were investigated in Ringer solution with H<sub>2</sub>O (RH<sub>2</sub>O) or D<sub>2</sub>O (RD<sub>2</sub>O 99, 87 or 43 per cent). The temperature at which ciliary movement ceases after 5 min is constant for each species. For *R. temporaria* it proved to be 41.8°–42.0°. 87 per cent RD<sub>2</sub>O increased this value by 1.8°–2.2°. Variation in the duration (0–24 h) of preliminary contact between cells and D<sub>2</sub>O before heating does not significantly affect the degree of stabilization. Moreover, even after a 1-sec heating of ciliated cells in 99 per cent RD<sub>2</sub>O without preliminary soaking, that is, when the contact of cells with D<sub>2</sub>O lasted only 1 sec, their thermostability proved to be higher than after a 1-sec heating in RH<sub>2</sub>O. In RH<sub>2</sub>O ciliary movement ceases after a 1-sec heating at 52.1° and in 99 per cent RD<sub>2</sub>O, at 54.2° C. If samples of mucosa are kept for 4 h in 99 per cent RD<sub>2</sub>O before 1-sec heating, the stoppage of ciliary movement occurs at the very similar temperature of 54.8° C.

(e) The resistance of ciliated cells to hydrostatic pressure is increased in RD<sub>2</sub>O. In RH<sub>2</sub>O ciliary movement is suppressed by 5 min at 1,650–1,700 atm., and in RD<sub>2</sub>O at 1,850–1,900 atm. Variation in the duration of preliminary contact between cells and D<sub>2</sub>O before the injurious action of pressure (from 0 to 24 h) does not influence the magnitude of the stabilizing effect. The effect of stabilization decreases when D<sub>2</sub>O concentration in the medium falls from 87 per cent to 43 per cent.

(f) Heating actomyosin at 40° C for 5 min causes a 63 per cent repression as compared with the initial value. The same heating in the medium containing 50 per cent D<sub>2</sub>O causes a 25 per cent repression of actomyosin activity.

(g) After 15 min action at a hydrostatic pressure of 1,300 atm., actomyosin activity was decreased by 74 per cent. Similar treatment of actomyosin in the media containing 50 per cent D<sub>2</sub>O caused only a 43 per cent decrease of activity.

(h) The resistance of ciliated epithelium models to heating for 5 min in solutions containing D<sub>2</sub>O (0.12 N KCl in 87 per cent D<sub>2</sub>O) increases from 39.4° in the control (heated in 0.12 N KCl in H<sub>2</sub>O) to 41.9° in the test. Decreasing the D<sub>2</sub>O concentration down to 45 per cent reduced the protective effect.

Detsherevsky and Kornienko<sup>9</sup> showed that the temperature which induced heat contracture of frog muscles increased by 5.6° C if H<sub>2</sub>O was replaced by D<sub>2</sub>O in Ringer solution. Variation in the time of D<sub>2</sub>O action from 5 min to 18 h did not affect the result. An increase in resistance was detected 60 sec after the immersion of muscles in the media containing D<sub>2</sub>O.

It has been shown that D<sub>2</sub>O protects cells of *E. coli* from γ-rays<sup>10</sup>. Poliovirus was found to endure the super-optimal temperature of 40° C better in D<sub>2</sub>O than in the medium with H<sub>2</sub>O<sup>11</sup>. Thus D<sub>2</sub>O increases the resistance of isolated proteins and of living cells to different injurious agents. This protective effect is not universal: in our experiments D<sub>2</sub>O did not change the resistance of plant cells to ethanol.

It is difficult to define the mechanism of this protective action of D<sub>2</sub>O. Some investigators are prone to explain the stabilizing effect of D<sub>2</sub>O on proteins by the replacement of hydrogen atoms in H-bonds in protein molecules by stronger D-bonds resulting in a greater stability of the native configuration of molecules. Our data indicating the independence, or relative independence, of the stabilizing effect of cells and proteins from the duration of their contact with D<sub>2</sub>O seem to contradict this. The replacement of H in H-bonds in protein molecules by D under normal conditions requires many hours. In these experiments, however, contact with D<sub>2</sub>O took place in the presence of denaturing agents. It seems quite probable that in such conditions deuterium exchange proceeds more rapidly and contributes to the stabilization in resistance to injury.

On the other hand, the replacement of H<sub>2</sub>O by D<sub>2</sub>O involves some changes in the structure of the water itself and in the dissociation constant of dissolved substances. This may affect many bonds, providing for a secondary, tertiary and quaternary structure of protein molecules and thus changing their stability to denaturing agents. A similar effect of D<sub>2</sub>O on the resistance of isolated proteins and living cells to injury is a new argument in support of the suggestion that the resistance of cells to various injurious agents is determined by the stability of the configuration of the protein molecules of the protoplasm. The stabilizing effect of D<sub>2</sub>O on cells and proteins goes with the capacity of D<sub>2</sub>O to repress the physiological activity of cells. There are, in the literature, numerous references to the repressive effect of D<sub>2</sub>O on the growth, reproduction and other manifestations of the metabolic activity of cells. In our experiments on the action of D<sub>2</sub>O we frequently observed a decrease in the rate of protoplasmic streaming in plant cells and of ciliary movement in animal cells. In 15 per cent D<sub>2</sub>O (before the

beginning of the enzymatic reaction the protein was kept for several minutes in the medium containing 50 per cent  $D_2O$  ATP activity of actomyosin was decreased by  $12.9 \pm 4.2$  per cent. The comparison of these facts permits the suggestion that the stabilizing effect of  $D_2O$  on the molecular structure of cell proteins is the basis of the suppressive effect of  $D_2O$  on vital functions of cells. At the present time the significance of conformational changes of proteins for the normal life of cells becomes more and more evident<sup>12,13</sup>. Decrease in the flexibility of molecules would be expected to inhibit their biochemical activity and, at the same time, increase their resistance. There is evidence to confirm that increased sensitivity of cells goes with a high level of metabolism. This idea forms a basis of Childe's theory about physiological gradients. Similar relationships were more than once observed in our laboratory when experimenting with cold and heat hardening of plant cells<sup>14</sup>.

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### Potential Significance of Free Taurine in the Diet

In previous investigations it has been shown that free taurine is present in the tissues of both invertebrate and vertebrate species<sup>1,2</sup>. However, published taurine analyses of specific food items or animal tissues, used as food, have been extremely limited<sup>3,4</sup>. Various reasons may be put forward to account for this lack of information. First,

in man, taurine has not been shown to have an active function, other than that of conjugation with bile-acids<sup>5</sup>. Secondly, more than forty years ago, Schmidt showed that the oral administration of taurine to human subjects resulted in the prompt urinary excretion of 60-87 per cent of the taurine sulphur in the organic form. It was presumed that the organic sulphur-containing constituent in the urine was free taurine<sup>6</sup>. Thirdly, until this investigation, the only reported toxic effect of taurine occurred in rabbits; while in man and other animals it has been shown to be non-toxic even in high dosage<sup>7,8</sup>.

During present examination of the effects of sulphur-containing compounds, from dietary sources, on patients suffering from psoriasis, it has been found that taurine induces a predictable response. After 2-g oral doses of taurine, intense itching occurs in about 1 h, and this persists for a variable time, usually 24-28 h. If skin lesions are present at the time of the test, these increase in redness and commence to scale profusely about 18 h after the taurine has been given. However, the initial itching response is independent of the extent of the dermatosis and may be elicited even in those in complete remission. When normal control subjects receive the test dose of taurine, no itching or other adverse effect has been demonstrated.

These clinical observations suggested that taurine had some function in the maintenance and possibly in the induction of the psoriatic state. It was conceived that in addition to the taurine produced endogenously by the catabolism of methionine and cystine<sup>9</sup>, free taurine might be ingested continuously in the diet. This line of deduction prompted an analysis of foods for their taurine content. Initially the investigation was limited to animal protein sources, but it is now being extended to include vegetable protein foods.

Preparation of the foods consisted in separation of the edible portion and dissection removal of fat from meats. The wet weight of each solid item was determined, and it was then homogenized with de-ionized water, using a Waring blender with a metallic semi-micro attachment. During the homogenization procedure, 10 ml. of de-ionized water was added for each 5 g of solid and the mechanical disruption was continued for 5 min. Aliquots of 20 ml. of the homogenate were de-proteinized by the Somogyi method, centrifuged and filtered through Whatman No. 42 paper. Selective passage of the zwitterion of taurine through both an anion and a cation exchange resin has been used as a basis for the subsequent assay. 4-ml. portions of the filtrate were passed through chromatographic columns, 30 cm x 10 mm in size, containing 'Dowex 50W-X4' (200-400 mesh) 4 ml. in the hydrogen form and 'Dowex 1-X8' (200-400 mesh) 2 ml. in the chloride form. The preparation of the columns was carried out according to Garvin's specification<sup>10</sup>. Each column was washed with two 1-ml. portions of de-ionized water. Quantitative analysis of taurine in the effluent was performed by Sörbo's method; utilizing the colour reaction between taurine and ninhydrin<sup>11</sup>. Each

Table 1. TAURINE CONTENT OF SELECTED FOODS  
(mg/100 g of edible portion as wet weight)

Item	Content of uncooked samples		Retention in baked samples		Retention in boiled samples		No. of samples analysed
	Mean	Range	Mean	Range	Mean	Range	
Beef (round)	36.2	15.0-47.2	13.3	9.6-12.5	6.0	5.8-6.3	9
Beef (liver)	19.2	14.4-27.0	14.1	6.8-18.4	7.3	3.6-9.5	9
Beef (kidney)	22.5	18.0-24.7	13.8	13.0-14.4	7.6	6.8-8.8	9
Lamb (leg)	47.3	44.6-51.0	25.7	22.0-28.4	12.6	9.1-18.4	11
Lamb (kidney)	23.9	12.8-44.0	15.4	8.1-29.0	5.1	4.7-5.5	8
Pork (loin)	49.6	39.4-69.0	21.9	7.0-10.0	11.8	9.1-18.4	9
Pork (liver)	16.9	11.0-22.8	8.5	12.6-39.0	4.3	3.0-5.4	12
Chicken (leg)	33.7	30.0-38.0	22.9	14.0-31.0	8.2	7.1-18.0	6
Cod (frozen)	31.4	23.3-39.6	29.4	26.0-32.8	16.1	12.5-19.8	16
Oysters (fresh)	69.8	39.0-123.8	26.4	21.7-30.8	8.9	5.9-12.2	10
Clams (fresh)	240.0	145.0-370.0	101.7	58.7-170.0	44.6	26.4-79.4	3
Milk* (homogenized)	15.1	10.4-20.0					

\* Liquid samples: taurine content expressed as mg/100 ml.

Table 2. LEACHING OF TAURINE FROM FOODS BY BOILING

Item	Sample No.	Content of uncooked food	Retention in boiled samples	Losses in cooking fluid
Beef (lean round)	1	46.4	5.8	20.4
	2	47.2	0.3	20.4
Pork liver	1	22.8	5.4	25.9
	3	11.0	3.0	8.4
Lamb kidney	1	15.0	5.5	2.3
	2	12.8	4.7	2.5
Oyster	1	123.0	11.4	195.0
	6	76.0	16.0	92.0
	7	88.0	9.0	84.0

The taurine content of the raw and boiled foods is expressed as mg/100 g of edible portion—wet weight. The losses in the cooking fluid refer to mg from 100 g of food boiled in 100 ml. of water for 15 min. In food samples, such as oysters, where the cooking fluid contains more taurine than the raw material, the excess represents taurine freed from chemical combination by the boiling procedure. The latter assumption has been verified by hydrolytic methods followed by chromatography.

analysis was checked for specificity by paper chromatography of concentrates of the column effluent. A one-dimensional descending system was used with a solvent of tertiary butanol:methyl ethyl ketone:pyridine:water in the volumetric ratio 40:40:1:20 (ref. 12). Taurine spots were identified on the paper by ninhydrin, 0.25 per cent in acetone, and also by the specific colour reaction between taurine and *O*-phthalaldehyde<sup>13</sup>. Liquid food samples were measured by volume and the analytical procedures were carried out as already described here, starting with the de-proteinization step.

In the case of foods, where the effect of cooking methods on the taurine content was being determined, the original sample was divided into three portions, each of which was then weighed. One portion was then homogenized, according to the method previously described; a second portion was boiled for 15 min in a known volume of de-ionized water and a third was baked in aluminium foil for 30 min at 350° F. The cooked portions were then homogenized and the taurine content of these samples and of the boiling fluid was assayed. The final taurine values were expressed as mg/100 g of edible food in the case of solids, and as mg/100 ml. in the case of liquids.

It can be seen from Table 1 that the highest taurine values have been found in sea foods from invertebrate phyla, but that other animal protein foods also contain significant quantities of the amino-acid. Apparently a part of the taurine is destroyed by heat; however, it is of greater interest that taurine is leached out by the boiling procedure. The extracted taurine can be recovered from the cooking fluid (Table 2). It is our intention to evaluate the effects of various food preservation techniques on the taurine contents of meats and fish, because it has previously been shown that, in the case of codling, 'icing' of the fresh muscle depletes the taurine, owing to the fact that it is leached out into the ice water<sup>14</sup>.

Our immediate concern in this investigation lies in the possibility that taurine may, in pathological circumstances, exert an active and toxic biochemical function in the human body. In patients with psoriasis, as indeed in normal persons, it has now been established that taurine may be ingested in large amounts in the daily diet. The actual amount will vary with food sources and with the cooking methods used. An investigation of taurine excretion and retention in normal and psoriatic subjects is in progress. The effects of high and low taurine diets are also being evaluated.

Several interesting questions arise from an examination of our preliminary data. Whereas it is known from the work of marine biologists and physiologists that taurine has active chemical and physico-chemical functions in the tissues of primitive phyla<sup>15</sup>, little is understood of its utilization in higher animals. In addition to the proposed function of taurine as a regulator of osmotic pressure in marine organisms<sup>16</sup>, phosphotaurine has been isolated from the marine worms and it is believed that this phosphagen, of low thermodynamic potential, may be utilized in the contraction of slow muscles. It is known

that taurine and its deaminated derivative isethionic acid are present in the squid axoplasm, and the synthesis of isethionic acid from taurine has been demonstrated in the dog's heart<sup>17,18</sup>. In the former case, the taurine may play a part in nerve transmission, and in the latter it is important in the transmission of cardiac impulses<sup>17,19</sup>. In the young chick, it appears that taurine is a growth factor, which is intimately associated with bone development<sup>20</sup>. It has been suggested that it donates sulphate for esterification into chondroitin sulphate, but that this role, in the chick, is temporary during the early weeks of life. We may ask ourselves whether taurine has any of these functions in man or whether it has other activities as yet undiscovered.

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### Effect of Vitamin E Deficiency on Ubiquinone Levels in Rat and Rabbit Liver

In a series of publications by Green and others<sup>1-3</sup> it was reported that the concentration of ubiquinone in animal tissues is depressed in tocopherol deficiency and that this vitamin (or a structurally related form) is specifically required for its maintenance or restoration. Investigations by Draper and others<sup>10-13</sup> indicated that all the well-characterized lesions observed in vitamin E deficiency are amenable to treatment with certain structurally unrelated synthetic antioxidants, notably *N,N'*-diphenyl-*p*-phenylenediamine (DPPD). These latter experiments led to the conclusion that functional displacement of tocopherol by DPPD occurs in metabolism and that the biological role of the vitamin is entirely attributable to its antioxidant properties. However, Green *et al.* reported that neither DPPD nor 'Santoquin' (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, another antioxidant which is active as a dietary substitute for tocopherols) was effective in maintaining ubiquinone levels<sup>4,7</sup> and they concluded that vitamin E has a specific non-antioxidant part in the metabolism of ubiquinone. In view of these reports we have investigated the effect of vitamin E deficiency and antioxidant treatment on ubiquinone concentrations in liver. It was chosen because it can be depleted of tocopherols and is known to show maximal change

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Three comparisons were made between vitamin E-deficient and control rats and one between normal and deficient rabbits. In experiments 1 and 2, control groups of 5-6 weanling female rats of the Sprague-Dawley strain were fed a basal vitamin E-deficient diet for 20 weeks. This diet contained the following ingredients (per cent): glucose 71.4, 'Labco' casein 20, salts 446 (ref. 14) 4, glycerol 2, vitamins in-glucose<sup>10</sup> 0.5, choline chloride 0.1, and 75 per cent methyl linoleate 2 (obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio; remaining 25 per cent primarily methyl oleate). Vitamins A and D were added at levels of 100 and 5 I.U./g, respectively. Females raised to sexual maturity on this diet, and mated, have uniformly exhibited a resorption gestation unless given supplementary vitamin E<sup>10</sup>. The treated groups received a supplement of 0.5 mg DL- $\alpha$ -tocopheryl succinate or 0.25 mg DPPD/g of diet.

In Exp. 3, 15 weanling females were fed a diet except for replacement of th

('Skelly Solve F', b.p. 30°-60° C). In the case of the rabbit livers the bulk of the sterols was removed by suction filtration after crystallization at -15° C from petroleum ether and the residue was chromatographed on 1.6 cm x 10 cm columns. Ubiquinone was eluted in 4 per cent diethyl ether, recovered in ethanol and estimated by reduction with NaBH<sub>4</sub> at 275 m $\mu$ . The  $\Delta E_{1\text{ cm}}^{1\%}$  value of 142 for ubiquinone-50 was used throughout, as comparative values were of prime interest, although a small error accrues from its use owing to the presence of a high proportion of ubiquinone-45 in rat tissues. Recoveries of pure ubiquinone-50 applied to the column were essentially quantitative (96-102 per cent).

The results of the three rat experiments, shown in Table 1, fail to provide any evidence for a relationship between vitamin E status and the ubiquinone concentration in the liver. The data reveal wide ranges in ubiquinone levels within groups, but in none of the comparisons were there significant differences within experiments which can be attributed to a large variability in the ubiquinone levels. This has been cited by other workers as being associated with the degree of fatness. To illustrate the

were presumably attributable to some characteristic of the amino-acid basal diet used during the recovery period. The fact that the gross symptoms of the deficiency were alleviated by DPPD under these conditions confirms the conclusion reached previously that this compound is capable of direct metabolic substitution for tocopherols.

The results of this investigation are in agreement with the findings of Morton and Phillips<sup>24,25</sup>, Moore<sup>26</sup> and Phillips<sup>17</sup>, who reported that the ubiquinone content of the liver of vitamin E-deficient rats remained unchanged. It is difficult to account for the discordant results of Green and associates<sup>1-9</sup>, other than to point to the multiple dietary factors which obviously influence the ubiquinone level and to the difficulty of establishing significant treatment effects in the face of wide fluctuations within groups. In some instances the differences observed by these workers were relatively small and in certain cases the values for the deficient animals were the same as (or actually higher than) those for the normal controls. The magnitude of the differences observed in the present study

genetic uniqueness of Bence Jones proteins is related to the individual antigenic specificity of single immunizing proteins<sup>2</sup>. Another variable determining at least a part of the immunological difference between Bence Jones protein and normal unreduced  $\gamma$ -globulin is related to the unmasking of hidden antigenic determinants of *L* chains when they are either not incorporated into or cleaved from the parent  $\gamma$ -globulin molecule<sup>3</sup>. It has been suggested that certain potential antigenic sites of *L*-polypeptide chains are inaccessible in the intact  $\gamma$ -globulin molecule due to steric hindrance of adjacent *H* chain<sup>4</sup>.

The work recorded here was undertaken to investigate the possibility that there are additional antigenic differences between Bence Jones proteins and normal  $\gamma$ -globulin which are over and above those related to individual specificity and unmasking of hidden sites in the native intact molecule.

Bence Jones proteins were typed<sup>25</sup> and the results



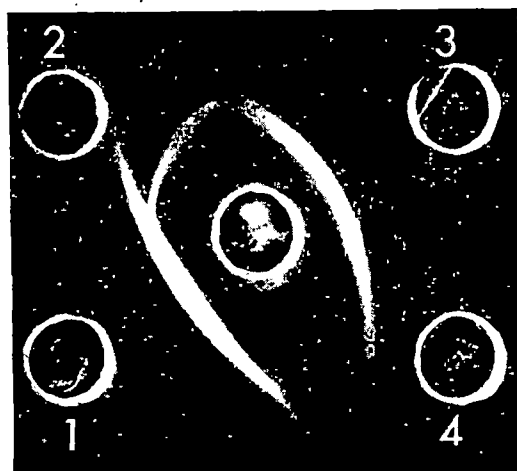


Fig. 2. Immunodiffusion. Comparison of group 1 Bence Jones proteins and normal *L* chains. Well No. 1, Bence Jones protein 1-40; No. 2, Bence Jones protein 1-41; No. 3, Bence Jones protein 1-42; No. 4, normal *L* chains. Centre well contained a specific group 1 Bence Jones (1-40) antiserum absorbed with 5 mg of normal pooled  $\gamma$ -globulin and 7.5 mg of normal *L* chains.

sites, then the autologous myeloma proteins with presumably intact *L* chains should not contain the same degree of antigenic uniqueness as do the free *L* chains of the Bence Jones protein. The possibility exists that the *L* chains of the myeloma protein are sterically arranged so as to make available an ordinarily occult site. This would presuppose a different quaternary arrangement of *L* and *H* chains in a myeloma protein as contrasted to the arrangement in a normal  $\gamma$ -globulin molecule. This has not been described.

In order to clarify further these antigenic relationships, group specific Bence Jones antiserum (anti BJ1-40) was absorbed with lyophilized normal *L* chains (Fig. 2). Reactivity persisted against the immunizing protein (BJ1-40); prominent cross-reactivity was noted with two proteins of the same immunological group but from different patients. No reactivity was noted with normal *L* chains. In the unabsorbed state, this antiserum gave a strong precipitin line with the normal *L* chains. Similar findings were noted when *L* chains and group 2 Bence Jones proteins were compared, using an *L* chain absorbed group 2 Bence Jones antiserum. These findings further demonstrate the antigenic uniqueness of Bence Jones proteins when compared with normal *L* chains. This uniqueness is not related to individual specificity for the *L* chain absorbed antiserum cross-reacted with Bence Jones proteins of the same group from different patients. In addition, the antigenic uniqueness appeared not to be related to the hidden antigenic sites shared by normal *L* chains and Bence Jones proteins; for the absorbed antiserum failed to react with the free *L* chains. Thus Bence Jones proteins contain antigenic properties which are not overtly present in the isolated *L* chain polypeptide sub-units of normal  $\gamma$ -globulin.

Investigations by Kunkel, Mannik and Williams<sup>9</sup> suggest that purified human antibody of a given specificity can differ sufficiently in structure from other molecules of  $\gamma$ -globulin to impart a characteristic unique antigenicity. This uniqueness was localized to the papain split *S* fragment of the antibody molecule. It is thus possible that the distinctive antigenic characteristics of Bence Jones proteins may have apparent counterparts in the *L* chains of isolated antibodies.

These observations again raise the possibility that Bence Jones proteins are qualitatively abnormal, due to primary structural differences which are not present in the *L* chain sub-units of normal  $\gamma$ -globulin<sup>2</sup>. The fact that isolated antibodies possess antigenic uniqueness which may in part be related to the *L* chain component, however, lends

more weight to the possibility that Bence Jones proteins represent quantitative abnormalities in the sense that they are hypertrophied molecular components normally buried in the heterogeneous  $\gamma$ -globulin population.

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### Labelled Tyrosinase from Labelled Substrate

It is well known that tyrosinase becomes inactivated when it oxidizes phenol or pyrocatechol but is much more stable towards substituted phenols and catechols, such as 4-5-dimethyl catechol<sup>1</sup>. We wish to report evidence which seems to support the view that this inactivation is due to the formation of a compound between the enzyme protein and the product of oxidation.

In the investigations reported here, 1-<sup>14</sup>C phenol (Radiochemical Centre, Amersham, Bucks, England) was used as substrate for mushroom tyrosinase (80 cresolase units per ml.). The enzyme was prepared according to the method of Frieden and Ottesen<sup>2</sup>, but omitting the final step of chromatography on diethyl amino ethyl cellulose.

Determination of levels of radioactivity were performed on a Nuclear Chicago planchette counter. The radioactive phenol was used as a solution of 0.5  $\mu$ mole per ml., which gave  $2 \times 10^5$  d.p.m./ml.

Reaction mixtures normally consisted of 0.5 ml. of a phenol solution, 1.0 ml. of 0.2 molar phosphate buffer (pH 7.0) sodium ascorbate and enzyme solution equivalent to 3 mg protein. Preliminary experiments demonstrated that when such a mixture was incubated at 25° C for 3-4 h, and the protein separated by vacuum dialysis or by boiling the reaction mixture, the protein was always radioactive. This radioactivity cannot be removed by washing successively with water, ether and acetone.

Enzyme which had been inactivated by heat did not become significantly radioactive when incubated with the reaction mixture and recovered as described here. In a typical experiment, the active enzyme gave 1,290 c.p.m. and the inactive preparation gave 90 c.p.m.

The possibility that the label was none the less produced by physical carry-over of radioactive material was finally removed by the following experiment. Identical flasks were prepared containing the usual reaction mixture, and were incubated for 2 h at 25° C. At the end of this time 10 mg of pyrocatechol was added to one of the flasks and incubation was continued for a further 5 h. Protein was then recovered in the usual way and plated out. The normal sample had a radioactivity of 2,049 c.p.m./mg; the catechol treated one had an activity of 1,612 c.p.m./mg. These results are more consistent with competition between the large excess of non-radioactive pyrocatechol and the minute amount of initially labelled material, for the remaining active sites than they are with dilution of carried-over catechol, since the latter process would be



expected to produce virtually non-radioactive protein with the great excess of non-radioactive catechol used here.

When a portion of the labelled protein was hydrolysed with 6 N hydrochloric acid, and the hydrolysate chromatographed with butanol:acetic acid:water (4:1:5) organic phase on Whatman No. 3 paper, examination of the chromatogram on the strip counter showed that all the radioactivity normally remained at the origin, although occasionally a small peak with an  $R_F$  of about 0.5 was observed. When a portion of the hydrolysate was freed of excess HCl, then shaken with 'Dowex 50' resin ( $H^+$  form), over 80 per cent of the radioactivity was retained on the resin, indicating the presence of a free basic group or groups on the radioactive molecule.

Hydrolysis of the radioactive protein with enzymes was next tested. Different portions were hydrolysed with trypsin and with pepsin, then chromatographed on paper using a solvent described by Waley and Watson<sup>3</sup>, namely, N-butanol/acetic acid/water/pyridine (30:6:24:70). This solvent also failed to move the radioactivity in an acid hydrolysate from the origin. The enzymic hydrolysates, however, gave a completely different picture. Each gave a rather broad diffuse area of material reacting with ninhydrin and a corresponding radioactive peak. These peaks were well removed from the origin. From similar chromatograms, not treated with ninhydrin, the radioactive areas were cut out and the soluble material was eluted with water. The solutions were clear and of a pale golden yellow colour. Both eluates were placed on Whatman No. 1 paper and chromatographed with butanol/acetic acid/water (4:1:5) (organic phase). The yellow-green colour of the spots of material on the paper was discharged completely by the acid chromatography solvent. The pepsin hydrolysate again gave a poorly defined peak. The tryptic hydrolysate gave a small radioactive peak at the origin, another small one just below the origin, and a large peak farther down the paper. This last peak was marked by the slow development of a brown colour on drying the paper. The large peak was eluted with water and hydrolysed with acid in the usual way. The hydrolysate was re-chromatographed with butanol/acetic acid/water solvent. As was to be expected, the rather inhomogeneous preparation yielded many amino-acids, and the radioactivity was present exclusively at the origin.

We have shown that when tyrosinase oxidizes radioactive phenol under conditions where the well-known substrate inactivation would normally occur, a radioactive protein can be obtained from the reaction mixture. The radioactivity cannot be removed by washing with water, acetone and ether in succession. Complete (acid) hydrolysis of the protein yields a radioactive material the chromatographic behaviour of which does not resemble that of phenol, pyrocatechol or o-benzoquinone, and which can be absorbed on to a weakly acidic ion-exchange resin—behaviour which one would not expect of phenol or its two derivatives. On mild (enzymatic) hydrolysis, however, radioactive components were obtained which are mobile in the chromatographic solvents tested.

While any consideration of the mechanisms involved must at this stage be purely speculative, it is reasonable to inquire whether reactions are already known to occur under physiological conditions which could account for these observations. We suggest that the newly formed quinone may, before leaving the active centre of the enzyme, undergo a Michael reaction with a free amino group of the enzyme, as in the cyclization of dopaquinone<sup>4,5</sup> during the conversion of tyrosine to melanin. The substrate thus becomes permanently attached to the active centre of the enzyme and prevents the enzyme from accepting and oxidizing other molecules of the catechol. This would account for the observation that homocatechol (4-methyl catechol) is less inhibiting than pyrocatechol, and the 4,5-dimethyl catechol is apparently devoid of

inhibitory action, since with the 4 and 5 positions blocked, the condensation would be completely impossible.

Attempts to investigate the mode of action of tyrosinase have so far been hampered by a lack of information about the nature of the active centre. The investigations described here offer a method of isolating, identifying, and investigating peptide fractions specifically derived from the active centre of the molecule.

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## PHYSIOLOGY

### Interactions between Active Sodium Transport and Active Amino-acid Transport in Isolated Rabbit Ileum

In previous publications dealing with ion transport across *in vitro* segments of rabbit terminal ileum, we have demonstrated that sodium is actively transported from mucosa to serosa and that the presence of an actively transported sugar in the mucosal solution increases the rate of active sodium transport by means of an interaction with the sugar transport *per se*<sup>1-3</sup>. In addition, we have presented a model for the sodium-sugar interaction<sup>3</sup> which is consistent with the demonstration of Bihler *et al.* that the entrance of actively transported sugars into the intestinal cell is dependent on sodium<sup>4</sup>. These findings, together with Csaky's observations that sodium is necessary for the active intestinal transport of amino-acids and pyrimidines, as well as active sugar transport<sup>5</sup>, led us to investigate the effect of actively transported amino-acids on sodium transport by rabbit ileum.

The short-circuit technique and other methods and procedures used in this investigation have been described previously<sup>1</sup>. The tissue was initially perfused with the buffer medium containing neither sugar nor amino-acid, and the amino-acid under examination was afterwards added to both the mucosal and serosal reservoirs simultaneously.

The effect of L-alanine on the short-circuit current ( $I_{sc}$ ) is shown in Fig. 1 (open circles). The increase in the  $I_{sc}$  (and the transmural potential difference) commences within 10 sec following the addition of the amino-acid, and the maximum level is usually achieved within 1-2 min. This increase is observed only if the amino-acid is added to the mucosal solution; addition to the serosal solution alone is ineffective. Furthermore,  $5 \times 10^{-4}$  M ouabain either prevents or diminishes the stimulation of the  $I_{sc}$  when added 10-15 min prior to the amino-acid and rapidly inhibits the  $I_{sc}$  when added after the amino-acid. This effect of ouabain is most marked when the glycoside is added to the serosal solution. As for the sodium-sugar interaction<sup>3</sup>, little or no effect of ouabain is observed when it is added to the mucosal solution alone.

Also shown in Fig. 1 (closed circles) are the results of a typical experiment in which the perfusion medium was initially sodium-free as the result of replacement of sodium with potassium. In the absence of sodium the  $I_{sc}$  does not differ significantly from zero and no effect is observed following the addition of L-alanine. The sub-

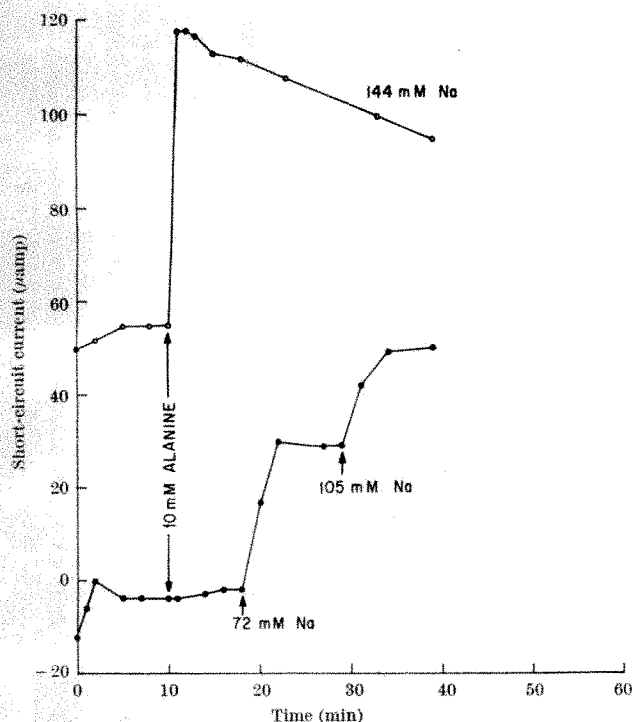


Fig. 1. The effect of 10 mM L-alanine on the  $I_{sc}$  in the presence of 142 mM sodium (open circles) and in the presence of a sodium-free medium (closed circles). Following the addition of alanine, the medium which was initially sodium-free was diluted with the 142 mM sodium medium to give final sodium concentrations of 72 mM and 105 mM. The times of these dilutions are indicated by arrows.

sequent addition of sodium to the medium (arrows) results in an immediate increase in the  $I_{sc}$  and the levels achieved approach those which would be predicted by a linear relationship between the  $I_{sc}$  and the sodium concentration of the medium<sup>3</sup>.

We have previously demonstrated that there is good agreement between the  $I_{sc}$  and the rate of active sodium transport by terminal rabbit ileum both in the presence and absence of actively transported sugars<sup>1,2</sup>. In Table 1 are shown the mucosa-to-serosa ( $\Phi_{ms}^{Na}$ ), serosa-to-mucosa ( $\Phi_{sm}^{Na}$ ) and net ( $\Phi_{net}^{Na}$ ) fluxes of sodium and the average  $I_{sc}$  in the presence and absence of L-alanine (10 mM). The agreement between  $\Phi_{net}^{Na}$  and the  $I_{sc}$  supports the conclusion that the increase in the  $I_{sc}$  following the addition of L-alanine may be attributed to an increased rate of active transport of sodium. Recently, Esposito *et al.*<sup>6</sup> have demonstrated that the rate of sodium transport by everted sacs of rat intestine is increased by both glucose and amino-acids in complete agreement with our findings.

Table 1. EFFECT OF L-ALANINE ON SODIUM FLUXES IN RABBIT ILEUM\*

	$\Phi_{ms}^{Na}$	$\Phi_{sm}^{Na}$	$\Phi_{net}^{Na}$	$I_{sc}$
			$\mu\text{mole}/\text{cm}^2 \text{ h}$	
Basic medium†	$9.5 \pm 0.2$ (42)	$6.7 \pm 0.3$ (33)	$2.8 \pm 0.4$ (75)	$2.6 \pm 0.6$ (75)
L-Alanine (10 mM)	$10.2 \pm 0.3$ (27)	$5.7 \pm 0.2$ (20)	$4.5 \pm 0.4$ (47)	$4.6 \pm 0.1$ (47)

\* All errors are expressed as standard errors of the mean. The number of determinations is given in parentheses.

† Electrolyte medium containing neither sugar nor amino-acid.

The relationship between the magnitude of the increase in the  $I_{sc}$  ( $\Delta I_{sc}$ ) and the concentration of L-alanine added to the perfusion medium is shown in Fig. 2. The curve is that predicted by a least-squares fit of the data plotted according to the method of Lineweaver and Burk<sup>7</sup> and indicates that the relationship between the  $\Delta I_{sc}$  and the concentration of L-alanine is consistent with Michaelis-Menten kinetics. The concentration of L-alanine which

elicits a half-maximal stimulation of the  $I_{sc}$  is 4.5 mM. Finch and Hird<sup>8</sup> have demonstrated that the uptake of L-alanine by rat intestine conforms to Michaelis-Menten kinetics and that the half-maximal rate of uptake is observed in the presence of 5 mM alanine. The similarities between our results and the kinetics of alanine transport reported here suggest that, barring major species differences with respect to the rate of alanine transport, there is a stoichiometric relationship between this rate and the increased rate of active transport of sodium.

The results described here are not limited to L-alanine. Significant stimulation of the  $I_{sc}$  has been observed following the addition of glycine, L-methionine,  $\alpha$ -amino-iso-butyric acid, L-lysine, L-glutamate (potassium salt), L-glutamine, as well as D-methionine, D-alanine and D-glutamate (potassium salt). Since we have not measured sodium fluxes in the presence of these amino-acids, the significance of these findings is uncertain. It is not improbable however that, as in the instance of L-alanine, the increase in the  $I_{sc}$ , particularly in the presence of neutral amino-acids, is attributable to an increase in active transport of sodium. Since the active transport of anionic or cationic amino-acids would by itself influence the  $I_{sc}$ , that portion of the  $\Delta I_{sc}$  which may be the result of an increased rate of transport of sodium cannot be defined in the absence of simultaneous determinations of both the amino-acid and sodium fluxes.

Finally, we have previously reported that once the  $I_{sc}$  has been maximally stimulated by an actively transported sugar the subsequent addition of another actively transported sugar does not elicit a further increase in the  $I_{sc}$ . This finding was interpreted as being consistent with the presence of a single, saturable carrier mechanism which is responsible for active intestinal sugar transport<sup>3</sup>. When, on the other hand, the  $I_{sc}$  has been maximally stimulated by the addition of either glucose or 3-O-methyl-glucose the subsequent addition of either L-alanine, L-lysine or L-glutamate results in a further large increase in the  $I_{sc}$ . This additive effect is also observed if the sequence of addition is reversed (that is, amino-acid, then sugar). Furthermore, the magnitude of the second rise in the  $I_{sc}$  usually approaches that which would be expected to follow the addition of the sugar or amino-acid alone. These findings are consistent with the evidence that the active transport of amino-acids and sugars are mediated by separate and parallel carrier mechanisms<sup>9</sup>, and suggest that the operation of each of these mechanisms results in an increased rate of entrance of sodium from the mucosal solution into the intestinal cell.

In recent years the finding that sodium is required for the active transport of sugars and/or amino-acids has been extended to many tissues other than mammalian intestine, for example, kidney slices<sup>11,10</sup>, leucocytes<sup>12</sup>

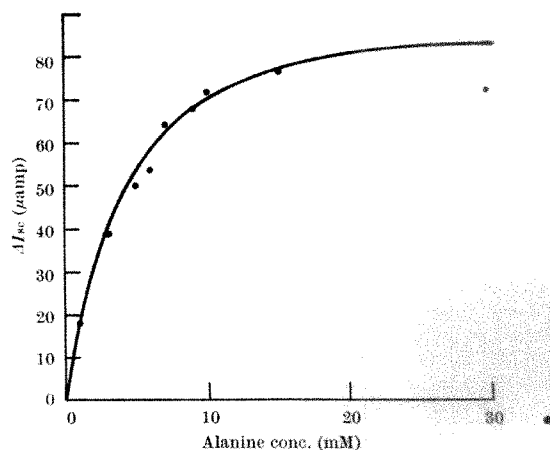


Fig. 2. The relationship between the concentration of L-alanine in the perfusion medium and the increase in the  $I_{sc}$  ( $\Delta I_{sc}$ ).



a marine pseudomonad<sup>13</sup>, Ehrlich ascites tumour cells<sup>14</sup>, pigeon erythrocytes<sup>15</sup>, striated muscle<sup>16</sup> and thymus nuclei<sup>17</sup>. Our findings that the active intestinal transport of sugars and amino-acids not only requires sodium but also brings about an increased rate of trans-mural sodium transport suggests that the role of sodium is not simply that of an activator of these transport processes. Instead, it is suggested that the active transport of both sugars and amino-acids is mediated by a ternary sodium-sugar or amino-acid-carrier complex and is dependent on the ability of the cell to maintain an intracellular sodium concentration lower than that in the surrounding medium. The inhibitory effect of ouabain on these sodium-dependent transport processes<sup>8,11,16</sup> may be solely the result of its inhibition of the active sodium extrusion mechanism which is responsible for the maintenance of the sodium concentration gradient. A more detailed description of this hypothetical model has been published<sup>9</sup>, and Vidaver has recently suggested an analogous model consistent with his findings concerning the sodium-dependence of glycine uptake by pigeon erythrocytes<sup>18</sup>.

The views expressed here are our own and do not necessarily reflect the views of the U.S. Air Force or the Department of Defense.

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### Inhibitory Synapses and Inflammation

B. RENSHAW<sup>1</sup> has shown that part of impulses from the motoneurons is not directed to the muscles; it spreads by way of a branch of the motoneurone axon through an inhibitory interneurone and then returns to inhibitory synapses covering the same motoneurone which originally evoked the impulse. Now it is known that nearly all neurones at all levels of the central nervous system possess a self-inhibitory mechanism of that kind.

J. C. Eccles *et al.*<sup>2</sup> have shown that strychnine and tetanotoxin block the inhibitory synapses and therefore increase the spinal polysynaptic reflexes.

Most diseases are revealed by inflammatory processes. Therefore it seems to be very significant that, as shown by us, the inhibitory synapses are also of great importance

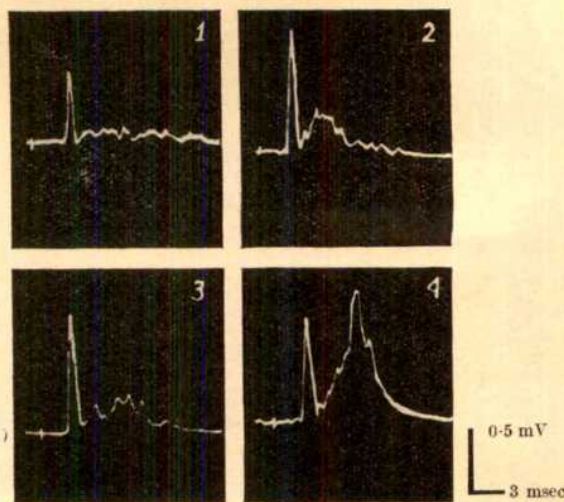


Fig. 1. Mono- and poly-synaptic reflexes from the damaged leg (3) are larger than the mono- and poly-synaptic reflexes from the undamaged paw (1). The injection of strychnine evokes a sharper increase of poly-synaptic reflexes from the damaged leg (4) than from the undamaged leg (2). The stimuli supernormal for the I group of fibres were applied to n. peroneus comm.

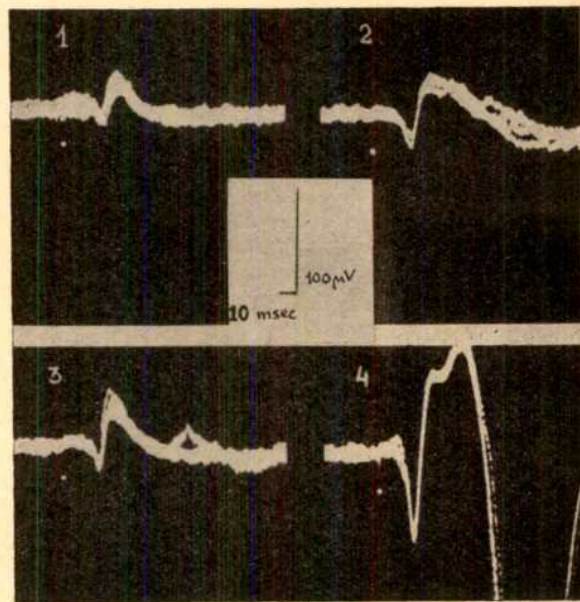


Fig. 2. Primary responses of the I sensory motor area from the damaged leg (2) are larger than from undamaged leg (1). The injection of strychnine evokes a sharper increase of the primary response from the damaged leg (4) than from the undamaged leg (3). Besides this a secondary response appears from the damaged leg (4). Each record has been formed by super-position of 5 traces. The white points mark the moment of stimulation. The stimulus (350 mV) is applied to n. peroneus superficiales

for the reactions evoked by the impulses from inflamed tissues. In our experiments the inflammation was evoked in one of the hind paws of a cat by means of cutaneous injection of turpentine (0.2–0.3 c.c.). Some hours or days later the strychnine-nitrate (0.05 mg/kg) was injected intravenously into the same animal. The spinal reflexes were evoked by stimulation of the n. peroneus comm. The strychnine acts on the reflexes of the animals with a damaged paw in the same way as it does on reflexes of healthy animals, but there is a very important peculiarity. On the side of the damaged leg the polysynaptic reflexes increase by 100–200 per cent as compared with the side of the undamaged one (Fig. 1).

A similar effect was established by investigation of the evoked potential of the brain. The injection of strychnine



increases the evoked potential both in the cortex (I and II somatosensory zones) and in the specific nucleus of thalamus (*VPL*), being much sharper in the hemisphere contralateral to the damaged leg than in the ipsilateral one (Fig. 2).

On the basis of these experiments it is obvious that the amount of neurones—at the level of spinal cord or higher, in the sub-cortex or cortex involved in the pathological reaction—depends on the activity of the inhibitory synapses. The blocking of inhibitory synapses has shown very clearly that in this case many more neurones could be involved in the pathological reaction and therefore it could become excessive.

In our previous experiments<sup>3</sup> it was shown that the compensation of disfunction of some damaged organs depended on the nervous system. It is possible that inhibitory synapses are the basis of the compensation.

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### Development of Acetylcholinesterase Activity at Muscle-tendon Junctions

In vertebrate skeletal muscles, besides a strong acetylcholinesterase activity at motor end-plates, a weaker but well-defined activity is found at muscle-tendon junctions<sup>1,2</sup>. Whereas the functional role of acetylcholinesterase at the end-plates seems to be well established, both the origin and function of the enzyme at muscle-tendon junctions remain obscure. The question was often raised whether the concentration of the enzyme at these sites could not be in some relation to the motor or sensory innervation of the muscle. However, acetylcholinesterase activity at muscle-tendon junctions appears to be unaltered after nerve section in adult animals<sup>3</sup>. On the other hand, in tissue culture no concentration of the enzyme develops either in the middle part or at the ends of muscle fibres<sup>4</sup>, and it has been suggested that this might be due to the absence of nerves<sup>5</sup>.

It seemed, therefore, of interest to test the possible influence of innervation, either motor or sensory, on acetylcholinesterase activity at muscle-tendon junctions in developing muscles.

The formation of acetylcholinesterase-active sites was examined histochemically<sup>6</sup> in the calf muscles of the rat during development. Traces of acetylcholinesterase activity were found at the ends of some muscles already in foetuses before birth. In new-born rats acetylcholinesterase activity is still very low at muscle-tendon junctions, whereas at the end-plate region the activity is fairly strong and well localized.

To determine the role of innervation in further development muscles were denervated at the early stage of incipient formation of muscle-tendon cholinesterase. The sciatic nerve was sectioned in new-born rats and acetylcholinesterase activity was examined in denervated calf muscles, mainly in the soleus muscle, from 3 days–1 month after the operation.

After the nerve section at birth, no acetylcholinesterase activity was formed at muscle-tendon junctions in denervated muscles. The small amount of acetylcholinesterase present at muscle ends at birth disappeared within 3–7 days and the fibre ends remained devoid of

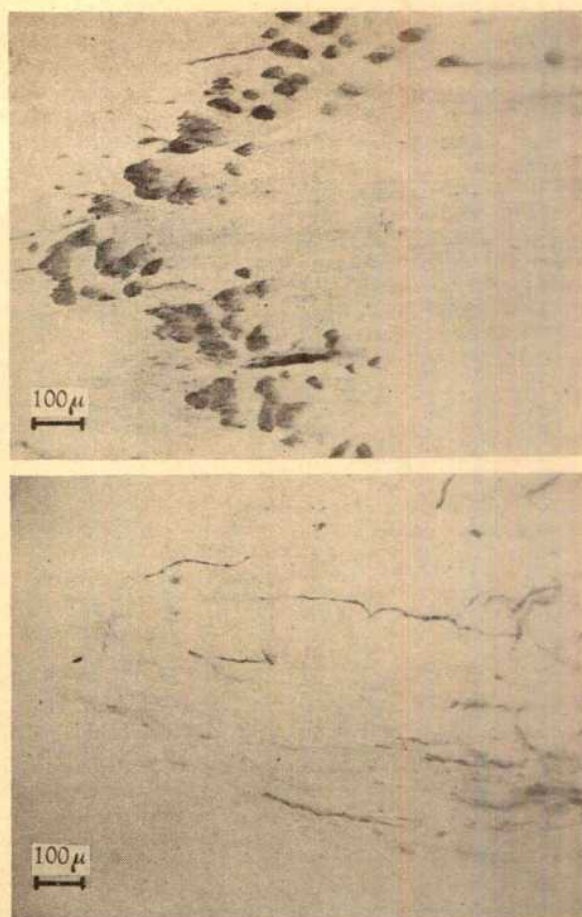


Fig. 1. Above, acetylcholinesterase activity at muscle-tendon junctions of the soleus muscle in normal rat three weeks old; below, absence of acetylcholinesterase activity at muscle-tendon junctions in the soleus muscle three weeks after motor denervation in litter-mate rat with spinal cord removed at birth, from the level  $L_1$  downward. Note richly branched cholinesterase-active sensory fibres. Method of Koelle and Friedenwald, slightly modified<sup>6</sup>. Frozen sections 25  $\mu$ , incubation 2 h at pH 6.2

the enzyme activity throughout the investigated period. No acetylcholinesterase activity could be detected at muscle-tendon junctions even when incubation with the substrate was prolonged to 2, 4 and 24 h. In the end-plate region, acetylcholinesterase activity decreased rapidly after denervation, and further development of the end-plates was arrested, as has been already described<sup>7</sup>. One month after denervation, acetylcholinesterase in the soleus muscle was scarcely detectable even at the end-plate region.

In the control muscles, acetylcholinesterase activity increased rapidly after birth, and within a few days the intensity of staining and shape of the active sites were similar to those of adult animals.

In the rat, motor nerve fibres supply the region of end-plates localized in the central parts of muscle fibres. Outside this region only polar zones of intrafusal fibres in muscle-spindles receive motor innervation. Some of the sensory fibres, however, reach the ends of the muscle fibres and innervate tendon organs or form free endings at the tendon. It was therefore important to find out whether the disappearance of acetylcholinesterase at muscle-tendon junctions was due to the loss of remote motor endings or of the nearby sensory innervation, or both.

To check these possibilities, another series of experiments was carried out in which the spinal cord was removed in new-born rats from the level  $L_1$  downward. This operation completely eliminates the motor innervation of muscles in hind limbs, whereas their sensory nerve



supply remains unaffected. After such motor denervation, acetylcholinesterase disappeared from muscle-tendon junctions as it did after the section of the nerve (Fig. 1). Only tendon organs frequently found in this region exhibited a strong cholinesterase activity, and richly branched acetylcholinesterase-active sensory fibres were well stained.

Although the mechanism leading to the accumulation of acetylcholinesterase at the muscle-tendon junction remains unknown, the decisive role of motor innervation in its development seems firmly established by these experiments.

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### Antagonism between $\text{Na}^+$ and $\text{Ca}^{2+}$ at the Neuromuscular Junction

THE quantity of transmitter released from presynaptic nerve terminals is in part determined by the amplitude of the action potential<sup>1,2</sup> invading the nerve terminals, and by the amount of a specific calcium-compound available at some strategic site<sup>3</sup>. Large reductions in external  $\text{Na}^+$  concentration, however, which would be expected to reduce the size of the propagated action potential<sup>4</sup>, do not greatly alter the output of acetylcholine at cholinergic nerve endings<sup>5,6</sup>. This might be explained if it were supposed that the reduction in  $\text{Na}^+$  concentration also increased the amount of available calcium-compound at the strategic site;  $\text{Na}^+$  deficient solutions might then maintain the release of transmitter in spite of the reduction of the amplitude of the action potential. On this hypothesis a  $\text{Na}^+$  deficient solution might be expected to increase the amount of transmitter released when the calcium concentration is low. Such an increase in transmitter release has been found in the experiments reported below.

By methods similar to those described by Fatt and Katz<sup>7</sup> intracellular records were made of evoked and spontaneous end-plate potentials from frog sartorii bathed in solutions which contained 0.23 mM  $\text{Ca}^{2+}$ , 2.0 mM  $\text{K}^+$  and various concentrations of  $\text{Na}^+$ ; tonicity being maintained by the addition of sucrose. The microelectrode was inserted into the end-plate region of a muscle fibre and approximately 100 evoked end-plate potentials followed by 50–100 spontaneous end-plate potentials were recorded. The microelectrode was left undisturbed while the concentration of  $\text{Na}^+$  in the bath was changed and allowed to equilibrate, after which the records were repeated. The mean number of 'quanta' of acetylcholine released per nerve impulse was calculated in each of the  $\text{Na}^+$  concentrations from the ratio of the mean evoked response to the mean spontaneous end-plate potential amplitude<sup>3</sup>. The amplitudes of the end-plate potentials were corrected for 'non-linear' summation<sup>8</sup>, the equilibrium potential for acetylcholine<sup>9</sup> being taken to be 50.1 and 57.3 mV in 23.1 and 38.5 mM  $\text{Na}^+$  respectively.

Records from a typical experiment are illustrated in Fig. 1. Evoked end-plate potentials from the end plate while bathed in normal  $\text{Na}^+$  (115.6 mM) and one-eighth normal  $\text{Ca}^{2+}$  (0.23 mM) are shown in Fig. 1A, and the spontaneous end-plate potentials in Fig. 1C. When the solution in the bath was changed to one-third normal

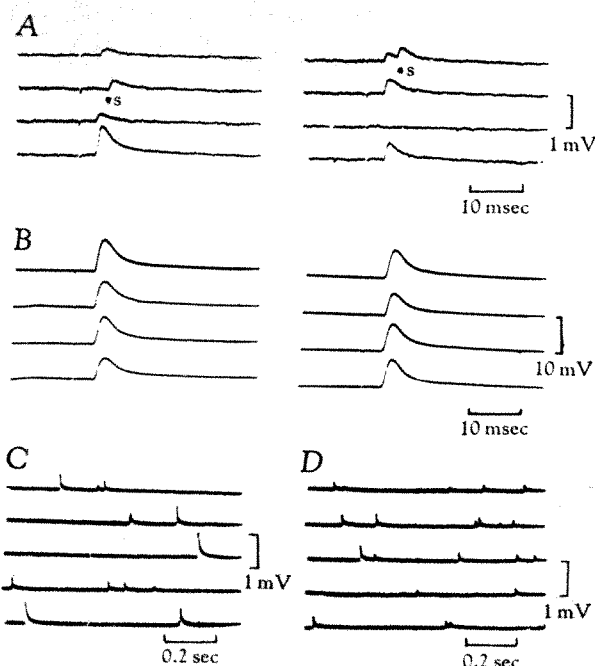


Fig. 1. Effect of reducing the concentration of  $\text{Na}^+$  on the evoked and spontaneous end-plate potential in 0.23 mM  $\text{Ca}^{2+}$ . A, selection of evoked end-plate potentials recorded in normal  $\text{Na}^+$ . The mean quantal content per nerve impulse was 0.9. Two of the responses were 'failures'; s indicates probable spontaneous end-plate potentials. B, responses recorded at the same end-plate in one-third normal  $\text{Na}^+$ . Note change in gain. The mean quantal content was 36. Spontaneous end-plate potentials recorded in normal  $\text{Na}^+$  (C) and in one-third normal  $\text{Na}^+$  (D).

$\text{Na}^+$  (38.5 mM) the evoked end-plate potentials were increased in amplitude (Fig. 1B), although the mean amplitude of the spontaneous end-plate potentials was reduced (Fig. 1D). In solutions containing one-fifth normal  $\text{Na}^+$  (23.1 mM) the amplitude of the end-plate potential was often sufficiently large to exceed the threshold required to initiate an action potential.

In Table 1 the results of 10 experiments show the marked increase of the number of quanta released per nerve impulse in low  $\text{Ca}^{2+}$  medium when the normal  $\text{Na}^+$  was replaced by  $\text{Na}^+$  deficient solutions. Conversely, there was a marked reduction when a  $\text{Na}^+$  deficient solution was replaced by normal  $\text{Na}^+$ .

These results suggest there is at the neuromuscular junction an antagonism between  $\text{Na}^+$  and  $\text{Ca}^{2+}$ , perhaps similar to that between  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  (ref. 10). An antagonism

Table 1. RESULTS OF TEN EXPERIMENTS SHOWING THE EFFECT OF CHANGING THE  $\text{Na}^+$  CONCENTRATION ON THE MEAN NUMBER OF QUANTA RELEASED PER NERVE IMPULSE

The  $\text{Ca}^{2+}$  concentration was kept constant at 0.23 mM. The normal  $\text{Na}^+$  concentration ( $\text{Na}^+ = 1$ ) was 115.6 mM

Experiment No.	Relative $\text{Na}^+$ concentration	Mean amplitude of corrected end-plate potentials (mV) ( $\pm$ S.D.)	Mean amplitude of spontaneous end-plate potentials (mV) ( $\pm$ S.D.)	Mean No. of quanta released per nerve impulse and fiducial limit
I	1	0.50 $\pm$ 0.34	0.25 $\pm$ 0.17	2.0 $\pm$ 0.9
	1/2	1.16 $\pm$ 0.73	0.23 $\pm$ 0.10	5.1 $\pm$ 0.7
II	1	0.56 $\pm$ 0.44	0.58 $\pm$ 0.58	0.9 $\pm$ 0.2
	1/3	4.93 $\pm$ 0.98	0.35 $\pm$ 0.21	14 $\pm$ 2
III	1	0.27 $\pm$ 0.24	0.32 $\pm$ 0.19	0.9 $\pm$ 0.2
	1/3	7.39 $\pm$ 1.11	0.20 $\pm$ 0.12	36 $\pm$ 4
IV	1	0.63 $\pm$ 0.47	0.29 $\pm$ 0.10	2.2 $\pm$ 0.7
	1/3	7.78 $\pm$ 1.07	0.22 $\pm$ 0.07	35 $\pm$ 3
V	1	1.38 $\pm$ 0.63	0.27 $\pm$ 0.11	5.0 $\pm$ 0.9
	1/5	4.30 $\pm$ 0.55	0.17 $\pm$ 0.05	25 $\pm$ 1
VI	1	0.13 $\pm$ 0.36	0.40 $\pm$ 0.14	0.8 $\pm$ 0.1
	1/5	5.87 $\pm$ 1.73	0.28 $\pm$ 0.09	21 $\pm$ 3
VII	1/2	1.91 $\pm$ 0.42	0.13 $\pm$ 0.01	15 $\pm$ 2
	1	0.11 $\pm$ 0.08	0.09 $\pm$ 0.06	1.2 $\pm$ 0.3
VIII	1/3	15.36 $\pm$ 1.18	0.15 $\pm$ 0.08	100 $\pm$ 8
	1	1.56 $\pm$ 0.73	0.41 $\pm$ 0.27	4 $\pm$ 2
IX	1/5	1.52 $\pm$ 0.18	0.12 $\pm$ 0.01	13.1 $\pm$ 0.4
	1	0.14 $\pm$ 0.13	0.17 $\pm$ 0.08	0.8 $\pm$ 0.2
X	1/5	7.01 $\pm$ 1.23	0.13 $\pm$ 0.03	52 $\pm$ 6
	1	0.21 $\pm$ 0.21	0.21 $\pm$ 0.04	1.0 $\pm$ 0.2

between  $\text{Na}^+$  and  $\text{Ca}^{2+}$  has been previously demonstrated at various other sites<sup>11-13</sup>.

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### Intracellular Postsynaptic Potentials in the Somatosensory Cortex of the Cat

CELLULAR activity in the somatosensory cerebral cortex has been investigated in detail with extracellular recording. Excitatory phenomena have dominated the findings in these experiments although inhibitory actions, mostly as 'surround inhibition', have been reported<sup>1-3</sup>. Since inhibition is abundant in the relay nuclei of the specific projection system<sup>4-6</sup> it has generally been assumed that the inhibition of cortical cells reflects inhibitory actions at sub-cortical level. The present results show, however, that powerful inhibitory potentials are generated in many cortical cells.

The experiments were performed on cats anaesthetized with 'Nembutal'. The intracellular activity was recorded from cells in the hindlimb area of  $S_1$  with glass micropipettes filled with 3 M potassium chloride or 2 M potassium citrate. Cortical movements were largely reduced by using a closed chamber system modified after Davies<sup>7</sup>.

In one type of experiment the dorsal part of the spinal cord was transected at the level of Th 12. The dorsal columns (DC), and the dorsal part of the lateral funiculus (DLF) contralateral to the investigated hemisphere, were dissected for electrical stimulation in ascending direction. In another group of experiments the cortical activity was investigated in preparations with intact spinal pathways and the animal was stimulated with single electrical shocks to the skin or with adequate stimulation of the skin. In this type of experiment some of the cortical cells could be classified with regard to the modality of the peripheral stimulus. In both types of preparations more than 100 cells were investigated intracellularly with regard to the postsynaptic potentials evoked by an afferent stimulation. The time of observation varied between one and 30 min. The mean resting membrane potential was 45 mV.

The most striking feature in the postsynaptic activity evoked by an afferent stimulus was a long-lasting hyperpolarization which was found in 80 per cent of the cells. Most commonly the inhibitory postsynaptic potential (IPSP) appeared initially, but in other cells the inhibition was post-excitatory. In the remaining 20 per cent of the cells only depolarization could be elicited. These principal types of synaptic actions were found in both types of preparations.

In cells being only hyperpolarized in response to stimulation the afferent volley elicited an IPSP with a steep rising phase and a slow decline (Fig. 1A). The mean latency was 9.8 msec and the mean duration 65 msec when the stimulus was applied to the dissected spinal fascicles at Th 10. In other cells the afferent stimulation elicited a depolarization followed by a prolonged hyperpolarization. The initial excitation had a mean latency of 9 msec when the stimulus was applied at Th 10. The duration of the depolarization as well as of

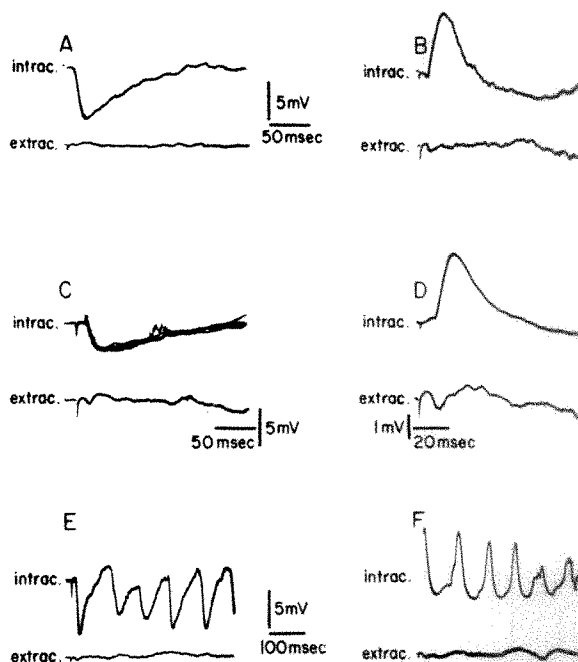


Fig. 1. Intracellular (intrac.) and extracellular (extrac.) records from the hindlimb area of  $S_1$  in response to a single afferent volley. Records A, B, D and F show the response to electrical stimulation at Th 10 of the dissected dorsal columns. Records C and E were obtained in a preparation with intact spinal cord. A, IPSP, resting membrane potential 63 mV. B, EPSP with postexcitatory hyperpolarization, resting membrane potential 70 mV. C, Hyperpolarization with depolarizing unitary potentials in response to tactile stimulation in a small skin area on the contralateral hindlimb, resting membrane potential 42 mV. D, EPSP, resting membrane potential 32 mV. E, Rhythmic inhibitory postsynaptic potentials elicited by an electrical shock to the contralateral hindlimb, resting membrane potential 63 mV. F, Rhythmic excitatory postsynaptic potentials elicited by a single electrical shock to the DC at Th 10, resting membrane potential 56 mV.

the hyperpolarization varied considerably between different cells. In one group of cells the excitatory effect had a similar duration to that in the cells showing only depolarization. In such cells postexcitatory hyperpolarization usually lasted less than 100 msec (Fig. 1B). In many cells the depolarization was very short, lasting only 5-10 msec and followed by a hyperpolarization, frequently with a duration of 100-150 msec. In the preparations with intact spinal cord some of these cells were identified as cells quickly adapting in response to bending hairs in a small contralateral receptive field. One group of cells responded to afferent stimulation only with an excitatory postsynaptic potential (EPSP). The membrane potential returned gradually to its resting value and there was no hyperpolarization (Fig. 1D). The mean latency of the EPSP was 9 msec and the mean duration 50 msec when the stimulus was applied at Th 10.

In the preparations with dissected spinal fascicles an EPSP or IPSP could be evoked in many cortical cells by an electrical shock to the DC as well as to the DLF. Other cells responded only to stimulation of the DC and in a few cells an EPSP was obtained only from the DLF.

In connexion with the synaptic potentials, unitary potentials were frequently observed. The amplitude of these potentials was usually one or a few millivolts. In most cells this activity appeared during the depolarization, but in some cells it appeared during the hyperpolarization (Fig. 1C). In some cells with a low membrane potential these unitary potentials were probably abortive action potentials and in these cells the spike arose from a primary depolarization. Most cells, however, had a constant resting membrane potential of 60-70 mV for many minutes and showed no injury discharge at impalement. Some of the cells could suddenly discharge with spikes of high amplitude which probably represented ordinary action potentials. It seems likely that the small unitary



potentials in these cells represent either the bombardment on the cell from excitatory presynaptic terminals or dendritic spike potentials not exciting the soma.

Regardless of the type of activation the initial response was frequently followed by repetitive after-discharges lasting for about 500 msec in response to one afferent stimulation. In other cells the initial response did not appear and there were only the late rhythmical discharges. Usually the following membrane potential oscillations were very similar to the initial response and consisted of rhythmical hyperpolarizations (Fig. 1E) or depolarizations (Fig. 1F). The interval between the initial response and the first after-discharge was about 100 msec, and the interval between the after-discharges was 60–80 msec. The rhythmical depolarizations probably represent the thalamic after-discharges now known to be due to post-inhibitory hyperexcitability in thalamic cells<sup>4</sup>. The rhythmical inhibitory potentials may have a similar origin or they may be due to a cortical event.

It is still too early to evaluate the relative significance of the inhibitory and excitatory activities evoked from the specific projection pathways in cells of the somatosensory cortex. It seems to be a reasonable working hypothesis that the inhibition eliminates stray excitation and thus subserves spatial discrimination.

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## HAEMATOLOGY

### Erythrocytic Enzymes of Various Animal Species

ENZYMES involved in the reduction of triphosphopyridine nucleotide (TPN) have been reported to be of primary importance in the resistance of erythrocytes to drug-induced hemolytic conditions<sup>1</sup>. A hereditary defect in man associated with erythrocytes sensitive to drugs (primaquine) has been described in which there is a deficiency of hexose monophosphate pathway enzymes in the erythrocytes. Evidence of a susceptibility to drug-induced hemolysis of erythrocytes has also been reported in the dog<sup>2</sup>.

The sheep and goat have been reported to be completely void of erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) activity by several investigators<sup>3,4</sup>, while others have reported very low activity<sup>5,6</sup>. There is, however, no reported evidence of increased drug sensitivity associated with these low or absent enzyme concentrations in sheep erythrocytes. Reports on the presence of G-6-PD in the erythrocyte of the pig<sup>5,7</sup> are also conflicting. The erythrocytes of most other domestic animals have been shown to contain about the same amounts of G-6-PD activity as do human erythrocytes<sup>5</sup>. Because of these conflicting reports concerning the pentose phosphate pathway enzymes and the fact that erythrocyte glucose metabolism can proceed alternatively via glycolysis to lactate the present study was undertaken to define the status of G-6-PD in relation to the glycolytic enzymes in several domestic animals.

All blood samples were collected from mature animals by venipuncture into heparinized tubes. Haemoglobin concentration was measured by the cyan-methaemoglobin method, and packed cell volumes (PCV) were obtained by the micro-haematocrit method. Haemolysates were pre-

pared from erythrocytes which had been previously washed with physiological saline, by addition of 10 or 20 volumes of distilled water. Haemoglobin concentration was determined in all haemolysates which were used for determination of enzyme activity. All enzyme measurements were conducted at 25° using a Gilford Model 2000 Multiple Absorbance Recorder which permitted the measurement of very small changes in absorbancy at 340 mμ in the presence of high levels of haemoglobin. Therefore, the separation of haemoglobin before the assay was not necessary as in experiments previously reported<sup>8</sup>. All enzyme activities are reported as μmoles of pyridine nucleotide reduced or oxidized per g of haemoglobin per h. Glucose-6-phosphate dehydrogenase was measured by a modification of the method of Glock and McLean<sup>9</sup>. Phosphohexoseisomerase was estimated by using fructose-6-phosphate, purified glucose-6-phosphate dehydrogenase, and TPN<sup>+</sup>. Aldolase and glyceraldehyde-3-phosphate dehydrogenase were assayed using DPN, arsenate, and fructose-1,6-diphosphate; in the case of the aldolase assay excess glyceraldehyde-3-phosphate dehydrogenase was added, and in the assay of the glyceraldehyde-3-phosphate dehydrogenase excess aldolase was added; in both cases DPNH production was measured. Lactate dehydrogenase was measured by a modification of a method described earlier<sup>10</sup>. All substrates, coenzymes, and enzymes were obtained from Sigma Chemical Company, St. Louis, Mo., U.S.A.

Table 1

	Species					
	Sheep	Pig	Horse	Dog	Monkey	Cow
No. of animals	12	8	4	4	6	4
PCV	37	43	36	44	45	37
Hb grams %	12.7	13.3	12.9	15.6	16.5	13.2
G-6-PD	24.7	414	602	237	173	231
PHI	828	984	1605	783	3978	984
Aldolase	37.2	84	490	75.6	81.5	43.8
G-3PD	768	715	1170	555	666	859
LDH	1381	1766	1170	1772	4626	637
G-6-PD/ALD						
ratio	0.66	4.93	1.23	3.13	2.12	5.04

Enzyme activities are reported as μmole of pyridine nucleotide reduced or oxidized per g of haemoglobin per hour

The results of these investigations are reported in Table 1 and indicate that G-6-PD activity is present in the erythrocytes of all species examined. The activity found in sheep erythrocytes was of such a low magnitude, however, that further studies were performed to determine whether the slow reduction of TPN was actually linked to G-6-PD. An ammonium sulphate fractionation of this enzyme resulted in a 12-fold increase in specific activity of the enzyme from sheep and a 7-fold increase from cattle from these findings. We would conclude that G-6-PD activity is present, though in small amount, in contrast to some earlier reports, in the erythrocytes of sheep. Since more enzyme could be used from these preparations, due to considerable haemoglobin removal, a 60-fold increase in activity per cuvette could be achieved and left no doubt as to the occurrence of G-6-PD in the erythrocytes of this species. The reported absence of activity may have been due either to the very low levels of enzyme present or to inefficient hydrogen acceptance by the dyes employed. The latter has been reported to occur in investigations with monkey erythrocytes<sup>11</sup>. The G-6-PD levels found in the other species are similar to the values reported by other investigators.

Of the glycolytic enzymes assayed, aldolase activity was the lowest and thus may be the rate-limiting enzyme of the glycolytic pathway. This is in agreement with other observations on human erythrocytes<sup>12</sup>. Glycolytic enzyme activities in the sheep, pig, dog and monkey in decreasing order were lactic dehydrogenase > phosphohexose isomerase > glyceraldehyde-3-phosphate dehydrogenase. This order does not apply to horse and cow erythrocytes. The G-6-PD/aldolase ratio suggests that among the various species there may be a marked variation in the relative importance of the glycolytic and pentose

pathways. Differences in the biochemical behaviour of the erythrocytes of various species may represent adaptations to different physiological needs and therefore may account for species differences in susceptibility to various haemolytic agents (such as copper poisoning in sheep). The presence of an inherited erythrocytic enzyme deficiency in animals is also possible and could similarly account for the unexplained susceptibility of individual animals to certain drugs. For example, the susceptibility to toxic effects of phenothiazine presents a peculiar dose problem because there is an extremely wide variation in the response of individuals of the same species to similar doses of the drug. Individual horses may also suffer a haemolytic crisis not shown by other animals<sup>13</sup>.

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## IMMUNOLOGY

### Specificity of Guinea-pig Antibodies and Delayed Hypersensitivity

THE pioneering work of Landsteiner<sup>1</sup> on the serological reactions of rabbit anti-hapten antibodies showed that these antibodies were highly specific for the simple haptenic molecule. Thus, antibody-hapten interactions usually depend on the chemical nature of the hapten rather than on that of the protein carrier to which the hapten is attached. While this hapten-directed specificity appears to be the general rule, it has been pointed out repeatedly in the past (most notably by Mutsaers and Gregoire<sup>2</sup> and Haurowitz<sup>3</sup>) that the rabbit may also produce antibodies the specificity of which extends to the hapten-protein linkage and may even encompass nearby portions of the protein carrier itself.

The immunological specificity of the delayed hypersensitivity response in guinea-pigs stands in direct contrast to the specificity of rabbit antibodies. As many authors have shown<sup>4-6</sup>, the elicitation of the delayed hypersensitivity reaction appears to involve an obligatory participation of the carrier protein as well as the haptenic moiety. Based on this specificity difference, it has been suggested that the delayed response recognizes and requires an appreciably larger antigenic determinant than does conventional circulating antibody. These specificity differences have also been cited in support of the suggestion that delayed hypersensitivity is unrelated to other forms of immunological response.

\* Since the results cited above were based on experiments involving two different animal species, it was of interest to examine the guinea-pig antibodies, frequently

detectable in the circulation a few days after the development of delayed hypersensitivity to a single injection of a hapten-protein conjugate. The specificity of these antibodies was compared with that of the delayed reaction on one hand and of rabbit anti-hapten antibodies on the other.

The haptens used for our experiments were *p*-amino-benzoate and *p*-nitroaniline, coupled to purified guinea-pig albumin (GpA) for immunization and testing and to crystalline hen ovalbumin ('Oval') for cross-testing. The haptens were attached to the carriers either by diazonium coupling forming 'azo' links mainly with the tyrosine and histidine residues, or by reacting their isocyanate or isothiocyanate derivatives with the ε-amino groups of the lysine residues of proteins to form respectively the carbamido or thiocarbamido conjugates.

Guinea-pigs were immunized with 10 μg of *p*-benzoate thiocarbamido-GpA or *p*-nitrobenzene carbamido-GpA, or with 100 μg of *p*-benzoate azo-GpA or *p*-nitrobenzene azo-GpA, injected intradermally in a complete Freund's adjuvant. The animals were bled daily from the 7th day after immunization. The sera obtained in this way were tested for antibodies by passive cutaneous anaphylaxis<sup>7</sup> using homologous antigens and also antigens heterologous with respect to either the hapten-protein linkage (azo vs. thiocarbamido) or to the carrier protein (GpA vs. 'Oval'). Control animals not employed in the antibody studies were skin-tested with 10 μg doses of the several antigens to assess the development of delayed sensitivity and its specificity.

In conformity with earlier observations, guinea-pigs sensitized in this manner developed typical delayed skin reactivity within 6-8 days after immunization. In every instance, the specificity was such that no cross-reactions were observed with either the homologous hapten on the different carrier protein, or with the homologous hapten linked differently to the same carrier protein employed for sensitization.

In each animal studied, the earliest antibody to appear in the circulation (usually on the 10th-14th day after immunization) was both carrier- and link-specific. Within 1-7 days thereafter, depending on individual variation, this was followed by the appearance of antibody which cross-reacted with antigens containing a heterologous link or carrier.

The variation in response among the different guinea-pigs was reflected not only in the time of appearance of cross-reacting antibodies but, curiously, in the temporal order of this appearance. The results outlined in Tables 1-3 are from individual animals selected to illustrate this point. In Table 1 are data from an animal which produced carrier- and link-specific antibody for only one day before cross-reaction for the heterologous link developed. One week later, antibody able to cross-react with the unrelated carrier protein appeared in the circulation. Table 2 shows an animal that formed very specific antibody for a full week before cross-reacting activity appeared first for the heterologous linkage and then for the heterologous carrier protein. On the other hand, the animal described in Table 3 developed a cross-reaction to the unrelated carrier protein some days before it formed antibody able to cross-react with the unrelated linkage. There appeared to be no consistent relationship between the order of decrease in antibody specificity and the type of hapten or linkage employed for immunization.

Preliminary experiments based on the known susceptibility of macroglobulins to reducing agents<sup>8</sup> suggest that the earliest antibody is mainly of the 19 S macroglobulin type. Treatment of some of the sera with 0.05 M 2-mercaptoethanol (buffered at pH 7.2) for 30 min at 37° C resulted in inactivation of the earliest, carrier- and link-specific antibody while affecting little or not at all the later, cross-reacting antibodies.

Some years ago we presented evidence<sup>9</sup> that the delayed hypersensitive guinea-pig might form anti-hapten anti-



Table 1. PASSIVE CUTANEOUS ANAPHYLAXIS WITH SERA FROM A GUINEA-PIG IMMUNIZED WITH BENZOATE THIO-CARBAMIDO-GPA\*

Days after immunization	Benzoate thio-carbamido-GpA	Test antigens	
		Benzoate azo-GpA	Benzoate thio-carbamido—(Oval)
9	—	—	—
10	+	—	—
11	++	+	—
15	++	++	—
16	++	++	—
17	++	++	+
18	++	++	++
21	++	++	++

Table 2. PASSIVE CUTANEOUS ANAPHYLAXIS WITH SERA FROM A GUINEA-PIG IMMUNIZED WITH p-NITROBENZENE CARBAMIDO-GPA\*

Days after immunization	p-nitrobenzene carbamido-GpA	Test antigens	
		p-nitrobenzene azo-GpA	p-nitrobenzene carbamido—(Oval)
9	—	—	—
10	++	—	—
11	++	—	—
16	++	—	—
17	++	+	—
18	++	+	+
19	++	++	++
20	++	++	++

Table 3. PASSIVE CUTANEOUS ANAPHYLAXIS WITH SERA FROM A GUINEA-PIG IMMUNIZED WITH BENZOATE AZO-GPA\*

Days after immunization	Benzoate azo-GpA	Test antigens	
		Benzoate thio-carbamido-GpA	Benzoate azo—(Oval)
11	—	—	—
14	++	—	—
15	++	—	+
16	++	—	++
17	++	—	++
18	++	±	++
21	++	±	++
24	++	++	++
29	++	++	++

\* Each serum was diluted two-fold with saline, injecting 0.1 ml. intradermally. Three hours later, 1 ml. of a solution containing 1 mg. of antigen and 1.5 per cent 'Pontamine Sky Blue 6BX' dye was injected intravenously, and the resultant reactions were graded 15 min thereafter, based on the diameter of the coloured spot<sup>7</sup>.

bodies with a specificity extending to the haptenic linkage and presumably also to the amino-acid to which the hapten is attached. The present data extend this observation and indicate that antibody with even more stringent requirements for antigen may be formed under similar circumstances. The specificity of this antibody involves a determinant large enough to include not only the hapten, but also a portion of the carrier protein molecule adjacent to it. Neither hapten nor carrier protein alone appears to provide sufficient interaction energy to mediate the passive cutaneous anaphylactic reaction. Such a specificity is typical of delayed hypersensitivity, and suggests that antibody formation and delayed hypersensitivity are not separable on the basis of specificity considerations alone. The demonstration that the carrier protein also partakes in the specificity of immunological tolerance<sup>9</sup> and of the anamnestic antibody response to hapten-protein conjugates<sup>10,11</sup> further emphasizes this point.

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## PATHOLOGY

### Simultaneous Infection of a Bovine Foetus by Two Fungi

RECENT investigations have shown that fungi are associated with bovine abortion in Hong Kong as in other countries. In each instance the probable pathogen was isolated and, except for one foetus, a single organism was considered to be the abortifacient. The exception, described here, occurred in a 6-month-old foetus which was infected by two fungi.

The material consisted of the foetal stomach and its contents, umbilical cord, heart and pieces of skin, and was examined and cultured within a few hours of abortion. It was examined macroscopically for visible symptoms of infection and also microscopically by squashing small pieces of tissue and by sections. To isolate the fungi small tissue slices were surface-sterilized by immersion in 95 per cent alcohol and then plated on to Sabouraud dextrose agar ('Oxoid'). Foetal stomach contents were extracted into a tube by pipetting through a sterile incision in the stomach wall; the fluid was then streaked on to the medium using a glass rod. All plates were incubated at 25° C and at ambient temperatures (approx. 30° C) and examined after 3 days when colonies which had developed directly from the tissues were subcultured for further examination. No bacterial tests were made as the parent had received routine protection against *Brucella abortus* and neither this bacterium nor *Vibrio fetus* had been isolated from foetuses in many other cases of abortion in the same herd.

Macroscopic examination of the skin and stomach contents indicated severe fungal infection of the foetus. Numerous discrete lesions were readily observed on the skin pieces. These were of variable size up to 5 mm diameter, pale in colour, and not restricted to any particular region. The stomach fluid contained visible aggregations of material and its consistency was considerably thicker than that from uninfected foetuses of the same age. This greater density was similar to that noted on examination of the stomach contents from other specimens of bovine mycotic abortion. No obvious lesions were seen on the other tissues examined.

Microscopic examination of squash preparations and sections showed numerous septate hyphae in the outer layers of skin tissue. No hyphae were seen in sections of the other tissues. Copious mycelium was found in the stomach fluid (Fig. 1). This had the broad, aseptate and branched structure typical of a phycomycete, and together with desquamated epithelial cells from the stomach wall constituted the visible aggregations.

No colonies developed from cultures of umbilical cord and heart tissue. However, cultures of stomach tissue and contents all yielded numerous colonies of *Mucor* after 3 days. These were sub-cultured and later iden-



Fig. 1. Hyphae of *Mucor dispersus* and desquamated epithelial cells in the stomach contents of an aborted bovine foetus



tified as *M. dispersus*, a species of this genus which does not appear to have been recorded previously as an abortifacient. No development of *Mucor* was recorded from any plate containing skin tissue. Fungus colonies also developed from each piece of cultured skin tissue but their rate of development was much slower. Sub-cultures from these yielded sterile grey-brown colonies of dematiaceous mycelium only. Although this organism grew readily on simple synthetic media all attempts to induce sporulation were unsuccessful.

Skin lesions are infrequent features of aborted foetuses in Hong Kong, although a survey elsewhere showed them to be present in almost half the cases examined<sup>1</sup>. In the foetus described it was not possible to ascribe the cause of abortion to either or both organisms. Numerous species of *Mucor* have been named as abortifacients and there have been many instances where fungi, although seen to be present by microscopic examination, have failed to develop and sporulate following abortion<sup>1</sup>. Simultaneous association of two fungi in bovine abortion is apparently of rare occurrence, and no reference to this phenomenon could be found in available literature. However, as the majority of fungal abortifacients are distributed both widely and abundantly, with concomitant opportunity to induce infection, it is possible that multiple infection occurs more frequently than the records suggest.

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### Effect of Age and Hormonal State on the Numbers of Deoxyribonucleic Acid Synthesizing Nuclei in Rat Adrenal Cortex

THE use of tritiated thymidine to label nuclei synthesizing DNA (S-phase) prior to mitosis has provided much new information on the pattern and magnitude of cell turnover in many tissues and organs. Less is known, however, about the relative frequency of cell turn-over in the adrenal gland. Accordingly the investigation described here was undertaken to determine whether the number of nuclei in DNA synthesis in the different zones of the rat adrenal cortex varied with age and with experimental procedures designed to alter its hormonal environment.

To investigate the effect of age, 3 groups of normal rats near 60, 175 and 250 g body-weight were examined. All were albino males of an inbred strain, maintained on a commercial pellet diet and tap water. Tritiated thymidine (1  $\mu$ Ci/g) was given as a single intraperitoneal injection at 10 a.m. and the rats were killed 4 h later by bleeding under ether anaesthesia. Constant labelling times were maintained throughout this and subsequent experiments to avoid diurnal fluctuations in mitotic rhythm and DNA synthesis<sup>1</sup>. Autoradiographs of the adrenals were prepared

with Kodak 'AR 10' film, as previously described<sup>2</sup>, and the percentage of labelled nuclei (labelling index) in zona glomerulosa, the outer and inner parts of fasciculata, and zona reticularis was measured. In assessing fasciculata labelling outer and inner layers 8 cells in width were selected. The labelling index was calculated from counts of at least 1,000 nuclei in each zone per adrenal and, as far as possible, fibroblasts and vascular endothelial cells of the reticular framework of the gland were excluded.

The results for normal rats are shown in Table 1. The labelling index was highest in the outer layer of zona fasciculata at all ages. Labels were slightly less frequent in zona glomerulosa, and the inner fasciculata and reticularis zones contained few radioactive nuclei. In all zones, apart from zona reticularis, the labelling indices were significantly higher in young 60-g rats, reflecting the new cell formation necessary for active growth at this age and paralleled by frequent labelling of nuclei in other organs such as kidney and liver. The differences in labelling of adrenals from 175- and 250-g rats were marginal and not statistically significant, and this was taken as evidence of the relative stability of adrenal cortical nuclei in more mature animals maintained in normal non-stressful laboratory conditions.

The effect of variations in the hormonal state on the tritium-labelling index of the various adrenal cortical zones was examined in 4 groups. The first comprised 4 rats given adrenocorticotrophic hormone (ACTH) (10 units) daily for 14 days. The second group contained 8 rats given hydrocortisone (5 mg) daily for 28 days. The third consisted of 20 rats sensitized by unilateral nephrectomy and drinking 1 per cent sodium chloride as described by Selye, Hall and Rowley<sup>3</sup>, and given microcrystalline deoxycorticosterone (50 mg/kg) at 10-day intervals for 6 weeks. The final group of 8 rats with one kidney removed and drinking saline for 6 weeks served as controls. All hormones were injected subcutaneously. Tritiated thymidine was given and autoradiographs were prepared as already described here.

The results are summarized in Table 1. ACTH caused marked adrenal enlargement and a significant increase in the labelling index ( $P < 0.001$ ) in outer zona fasciculata when compared with normal rats of similar body-weight (173 g). Labelling of the other cortical layers was only slightly increased by ACTH and did not reach the limits required for statistical significance. Hydrocortisone induced adrenal cortical atrophy which obscured the junction between the glomerulosa and fasciculata zones and made certain identification of individual cell types less reliable. Accordingly counts of labelled nuclei in zona glomerulosa and the outer fasciculata layer were combined in the hydrocortisone-treated group ( $0.2 \pm 0.05$ ) and compared with a similar composite zone in normal 173-g rats ( $1.1 \pm 0.16$ ). Hydrocortisone significantly depressed the labelling index in this outer cortical layer ( $P < 0.001$ ), but had no effect on the inner fasciculata and reticularis zones. Sodium chloride, either alone or combined with deoxycorticosterone, significantly depressed the labelling index in zona glomerulosa ( $P < 0.001$ ) when compared with mature normal rats (247 g). The administration of deoxycorticosterone in these circumstances

Table 1. FREQUENCY OF TRITIATED-THYMIDINE LABELLED NUCLEI IN ADRENAL CORTIX OF NORMAL AND HORMONE-TREATED RATS (Means  $\pm$  S.E.)

Group	No. of rats	Body-wt. (g)		Left adrenal (mg)	% Labelled nuclei in zones			
		Initial	Final		Glom.	Outer fasc.	Inner fasc.	Retic.
Normal	4	—	58 $\pm$ 1.4	9.5 $\pm$ 0.27	2.1 $\pm$ 0.23	3.5 $\pm$ 0.20	0.9 $\pm$ 0.09	0.4 $\pm$ 0.14
Normal	4	—	173 $\pm$ 4.3	19.1 $\pm$ 1.05	0.8 $\pm$ 0.13	1.1 $\pm$ 0.14	0.3 $\pm$ 0.05	0.5 $\pm$ 0.08
Normal	6	—	247 $\pm$ 4.2	21.2 $\pm$ 0.90	0.6 $\pm$ 0.05	0.8 $\pm$ 0.13	0.2 $\pm$ 0.04	0.3 $\pm$ 0.04
ACTH	4	165 $\pm$ 0.7	147 $\pm$ 9.8	69.0 $\pm$ 6.02	1.0 $\pm$ 0.17	4.4 $\pm$ 0.45	0.8 $\pm$ 0.23	0.3 $\pm$ 0.17
Hydrocortisone	8	148 $\pm$ 1.0	189 $\pm$ 5.3	12.2 $\pm$ 0.30	—	0.2 $\pm$ 0.05*	0.3 $\pm$ 0.16	0.4 $\pm$ 0.14
Deoxycorticosterone + saline	20	213 $\pm$ 1.7	264 $\pm$ 6.5	35.0 $\pm$ 1.67	0.3 $\pm$ 0.06	0.7 $\pm$ 0.09	0.2 $\pm$ 0.03	0.1 $\pm$ 0.03
Saline	8	215 $\pm$ 3.0	260 $\pm$ 7.8	24.1 $\pm$ 0.95	0.2 $\pm$ 0.04	0.5 $\pm$ 0.08	0.2 $\pm$ 0.05	0.1 $\pm$ 0.03

\* Assessed from combined glomerulosa and outer fasciculata.

causes hypertension in a proportion of animals<sup>2</sup>, but the drinking of 1 per cent saline alone has little effect on the blood pressure. Since the reduction in glomerulosal labelling was equivalent in both groups it would appear that sodium chloride excess rather than hypertension was the important factor influencing cell turnover in zona glomerulosa in these experiments. Deoxycorticosterone and/or saline did not alter the labelling index in zona fasciculata but both reduced uptake in reticularis.

The use of tritiated thymidine as a flash-label<sup>4</sup>, as in the investigations recorded here, permits reliable measurements of the effects of age and hormones on cell turnover in the rat adrenal cortex. More extended investigations to trace the migration pathways and fate of labelled cortical cells might resolve at least some of the controversy concerning the zonal, centripetal, and transformation theories of adrenal cortical cell origin<sup>5</sup>. It is clear at present, however, that zona reticularis cannot be regarded as a completely senescent zone in the rat, for flash-labelled nuclei were present in all experiments. It is also apparent that prolonged treatment with either deoxycorticosterone or hydrocortisone, both of which have a well-recognized effect on adrenal morphology and biosynthetic capacity, does not thereby completely inhibit DNA synthesis in all nuclei in the appropriate cortical layer. Possibly the results we have obtained after such treatment approach the basal values of cell replacement that are necessary if the structural integrity of the adrenal cortex is to be maintained, even in an altered form.

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### A Cytolytic Parasite in the Cells of Transplantable, Malignant Tumours

At this institute two transplantable ascites tumours, the Yoshida rat sarcoma and the Ehrlich mouse carcinoma, have for a number of years been kept alive by intraperitoneal passages and used in immunological research. Through these years each tumour after injection in rats and mice of the same strain has performed very consistently with a very low survival rate—about 5 per cent—and with uniform survival times before death from malignant ascites.

Testing another specimen of the Yoshida sarcoma we found two years ago a much higher survival rate—about 50 per cent—and much longer survival times after transplantation to our rats. Microscopic examinations of the ascitic fluid revealed some peculiar changes of the sarcoma cells. Further observations have shown these to be due to an intracellular infection by a parasite, which when found outside the cell shows a long flagellum.

The parasite is found, usually in great numbers, in the cytoplasm of the cells, most often as clusters filling vacuoles, or 'cysts' (Fig. 1), either in one large or in several small cysts. The number of parasites in each cyst ranges from three to about forty. Rarely, a few parasites are found scattered singly in the cytoplasm, and sometimes one or two parasites can be seen in the nucleus. In smears stained by Giemsa's method the parasite is small, measuring c. 1–2 $\mu$ . The size is rather uniform, the shape rounded or oval, although a few larger, elongated forms can be found, and each parasite displays some structural

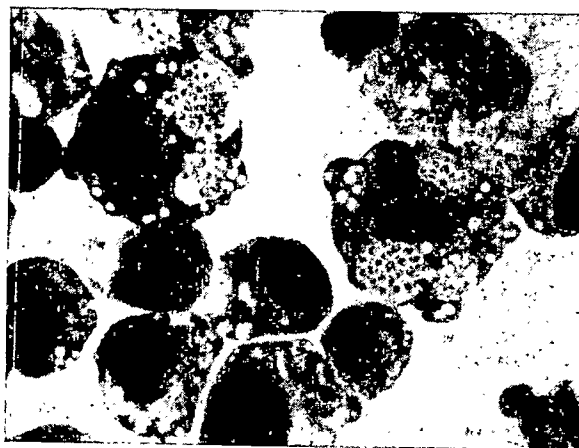


Fig. 1

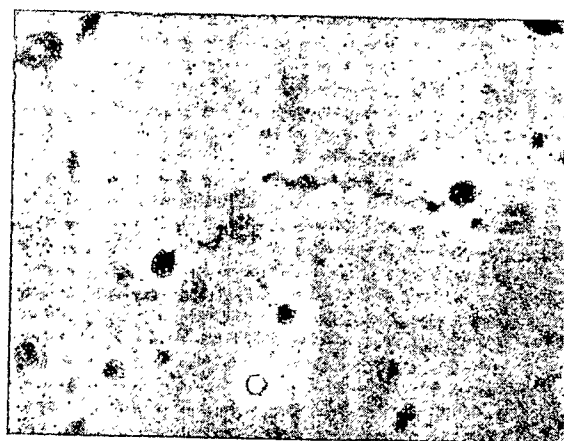


Fig. 2

differentiation. An unstained area occupies a large part of the cell, and a distinct, nucleus-like structure, taking a reddish-purple stain, is found in the periphery of this region, while the rest of the cell is evenly blue.

During the two years we have transferred the infected sarcoma a gradual adaptation between the parasite and the sarcoma cells has taken place, and the result of a peritoneal transfer now differs only slightly from what happens after injection of the uninfected Yoshida sarcoma. By now the survival rate closely approaches the low percentage of the uninfected sarcoma and the survival times are but slightly longer. When first observed the animals often produced large amounts of ascitic fluid with rather few sarcoma cells but now the fluid production is less severe, the cell counts having approached those of the uninfected sarcoma. The percentage of infected cells is rather constant, being about 25 per cent. Whereas subcutaneous injection of the uninfected sarcoma shows the same high percentage of 'takes' as intraperitoneal injection, the infected sarcoma does not produce any solid tumour when injected subcutaneously.

Rats surviving intraperitoneal transplantation are resistant to subsequent transplantation of the uninfected tumour. When killed at various intervals after injection of the infected tumour, however, a severe fibrinous peritonitis can be found leading to complete obliteration of the peritoneal cavity by fibrous adhesions. Attempts to cultivate the parasite in various bacteriological media have been unsuccessful.

While intraperitoneal injection of the uninfected rat tumour in mice produces no apparent ill-effects the infected rat tumour will by this route kill the majority of mice within six to ten days. In the mouse peritoneum the parasite multiplies rapidly and may readily be

observed by the phase-contrast microscope. Fig. 2 shows the appearance of an extracellular parasite, the flagellum being noticeable. Rats made resistant to transplantation by the intracoeal vaccination of Lund<sup>1</sup> are clinically unaffected by intraperitoneal injection of the parasitized tumour, so the rats do not show a susceptibility to the parasite itself as found in mice. At present it seems probable that the parasite can be cultivated by intraperitoneal passage in mice, although in small numbers.

By intraperitoneal transplantation of the Ehrlich carcinoma and subsequent introduction of the parasite I have recently been able to develop an infected, transplantable Ehrlich carcinoma in mice, the tumour cells, however, disappearing after nine passages.

The establishment of a transplantable, parasitized, malignant tumour in each of two species of animals and the cytolytic property of the parasite towards the cancer cells make identification desirable. A parasite has recently been described by Nelson<sup>2</sup> and by Werner and Pierzynski<sup>3</sup>, associated with ascites in white mice, which shows important similarities in morphology and behaviour to a possible point of identity with the parasite described in this communication. In none of these cases, including that now described, has the source of the parasite been found, but even the parasite described by Nelson was connected with experimental cancer research, as it was found in mice examined because of an atypical response to certain chemical carcinogens. Nelson suggests classification in the protozoan order Microsporidia. The mutual adaptation between tumour and parasite may be overlooked and might be misleading, in the experimental use of transplanted tumours.

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## Role of the Fibrous Capsule in Carcinogenesis by Plastic Film

THE ability of materials in the form of a film to induce sarcomas at the site of implantation was first shown by Turner in 1941<sup>1</sup> when he implanted disks of 'Bakelite' subcutaneously in rats. Oppenheimer and his associates re-discovered this phenomenon in 1948<sup>2</sup>. Later, they extended their work to show that the same applied to mice and that many different kinds of plastic film, metal foils, and glass cover slips were capable of inducing sarcomas<sup>3,4</sup>. Few tumours developed when the same materials were implanted in the form of powders, granules, or sponges<sup>5</sup>.

The importance of the physical form of the materials for tumour-induction has been generally recognized, but the mechanism by which the film acts is still poorly understood. Oppenheimer *et al.* carried out histological studies comparing the reaction around a piece of film with the reaction around a textile of the same material<sup>6</sup>. The film became surrounded by a fibrous capsule which by six months had become avascular and relatively acellular. Connective tissue fibres penetrated the textiles, but no capsules formed around them. At varying periods after six months, renewed fibroblastic activity, which they felt was a pre-sarcomatous stage, was seen in the inner aspects of some capsules. They found that the plastic film could be removed from the capsules after six months without affecting the number of tumours which developed. Removal before this time prevented sarcoma formation.

Since the discovery by Malmgren *et al.*<sup>7</sup> that a number of carcinogenic chemicals suppressed antibody formation in mice, and the discovery by Foley<sup>8</sup> and by Prehn and Main<sup>9</sup> that methylcholanthrene-induced sarcomas in

mice contain new antigens which are not present in the normal tissues of the host, there has been speculation that carcinogenic activity may be directly related to the ability of a substance to suppress the immune mechanism. The fact that the plastic film no longer had to be present after six months in Oppenheimer's experiment, and that by this time the fibrous capsule around the film had become dense and avascular, suggested that in this type of carcinogenesis as well, suppression of the immune mechanism might be an important factor. The dense fibrous capsule might act as a barrier to immunologically competent cells, thus functioning in the same manner as a 'Millipore' diffusion chamber<sup>9</sup>; or the avascularity of the inner layers of the capsule might result in delayed vascularization of the developing tumour<sup>10</sup>. In this protected environment, it is possible that antigenically altered 'mutant' cells could survive and proliferate, whereas they might be destroyed in a site more accessible to the host's immune mechanism. This work was designed to determine whether the interior of the capsule is a preferred site for survival of antigenic tumours.

Three separate experiments were performed. In each, the skin on the dorsum of *BALB/cAn* mice six to twelve weeks old was incised, and a round piece of plastic film was placed in the subcutaneous tissue beneath the panniculus carnosus. Approximately nine months after implantation of the film, the skin was incised and lifted away from the body until the film and its surrounding fibrous capsule were found. In the experimental animals, a small slit was made at one edge of the capsule, and a piece of a transplanted fibrosarcoma which had been induced by 3-methylcholanthrene in a *DBA/2* mouse was injected into the space beside the film through a 21-gauge trocar. The trocar was inserted through the slit in the capsule, and the tumour was placed at the side of the capsule opposite the slit. In the control animals, the tumour was injected into the subcutaneous tissue adjacent to, but not inside, the fibrous capsule.

The three experiments varied somewhat with respect to the sex of the mice, the transplant generation of the tumour, and the size and material of the plastic. In Experiment 1, there were six females and ten males which were allocated equally between experimental and control groups. A first transplant generation tumour was used for inoculation. The film consisted of 'Cellophane' dialysing membrane 8 mm in diameter. In Experiment 2, 18 males received film consisting of 'Millipore' filters which had been fused with acetone. These were 20–23 mm in diameter. A second-generation tumour was transplanted. In Experiment 3, 'Cellophane' dialysing membrane 14 mm in diameter was used. The tumour was in the second generation when it was transplanted.

The animals were checked at weekly intervals for growth of the transplanted tumours. In several cases, there appeared to be growth during the first three or four weeks after transplantation, followed by regression. Table 1 summarizes the results. In the column headed 'Tumours grew progressively' are included tumours which showed progressive growth for more than two months and reached a size of at least 12 mm in diameter before the animals were killed. The column headed 'Tumours grew > 5 mm in diameter' includes both the tumours which grew progressively and those which grew to at least 5 mm in diameter as measured by palpation through the skin, but which then regressed. Nodules

Table 1. SURVIVAL OF HOMOLOGOUS TUMOURS INSIDE AND OUTSIDE FIBROUS CAPSULES

Experiment	Tumours transplanted		Tumours grew progressively		Tumours grew > 5 mm in diam.	
	Inside capsules	Outside capsules	Inside capsules	Outside capsules	Inside capsules	Outside capsules
1	8	8	4	2	6	2
2	0	9	5	1	7	2
3	15	15	5	2	12	4
Total	32	32	14	5	25	8

$\chi^2 = 4.7906$   
 $P < 0.05$

$\chi^2 = 16.0156$   
 $P < 0.001$



less than this size were not included because of the difficulty in determining whether growth had really occurred. Since the plastic film itself induces sarcomas, it was necessary to determine whether the progressively growing tumours were of *BALB/c* or *DBA/2* origin. In Group I, it was assumed that all were of *DBA/2* origin since in a control group of fifty *BALB/c* mice implanted with the same type and size of film at the same time, the first tumour did not arise until 17 months after implantation. In Groups II and III, however, a few tumours had developed at the site of implantation before the *DBA* tumours were inoculated. With these groups, the strain of origin of each progressively growing tumour was determined by evaluating its growth on transplantation to *DBA/2* and (*BALB/cAn* × *C<sub>3</sub>H/He*)*F<sub>1</sub>* hybrids.

These results demonstrate that the fibrous capsule which forms around a piece of plastic film inserted subcutaneously is a preferred site for survival and growth of moderately antigenic tumours. This is indicated best by the figures related to progressive growth of the transplanted tumours. The figures which include 'tumours' which grew for a period and then regressed must be viewed with caution because those tumours were not studied histologically. It is possible that some were bacterial abscesses resulting from contamination at the time of inoculation. The reason for the preferential survival within the capsules is not demonstrated. The tumour which was transplanted was antigenically different from the host, though it did not differ with respect to the strong *H-2* antigens. Even so, the tumour was probably more strongly antigenic with respect to its homologous host than plastic film-induced tumours appear to be in isologous hosts<sup>11</sup>. The results are thus compatible with the hypothesis that the capsule acts as a barrier to the immune mechanism, but they do not prove it. Tumours may grow better within the capsule for other reasons. If it can be shown that isologous tumours which are not antigenic for their strain of origin do not grow better within the capsule than outside, the hypothesis that the capsule acts as a barrier to the immune mechanism will be more firmly established.

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## HISTOLOGY

### Histochemical Demonstration of Conjugated and Unconjugated Bilirubin using a Modified Diazo-reagent

• Most of the methods proposed for the staining of bilirubin in tissues involve oxidation of the pigment with production of coloured compounds such as biliverdin<sup>1-5</sup>. Only conjugated bilirubin appears to be stained

by such techniques, and negative results have been reported with brain tissue from infants with kernicterus. The diazo reaction also has been used to stain bilirubin in tissues<sup>6,7</sup>, but attempts to show different types of bilirubin have not met with success<sup>8</sup>.

This communication describes a method for a van den Berg reaction at a histochemical level. Unfixed cryostat sections stained with diazotized dichloraniline demonstrate the presence, in tissues, of conjugated bilirubin, which gives a 'direct reaction' in aqueous solution, and unconjugated bilirubin, which only reacts in the presence of a suitable accelerating solution. The use of dichloraniline was proposed by Rand and Di Pasqua<sup>9</sup> for serum bilirubin estimations.

The stock solutions were: *A*, dissolve 200 mg 2,4-dichloraniline in 100 ml. water containing 2.5 ml. conc. HCl and filter; *B*, 1 per cent sodium nitrite (both solutions *A* and *B* should be freshly prepared every two weeks and kept in the dark in a refrigerator); *C*, dissolve 6 g caffeine, 10 g sodium benzoate and 10 g urea in 100 ml. water. To 35 ml. of this solution add 25 ml. 40 per cent formalin and make the volume up to 85 ml. with water, then add 15 ml. methyl ethyl ketone.

The reagents should be freshly prepared and kept in an ice bath: (1) aqueous diazo reagent—add 1 vol. solution *B* to 50 vol. solution *A* and leave for 20 min in an ice bath before use; (2) accelerator-diazo reagent—add 1 vol. of aqueous diazo reagent to 2 vol. of solution *C*.

Tissue is rapidly frozen and 6μ cryostat sections are cut, mounted directly on to slides and allowed to dry for 1 h, in the dark, at room temperature. If necessary, sections can be kept in a cool dark place for several weeks without loss of staining properties.

(1) To demonstrate conjugated bilirubin ('direct' reaction): (i) immerse slides for 10 min in aqueous diazo reagent; (ii) wash for 3 min in running tap water; (iii) fix for 3 min in 10 per cent formol saline; (iv) counter-stain with haematoxylin, if desired, and mount in Apathy's syrup. Conjugated bilirubin is stained blue-violet.

(2) To demonstrate both conjugated and unconjugated bilirubin: untreated sections or sections previously stained by the aqueous diazo reagent, but not fixed, can be used. (i) immerse sections for 8 min in the accelerator-diazo reagent; (ii) wash in running tap water for 3 min; (iii) counter-stain with haematoxylin if desired, and mount in Apathy's syrup. Bile pigments in the section are stained red. Other pigments, such as lipofuscin, remain unstained.

With this method it has been possible to demonstrate in human liver and kidney from patients with cirrhosis and obstructive jaundice the presence of a yellow pigment, mainly localized in bile canaliculi (Fig. 1), which gives a 'direct' reaction with an aqueous solution of dichloraniline. This reaction is not given by the pigment present in livers of Gunn rats, whose only bile pigment is unconjugated bilirubin. It therefore seems likely that the pigment which has been stained is conjugated bilirubin, the major part of which is bilirubin glucuronide<sup>10</sup>.

Although extension of the staining time does not increase the amount of pigment stained, it is possible that, for technical reasons, some of the conjugated bilirubin in the tissue remains unstained by the aqueous diazo reagent. There seems, however, little doubt that, qualitatively, a positive reaction is indicative of the presence of bilirubin glucuronide in the liver cells and bile canaliculi.

The accelerator-diazo reagent gives extensive staining of liver cells, Kupffer cells and bile canalicular contents. When added to tissue which has previously been stained with the aqueous diazo reagent, further staining immediately occurs (Fig. 2), sufficiently intense to be seen with the naked eye. This reaction will be given both by any conjugated bilirubin which has not been previously stained, and by the unconjugated bilirubin. It stains the pigment of Gunn rat liver.



Fig. 1



Fig. 2

Figs. 1 and 2. Needle biopsy of liver from a patient with large bile duct obstruction

Fig. 1. Stained with aqueous diazo reagent showing the conjugated bilirubin in blue violet (shaded black in picture) ( $\times c. 385$ )

Fig. 2. The same field stained with accelerator diazo reagent, showing conjugated and unconjugated bilirubin in red (shaded black in picture). Note staining much more intense than in Fig. 1, especially within the cells ( $\times c. 385$ )

With this procedure it is possible to examine sections stained for the bile pigments within a few minutes of receiving a specimen. The application of the technique to liver biopsy material is at present under investigation.

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## BIOLOGY

### Increase in Fruit Set of *Vitis vinifera* by Treatment with Growth Retardants

FRUIT set of seedless cultivars of the grape (*Vitis vinifera* L.) has been improved by treatment with auxins<sup>1,2</sup>, gibberellins<sup>3</sup>, and kinins<sup>4</sup>. By contrast, fruit set of seeded cultivars has not been improved by treatment with these growth regulators. For two reasons, the set of seeded and seedless grapes has been examined further: first, to examine the effect of pre-anthesis treatments—previous

experiments have for the most part been confined to application at or soon after anthesis, whereas earlier treatments have been shown to increase fruit set in pears<sup>5</sup> and apples<sup>6</sup>; secondly, to compare regulators which have been classified as auxins and gibberellins with anti-auxins and anti-gibberellins (or growth retardants); the latter group has not previously been tested on grapes.

Single inflorescences were treated on vines growing in the field by a momentary dip in the appropriate aqueous solution containing 0.05 per cent v/v 'Tween 20'. All treatments were applied to one vine as a replicate, and there were nine replicates. Three times of treatment were compared: 2–3 weeks pre-anthesis, anthesis, and 2–3 weeks post-anthesis. When mature, the inflorescences were gathered and measurements made of the number of berries set, and other variables.

The two substances classed as growth retardants, 2-chloroethyltrimethylammonium chloride (CCC or 'Cycocel') and tributyl-2,4-dichlorobenzylphosphonium chloride ('Phosfon-D'), when applied before anthesis, considerably increased the number of berries set on three cultivars (Table 1). There were differences between these three cultivars in the response obtained, and the fourth cultivar, 'Doradillo', did not respond to either. In no case was set increased significantly by treatment with these substances at, or after, anthesis.

Besides the growth retardants, gibberellic acid (GA<sub>3</sub>) also altered the set and development of grape berries when applied before anthesis. The effects of CCC and GA<sub>3</sub> on the seeded cultivar, 'Muscat of Alexandria', are compared in Table 2 and Fig. 1. CCC increased fruit set by increasing the number of seeded berries per cluster, with a commensurate increase in cluster weight. Its only other effect was to increase slightly the size of seedless berries. The rachis, pedicel, seed and flesh tissue appeared

Table 1. Effect of dipping grape inflorescences in solutions of CCC and 'Phosfon-D' (100 mg/l.) 2 to 3 weeks before anthesis, on the total number of berries set per inflorescence, measured when ripe

Cultivar	Expt. No.	Untreated	CCC	'Phosfon-D'
'Zante currant'	1	244	403*	279
	2	276	581*	588*
'Sultana'	3	231	299	408*
'Muscat of Alexandria'	4	88	138†	128*
'Doradillo' (syn. 'Jaen')	5	227	226	172

\*, † Values significantly greater than untreated at probabilities > 1 per cent and 0.1 per cent respectively.

Table 2. Effects of dipping 'Muscat' inflorescences in solutions of CCC (100 mg/l.) and GA<sub>3</sub> (20 mg/l.) 18 days before anthesis, on the fruit measured 130 days later (Experiment 4)

	Untreated	CCC	GA <sub>3</sub>
Cluster fresh weight (g)	241.0	406.0†	223.0
Cluster length (cm)	19.8	19.9	25.4†
No. of berries per cluster	seeded	68.0	120.0†
	seedless	20.0	18.0
	total	88.0	138.0†
Percentage of seedless berries	23.0	13.0‡	83.0†
Seeded berries	fresh wt. (g)	3.86	3.67
	length (mm)	19.6	18.9
	width (mm)	18.0	17.9
Seedless berries	fresh wt. (g)	0.93	1.24*
	length (mm)	11.2	12.7*
	width (mm)	10.4	12.2†
Juice sugar content by refractometer (%)	22.5	23.0	25.2†

\*, †, ‡: values significantly greater than untreated at probabilities > 5 per cent, 1 per cent and 0.1 per cent, respectively. (‡, †: significantly less than untreated).

to be unaltered. GA<sub>3</sub> also increased fruit set, but most of the berries were seedless; cluster weight remained the same as untreated because of the lighter weight of seedless berries. The berries were longer and narrower than untreated, and also sweeter. All parts of the inflorescence framework were elongated and the rachis became 'wiry' (Fig. 1).

Subsequent trials have confirmed the effect of CCC in increasing grape set, particularly in 'Muscat'. To be effective, it may be applied as a cluster dip or a vine spray, but it must be applied before anthesis. Frequently the treatment results in smaller berries, although cluster weight is still increased because of the large increase in set.

An interesting aspect of these results is that the three cultivars which responded to growth retardants have different types of berry development: 'Zante Currant' is parthenocarpic, 'Sultana' is stenospermocarpic (that is, the seeds abort when half grown), and 'Muscat of Alexandria' is seeded. So far as I am aware, these are the first chemical treatments which have caused a large increase in the set of grapes with seeds.

This effect of growth retardants seems unusual when compared with their other effects. For example, they retard stem growth and internode elongation, fatten stems, slow leaf blade expansion, make leaves darker green and delay seed germination<sup>7</sup>. Many of these effects are opposite to the effects of gibberellic acid and have led to their designation as anti-gibberellins. In fact, it has been shown that simultaneous treatment with gibberellic acid prevents these substances from retarding stem



Fig. 1. Typical 'Muscat' clusters from the experiment described in Table 2: left, CCC; middle, untreated; right, GA<sub>3</sub>.

elongation<sup>8</sup>. From the results in Table 2 the only variables where GA<sub>3</sub> and CCC show opposite effects are the number of seeded berries per cluster and the percentage of seedless berries. It remains to be seen whether these effects are related or whether the substances interact when applied together. Nothing is known about the mechanism of this effect on set, but, in view of the effects of these compounds on other plant growth processes<sup>9,10</sup>, it is likely that an investigation of the mechanism will aid our understanding of the fruit setting process.

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### Lateral Movement of Inorganic Solutes in Plants

PREVIOUS work in inorganic solute movement in plants has suggested that the minerals follow a specific polar path from the roots which absorb them to the leaves in which they are deposited<sup>1,2</sup>. Recently, evidence supporting polar transport has been provided by Caldwell using a split root technique<sup>3</sup>. It was established that when the root systems were placed half in rich loam and half in sand the nutrient status of the two halves was reflected in the development of the foliage directly above. This result was interpreted as a polar movement of nutrients. However, the important issue is not whether polar movement can occur, but whether it must occur, owing to the absence of lateral transport systems.

*Coleus* is particularly suitable for an investigation of this kind because the main vascular bundles are situated at the angles of the square stem. We used Caldwell's split root technique on *Coleus frederici*. Cuttings were taken and a fortnight later the rooted stumps were carefully split from the base upwards, and the plants potted so that each half of the root system was contained in a separate pot (Fig. 1). In this way the nutrition and water supply of the two halves could be varied independently.

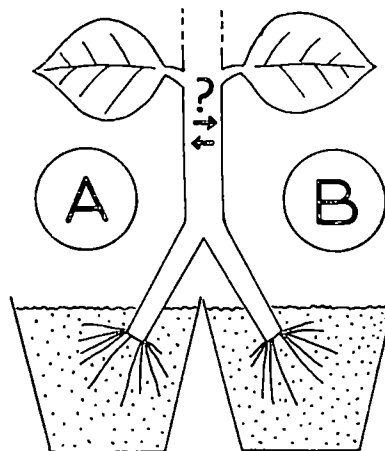


Fig. 1. The split root system technique and possible lateral transport system



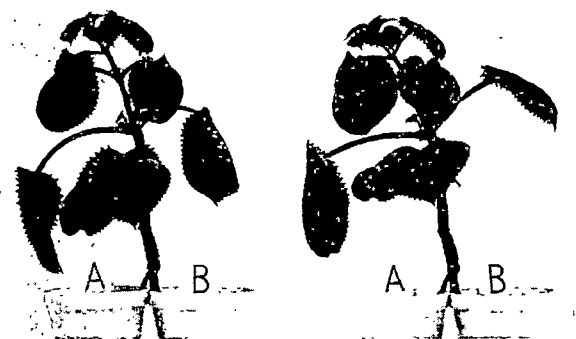


Fig. 2. The plant which has wilted (left) recovers completely in a few hours (right) after watering side *A* only

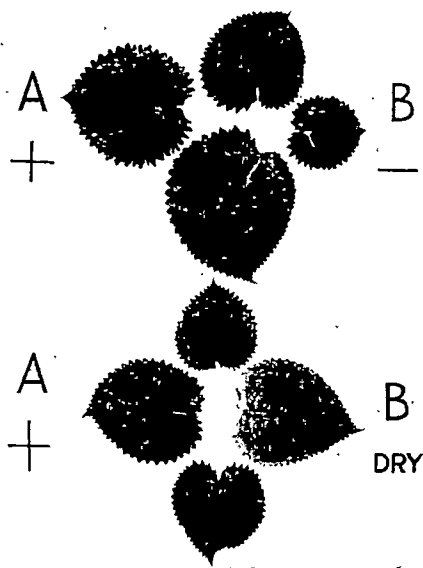


Fig. 3. Typical foliage from plants with differing nutrient and water status, sides *A* and *B*. +, Complete nutrient solution; -, nutrient solution deficient in phosphate

First, experiments were conducted with plants rooted (*A* and *B*) in loam. When side *A* was liberally watered and side *B* allowed to dry out no visible signs of stress could be induced in the foliage above *B*, indicating the ready lateral movement of water. This deduction was confirmed in separate experiments by allowing *A* and *B* to dry until all the foliage wilted. On watering one side (*A*) only, rapid recovery occurred in the foliage of both sides (*A* and *B*) (Fig. 2).

It was then postulated that salts might be carried along with this lateral movement of water. This was investigated by rooting the cuttings in sand cultures, and giving both sides only de-ionized water before the addition of nutrients. When side *A* was then fed complete nutrient and side *B* on phosphate-deficient solution, the treatment resulted in visible deficiency symptoms of the foliage on side *B*, confirming Caldwell's experiment. In another series of experiments side *B* was given no nutrient solution, and was allowed to dry out while side *A* was fed on a complete nutrient solution. With this treatment the foliage of *A* and *B* was healthy and normal (Fig. 3).

Dry weights were obtained of all leaves and axillary branches on the orthostichies above the sand. Alternate

leaves (from the median orthostichies) were rejected. These results indicate that lateral movement of phosphate can occur in response to a change in the water potential gradient. Polar transport is not obligatory. Experiments involving partial defoliation with lateral incisions provided further confirmation for the conclusion that certain solutes are transported by the transpiration stream irrespective of its path.

In whole plants the water tensions exerted by foliar evaporation cause the transpiration stream to move through the vascular xylem which offers least resistance to flow. Lateral tensions are usually small in plants and hence solutes carried by the transpiration appear to follow a specific polar path. However, after appropriate treatment lateral tensions develop in the plant which induce lateral movement of both water and solutes, thus proving the existence of a lateral transport system.

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### Effect of Cobalt on the Non-nodulated Legume

It has been demonstrated on several occasions that the addition of small quantities of cobalt as a nutrient has a stimulating effect on the growth of nodulated legumes<sup>1-3</sup>, whether they are grown in water culture, in sand culture or in the field. This has generally been attributed to an effect on the symbiotic fixation of nitrogen by the nodule, and the discovery that cobamide co-enzymes are present in the nodules of several leguminous species, as well as in the nodules of certain non-legumes<sup>4</sup>, enhances the probability of this hypothesis.

We have recently examined further the possibility that the legume itself, as distinct from its symbiont, has a requirement for cobalt, and this has been found to be the case. The relatively high degree of contamination by cobalt from the atmosphere has caused some difficulties. In several experiments using subterranean clover as the test plant, measurement of maximum radius<sup>5</sup> of the plants showed a clear-cut difference in the early stages which disappeared completely as the plants aged, and this effect was worse in the constantly disturbed air of a growth room than in the quieter air of a glasshouse. We have now been able to obtain a response on a sufficient number of separate occasions to leave no room for doubt. One set for plants grown in water culture in nutrient containing 5,000  $\mu\text{M/l}$ . nitrate nitrogen, and harvested at 50 days old, was as follows:

Co added  $\mu\text{M/l}$ . 0.0 0.02 0.20 2.00

Dry wt. 0.80 0.92 1.03 0.06

Difference for significance in dry wt. at 5 per cent 0.15, at 1 per cent 0.22.

An analogous stimulation has also been found in plants nodulated with an ineffective strain<sup>6</sup>. Since radioautographs using <sup>60</sup>Co suggested that cobalt was concentrated in the root tips, as well as in the nodules, some investigation has been made of the metabolism of the different parts of plants growing in nutrient solutions with and without added cobalt. Several differences have been observed, but perhaps the most notable is that, whereas no effect is shown by the leaves, the nodules and root tips of the +cobalt plants show a higher respiration rate than those of the -cobalt plants, when expressed on a dry matter basis. When calculated on the basis of total nitrogen content, however, the difference between the nodules of the two treatments became non-significant.

Table 1. DRY-WEIGHT OF LEAVES AFTER VARIOUS TREATMENTS

Dry-weight (g)	<i>A</i> Complete	<i>B</i> Deficient	<i>A</i> Complete	<i>B</i> Dry
Replicate (1)	2.492	0.668	1.364	1.610
(2)	1.294	0.656	1.106	1.618
(3)	1.610	0.710	1.456	1.563
Means	1.799	0.678	1.309	1.597

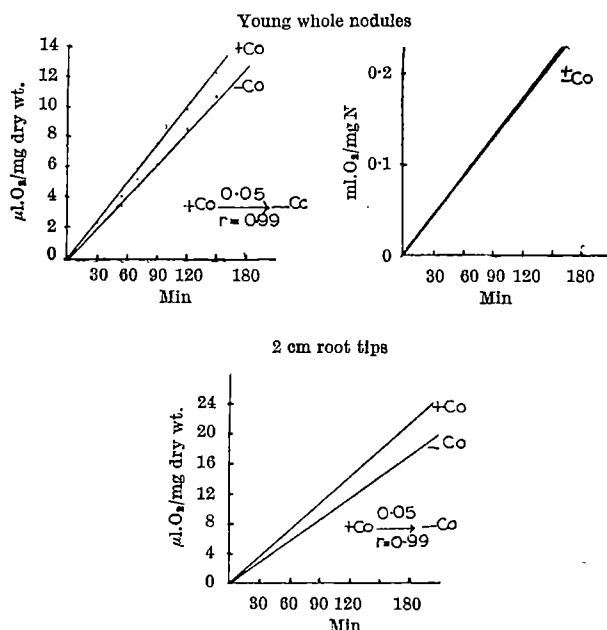


Fig. 1. Respiration of nodules and root tips (50-day plants)

The respirometer curves for the nodules and root tips are given in Fig. 1.

Apart from the general interest in the possible role of cobalt in the metabolism of a plant, this appears to be the first time that a differential requirement for a nutrient has been found, when the leaf tissues of a plant show no response to an element which has a marked and characteristic effect on the metabolism of the roots.

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## MICROBIOLOGY

### Preparation of <sup>14</sup>C-D(—)-β-hydroxybutyric Acid from <sup>14</sup>CO<sub>2</sub> using 'Knallgas' Bacteria (*Hydrogenomonas*)

ORGANISMS which fix carbon dioxide can be used to advantage for the preparation of radioactive labelled compounds. During growth, radioactive carbon from <sup>14</sup>CO<sub>2</sub> can be incorporated into all cellular substances. If growth is inhibited, assimilated carbon is bound primarily in the form of reserve material. Several 'knallgas' bacteria of the *Hydrogenomonas* type incorporate <sup>14</sup>CO<sub>2</sub> into poly-β-hydroxybutyric acid (PHBA) in a hydrogen-oxygen atmosphere in the absence of a source of nitrogen<sup>1,2</sup>. PHBA can be depolymerized to D(—)-β-hydroxybutyric acid (HBA) by using either hydrazine<sup>3</sup> or enzymatic hydrolysis<sup>4,5</sup>.

<sup>14</sup>C-PHBA is prepared using the apparatus shown in Fig. 1. Chemolithotrophically grown cells<sup>6</sup> are gathered, washed, and suspended (0.5 mg dry weight/ml.) in a nitrogen-free mineral medium. From this suspension 100

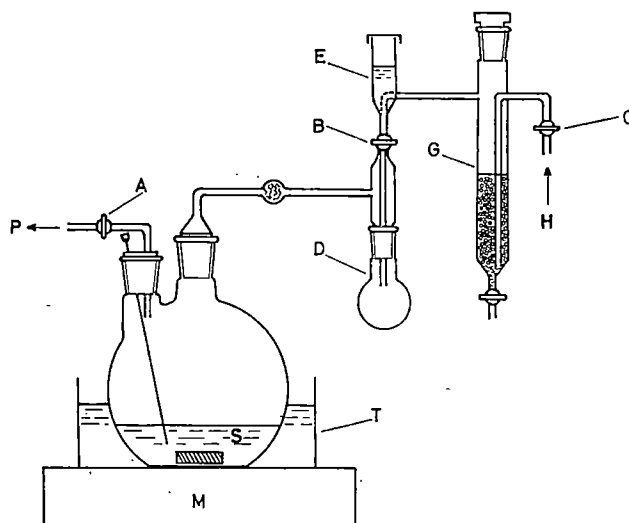


Fig. 1. Apparatus for the preparation of <sup>14</sup>C-poly-β-hydroxybutyric acid. P, connexion to vacuum pump; S, bacterial suspension; M, magnetic stirrer; T, constant temperature bath; D, flask containing barium carbonate-<sup>14</sup>C; E, 10 per cent perchloric acid; G, washing tower containing 20 per cent KOH; H, hydrogen-oxygen mixture

ml. are pipetted into the 2 l. round flask and stirred at a temperature of 30° C. After opening stopcocks A, B, and C, 5 l. of carbon-dioxide-free 'knallgas' (70 per cent H<sub>2</sub> + 30 per cent O<sub>2</sub>) are allowed to flow through the apparatus. After closing stopcock A, the suspension is stirred for a further 30 min, stopcock B is then closed and the main reaction flask evacuated through stopcock A. Perchloric acid, 15 ml. of a 10 per cent solution, is then allowed to flow into flask D, which contains barium carbonate-<sup>14</sup>C (5 mc.). By opening stopcock B and C the <sup>14</sup>CO<sub>2</sub> which has been generated is carried over into the main reaction flask by the incoming 'knallgas' until the pressure is equilibrated. Stopcock B is now closed and the suspension stirred for 3 h. At the end of this time, the cells are killed with formic acid, washed and lyophilized. PHBA is isolated by using a sodium hypochlorite solution<sup>7</sup> or by means of chloroform extraction and is then hydrolysed. From 5 mc. of barium carbonate-<sup>14</sup>C (spec. act. 20–26 mc./m.mole), a yield of 3–3.5 mc. of HBA (60–70 per cent theoretical) with a specific activity of 12–20 mc./m.mole was obtained in a number of experiments.

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### Growth of 'Knallgas' Bacteria (*Hydrogenomonas*) using Direct Electrolysis of the Culture Medium

IN view of the recent discussion pertaining to the use of the 'knallgas' bacteria for the regeneration of exhaled air<sup>1–3</sup>, we should like to point out that it is possible to produce the oxygen-hydrogen mixture directly in the culture vessel by electrolysis of the mineral medium. Since the 'knallgas' bacteria of the *Pseudomonas* type (*Hydrogenomonas* strains H 16 and H 20) grow in chloride-

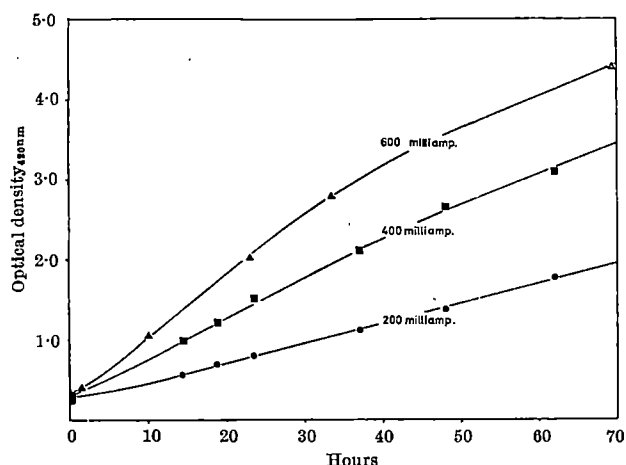


Fig. 1. Growth of *Hydrogenomonas* strain H 16 using electrolysis of culture medium for production of hydrogen and oxygen. Mineral medium (1 l.) was contained in a 2-l. culture vessel whose contents were magnetically stirred. Hydrogen and oxygen were produced using 2 platinum electrodes, each 16 cm<sup>2</sup>, at currents of 200, 400 and 600 m.amp. Turbidity of the cell suspension properly diluted (ext. < 0.3) was measured at 420 nm with 1-cm cuvettes (Zeiss Elko III)

free medium, oxygen and hydrogen can be produced in such a medium without interfering with side-effects using platinum electrodes with a relatively large surface area. Carbon dioxide is led directly into the culture vessel and removes excess oxygen. By using currents between 0.1 and 1.2 amp and at voltages not exceeding 5.3–5.5 V, good growth is achieved<sup>1,4</sup>. At low current-levels, growth is proportional to, and limited by, the hydrogen produced.

During growth, hydrogen, oxygen and carbon dioxide are consumed in the ratio of 8 : 3 : 1. Bacteria grown under these conditions can withstand a very high partial pressure of oxygen and contain very little reserve material (poly- $\beta$ -hydroxybutyric acid). Since hydrogen and oxygen are directly produced and consumed in the culture vessel, the electrolysis-culture method is simpler and less dangerous than other methods for the autotrophic growth of 'knallgas' bacteria. This method offers a number of distinct advantages for automatic continuous cultures.

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## ENTOMOLOGY

### Temperature Regulation in the Sphinx Moth during Flight

THE ability of some insects to raise the thoracic temperature by muscular activity above that of the surrounding air is well known<sup>1</sup>. However, we have demonstrated recently that the internal heat produced is regulated during periods of activity<sup>2</sup>. The investigation reported here shows that the moth, *Celerio lineata*, maintains a relatively constant body temperature during flight over a range of ambient temperatures.

We previously described a method of implanting thermistors and recording the body temperature of moths without hindering normal behaviour<sup>2</sup>. The same techniques were used here, but, to obtain extended periods of continuous flight, the moths were hung on the probe leads and stimulated to make flight movements. Tem-

peratures were recorded of moths subjected to ambient temperatures ranging from 10° C to 35° C.

The moths maintained thoracic temperatures between 32° C and 40° C. Rapid changes in ambient temperature during tethered continuous flight failed to produce correspondingly large changes in thoracic temperature. The thoracic temperatures in Fig. 1 represent levels maintained for longer than 15 sec during flight. A few values are included for the maximum temperature after increases in thoracic temperature in cases where equilibrium was not established; these demonstrate achievable levels at low ambient temperatures. If heat production during flight were constant, independent of ambient temperature, as in the migratory locust<sup>3,4</sup>, the thoracic temperature would be parallel to the isothermal line. However, our data suggest that heat production increases at low and decreases at high ambient temperatures. The thermal levels maintained during flight are consistent with the levels reported by Adams and Heath<sup>2</sup> during voluntary mixed activity.

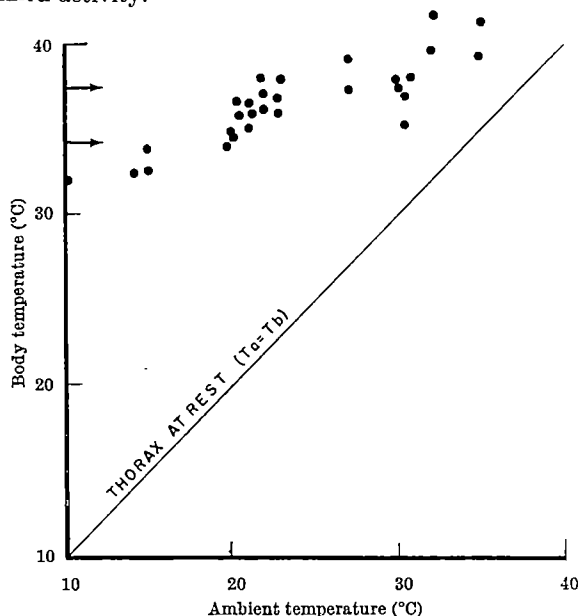


Fig. 1. Thoracic temperatures maintained by flying *Celerio lineata* plotted against ambient air temperatures. The arrows show the mean regulated range during voluntary mixed activity (ref. 2)

Flight at low ambient temperature is vigorous, but it becomes intermittent at higher temperatures where even the minimal output of flight energy places the animal under heat stress. At air temperatures above 30° C, the moth may regulate behaviourally by periodic landing and cooling. These results are exactly contrary to those of Church, showing that the migratory locust generates a constant body temperature excess of 6° C over an 11-degree range of ambient temperature during flight<sup>4</sup>. This difference may be related to the biology of the two animals. The nocturnal moth, lacking radiant heat sources, must rely on its own heat production to achieve and sustain the high body temperature required for activity, while the diurnal locust can regulate its body temperature by appropriate behavioural responses to radiant heat sources. Under high day-time temperature conditions the temperature excess resulting from flight in the locust may on occasion cause overheating. Activity may then be curtailed until more equable thermal conditions obtain. On the other hand, the usually cool night-time temperatures encountered by large moths allow the establishment of a favourable gradient to the environment, so that overheating might restrict activity only on nights when the air temperatures are above 30° C.

This evidence further substantiates the idea that at least some insects may be nearly homeothermic during



periods of activity. It further indicates that *Celerio lineata* is capable of distinguishing differences in thoracic temperature and of adjusting the heat output of the flight muscles during flight to compensate for changing external thermal stress.

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## VIROLOGY

### Mutual Precipitation of Two Viruses

SOME, but not all, pairs of proteins which are oppositely charged combine with and precipitate each other when mixed in salt-free solutions<sup>1</sup>. The precipitates usually dissolve when salt is added, presumably because anions and cations of the salt substitute the negatively and the positively charged proteins, respectively, in the compound formed by electrostatic attraction.

Until recently it seemed improbable that pairs of viruses would precipitate each other by electrostatic attraction, for those examined mostly had iso-electric points near pH 4.5 with deviations from this value by less than two pH units. The possibility became evident with the knowledge that bromegrass mosaic virus (BMV) is iso-electric at about pH 8.0 (ref. 2) and satellite virus (SV) (ref. 3) at about pH 7.0 (Fig. 1). To investigate the possibility, tests were made to see whether these viruses would precipitate with tobacco mosaic virus (TMV), because there is a wide range of pH values at which they are positively charged and TMV is negatively charged (Fig. 1). At any pH value within this range BMV is considerably more charged than SV and thus appeared more likely to precipitate with TMV than SV.

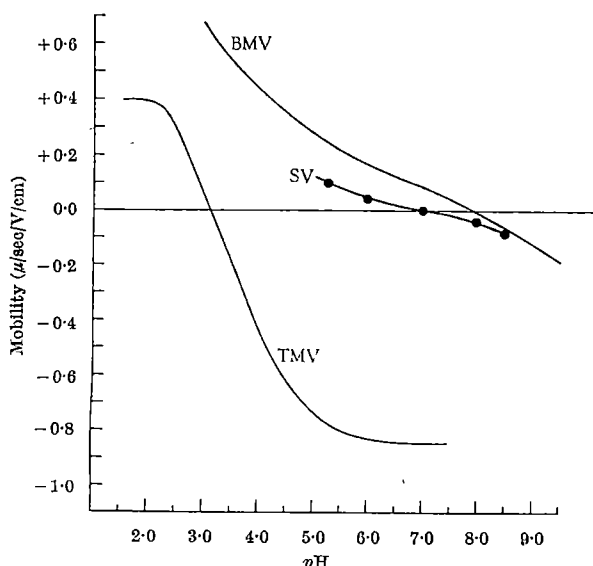


Fig. 1. Electrophoretic mobilities of TMV, BMV and SV at different pH values. The curve for TMV was taken from Kramer and Wittmann<sup>4</sup> and that for BMV from Bockstahler and Kaesberg<sup>3</sup>. They were obtained in buffers of ionic strength of 0.075 and 0.1, respectively. The curve for SV was obtained in the present work in M/15 phosphate buffer using a Perkin-Elmer electrophoresis apparatus. As isoelectric points and relative charge densities can depend on the presence of salt, any comparisons between TMV, BMV and SV, based on the curves shown above, apply only approximately to salt-free solutions.

When equal volumes of salt-free solutions of BMV and TMV at 1 g/l., adjusted to pH 5.0 with hydrochloric acid or sodium hydroxide, were mixed, a precipitate formed immediately, and when it had settled neither virus was detectable spectrophotometrically in the supernatant fluid. Precipitation occurred at all pH values between 4.0 and 6.5 and was most rapid at about pH 5.0. Adding sodium chloride to a concentration of 0.4 per cent or more immediately dissolved the precipitate.

By contrast, mixing salt-free solutions of SV and TMV in similar conditions did not produce a precipitate, and no interaction between the two viruses was detected when the mixture was examined in an analytical ultracentrifuge.

TMV and SV probably failed to interact because the charge density of SV was too small; at pH 5.3 (which is near optimal for precipitation between TMV and BMV) the electrophoretic mobility of SV (measured at 1 g/l. in M/15 phosphate buffer) is only about +0.1 μsec/V/cm, whereas that of BMV is about +0.3 μsec/V/cm. The mobility of BMV is slowed to about +0.1 μsec/V/cm when the pH is raised to just over 6.5, and at this pH BMV also fails to precipitate with TMV in spite of the fact that the raising of the pH slightly increases the negative charge density of TMV.

Salt-free solutions of SV and of yeast nucleic acid (1 g/l.) also failed to precipitate when mixed at pH 5.0, whereas solutions of BMV and of the nucleic acid immediately produced a precipitate that dissolved when sodium chloride was added to a concentration of 2 per cent. pH 6.0 was the upper limit at which a precipitate formed.

In salt solutions in which TMV and BMV do not precipitate one another they also fail to combine, for when a mixture of the two viruses in M/15 phosphate buffer at pH 5.3 was subjected to electrophoresis, the mobility of each virus and the size of the peak formed by it were the same as when it was used alone.

A suspension of the precipitate formed by mixing equal amounts of TMV and BMV caused numbers of local lesions on leaves of *Nicotiana glutinosa* similar to those caused by an equal amount of TMV inoculated alone.

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### Inhibition of Virus Growth by a Toxic Factor from Asbestos Pad and Cellulose Acetate Membrane Filters

HOUSE<sup>1</sup> has shown recently that a factor which is toxic for baby hamster kidney cells (BHK 21, ref. 2) is eluted from asbestos pads by Eagle's tissue culture medium. This toxic factor inhibits to a marked degree the cloning efficiency of the clone C 13 which he used.

This observation by House appeared to be related to observations we had made previously during the preparation of a phosphate-low medium which we required for the growth of phosphorus-32-labelled foot-and-mouth disease virus<sup>3</sup> in both pig kidney and baby hamster kidney cells. Using a formula based on Hanks's saline, but containing *tris* buffer instead of phosphate, we found in preliminary experiments that the yields of virus were the same as those in unmodified Hanks's saline. Unexpectedly, filtration of the phosphate-low medium through a Seitz E.K. asbestos pad resulted in a considerable reduction of virus yield. In view of House's observations we have now carried out further experiments to determine whether a toxic factor was responsible for the reduction in virus yields which we encountered in our preliminary experiments. Through Mr. House's co-operation we have been able to

Table 1. EFFECT OF FILTRATION OF MEDIUM ON GROWTH OF FOOT-AND-MOUTH DISEASE VIRUS IN PIG KIDNEY AND BABY HAMSTER KIDNEY CELLS

Medium	Filter	Cell and virus type	Virus yield (ml. 1/30 C' fixed by 1 ml.)	Infectivity titre (mouse $ID_{50}$ /0.03 ml.)
Phosphate-low	Unfiltered	Pig kidney C 997	0.31	5.5
	Seitz E.K.		0.04	3.5
	Ford's 'Sterimat'		0.08	—
	'Millipore'		0.07	—
	Unfiltered	Baby hamster kidney C 997	0.60	—
	Seitz E.K.		None detected	—
Eagle's	Unfiltered	Pig kidney C 997	0.54	5.5
	Seitz E.K.		0.27	5.1
	'Millipore'		None detected	3.9
	Unfiltered	Baby hamster kidney C 997	1.9	—
	Seitz E.K.		1.2	—
	'Millipore'		1.9	—
	Unfiltered	Baby hamster kidney O 1	2.0	—
	Seitz E.K.		1.4	—
	'Millipore'		2.0	—

include batches of his filtered media in some of these experiments.

Monolayers of pig kidney cells or baby hamster kidney cells (BHK 21, clone 13) were infected with foot-and-mouth disease virus of type C (strain 997) or type O (strain 1), washed once with the medium to be tested and then incubated at 37° until the cell sheet was removed from the glass by the action of the virus. The yield of virus was usually measured by determining the amount of complement (C') fixed with the homotypic antiserum, but in some experiments the amount of infective virus was also determined. The medium in which the infected cell sheets were incubated was filtered through a Seitz E.K. or Ford's 'Sterimat' asbestos pad or through a cellulose acetate membrane (Millipore Filter Corporation, Bedford, Mass.). In order to standardize conditions, 100 ml. of medium were filtered through an asbestos pad of 5 cm diameter or a membrane filter of 2.5 cm diameter.

The results in Table 1 show clearly that filtration of either phosphate-low or Eagle's medium through a Seitz or Ford's asbestos pad results in a considerable reduction of virus yield in both pig kidney and baby hamster kidney cells. In contrast, filtration of Eagle's medium through a cellulose acetate membrane filter had no effect on the virus yield in baby hamster kidney cells. Virus yields in pig kidney cells incubated in this medium, however, were as low as or even lower than those obtained with Eagle's medium which had been filtered through asbestos. This considerable reduction in virus yield in pig kidney cells was also observed with cellulose acetate filtered medium provided by Mr. House.

That these reductions were due to a toxic factor rather than to the removal of a necessary nutrient was shown in the virus yields obtained when the infected cell sheets were incubated in mixtures containing equal proportions of unfiltered and filtered Eagle's medium (Table 2). Although dilution of the unfiltered Eagle's medium with an equal volume of 0.15 M sodium chloride had only a small effect on virus yield, dilution with an equal volume of filtered medium reduced the yield considerably. By taking successive samples of filtrate from one asbestos pad House showed that the cloning efficiency of baby hamster kidney cells was not reduced by medium which passed through the

pad following adequate washing with the same medium. Similarly, using successive filtrate samples provided by Mr. House, we found that, whereas the medium passing first through the asbestos pad considerably inhibited virus growth, later samples had no inhibitory effect.

The nature of the toxic factor or factors has not been examined. The results illustrate, however, that care must be exercised in the use of filters as a means of sterilizing cell culture solutions which are to be used for virus growth.

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## GENETICS

### Interphase Deoxyribonucleic Acid Condensation, Late Deoxyribonucleic Acid Replication, and Gene Inactivation

A FEW years ago, Ohno<sup>1,2</sup> discovered that one of the two X chromosomes of female mammals remains condensed in somatic interphase nuclei, and Park showed that this phenomenon becomes apparent during the first three weeks of embryonic life<sup>3</sup>. Based on these findings and on the mosaicism observed for X-linked factors in female mice, Lyon<sup>4,5</sup> proposed a relationship between DNA condensation and metabolic inactivation of one of the two X chromosomes of females.

In agreement with this idea, it is now known that X chromosomes in excess of one become highly condensed in interphase somatic nuclei during early embryogenesis and produce little phenotypic effect<sup>6</sup>. Furthermore, normal males (XY) and females (XX) as well as individuals with as many as 5 X chromosomes have similar amounts of an X-linked enzyme<sup>6,7</sup>.

A further characteristic of the X chromosome was reported by Taylor<sup>8</sup> and Morishima *et al.*<sup>9</sup> when they found that the X chromosome that forms the sex chromatin body has a characteristic late DNA replication pattern.

An analogy to the heterochromatic X may be presumed for the Y chromosome since it shows positive heteropyknosis in early prophase<sup>1</sup>, has late DNA replication<sup>10,11</sup>, and extra Y chromosomes produce no conspicuous phenotypic effects<sup>11</sup>.

It is noteworthy that out of 22 pairs of autosomes, only three viable trisomies are known: trisomies  $D_1$ , 18 and  $G_1$  (ref. 11). Because of the small number of viable autosomal trisomies and the existence of autosomes with smaller DNA mass than the  $D_1$  and 18 chromosomes, it seems reasonable to assume that a large portion of the DNA of chromosomes  $D_1$ , 18 and  $G_1$  is condensed in somatic interphase nuclei and, therefore, physically unable to produce RNA. Furthermore, due to the multiple phenotypic effects and the lack of conspicuous interphase DNA condensation of the chromosomes involved in the viable autosomal trisomies, it can be again assumed that such autosomes would be less condensed and not as late replicating as the heteropyknotic X.

Work from my laboratory has recently shown that the chromosomes involved in the only three viable autosomal trisomies are largely late replicating ones, although not as much as the heterochromatic X chromosome<sup>12-14</sup>.

Since autosomes other than  $D_1$ , 18 and  $G_1$  have relatively large late DNA-replicating segments, the hypothesis can be extended to predict that autosomal partial trisomies and partial deletions will be found and would correspond to large and mostly late replicating or very small and early replicating chromosome segments. In this direction

Table 2. EFFECT OF MIXING FILTERED AND UNFILTERED EAGLE'S MEDIUM ON THE YIELD OF FOOT-AND-MOUTH DISEASE VIRUS IN PIG KIDNEY AND BABY HAMSTER KIDNEY CELLS

Medium	Cell and virus type	Virus yield (ml. 1/30 C' fixed by 1 ml.)
Eagle's—unfiltered	Pig kidney C 997	0.63
Seitz E.K. filtered	C 997	0.30
unfiltered + Seitz filtered (50 : 50)		0.32
'Millipore' filtered		0.10
unfiltered + 'Millipore' filtered (50 : 50)		0.16
unfiltered + 0.15 M NaCl (50 : 50)		0.55
unfiltered	Baby hamster kidney O 1	2.0
Seitz E.K. filtered		1.5
unfiltered + Seitz filtered (50 : 50)		1.6

it is interesting to know that partial trisomy for a sizable segment of the long arm of a C chromosome<sup>13</sup> is now known to be viable and involves late DNA replicating chromosome segments. Furthermore, deletions of the short arm of a No. 5 (refs. 16 and 17) and No. 18 (ref. 18) chromosomes have been found in the human population and correspond to absent late DNA replicating chromosome segments.

Because all the viable human congenital chromosomal abnormalities so far tested have been found to be composed of late replicating DNA (triploid/diploid individuals and trisomy mosaics for chromosomes other than  $D_1$ , 18 and  $G_1$  have been observed; in these individuals, it is suggested that life is sustained by a sufficient admixture of diploid cells<sup>19</sup>), it is postulated that these chromosomes are not as deleterious as early replicating ones when present in excess or defect, because late replicating DNA may be an expression of complete or partial metabolic gene inactivation<sup>11,20</sup>.

Furthermore, in accordance with the heteropyknotic X, it is suggested that late replicating autosome segments become condensed in somatic interphase nuclei during early embryogenesis.

The possible role of arginine-rich histone in the causation of late replicating DNA and interphase DNA condensation is now being investigated here.

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### Induction of Sex-linked Recessive Lethals and Visible Mutations by feeding X-irradiated DNA to *Drosophila melanogaster*

ATTEMPTS to induce genetic changes by chemical means go back to early days of modern genetical research. Even when Muller's<sup>1</sup> discovery of the mutagenic effect of X-rays had opened up a new and exceedingly fruitful field of research, the search for chemical mutagens was not abandoned. In fact, during the last two or three decades, hundreds of chemicals have been tried for their mutagenic effect; however, most of these are highly toxic and are not found free in Nature. The search for a specific mutagen led Gershenson<sup>2,3</sup> to try calf-thymus nucleic acid for its mutagenic effect. Gershenson reared flies upon food to which a large amount of (5 per cent) thymonucleic acid was added and found that a considerable number of the imagoes, derived from the larvae

that had had this food, showed various characteristic abnormalities. When these flies were bred, similar abnormalities were often found among their progeny. A large number of wing-mutations were claimed to have been induced by this treatment and this led him to claim that mutations had been produced *en masse*. Gershenson, employing *ClB*-technique, could not find any increase in the induction of sex-linked-lethals (s.l.l.). Rapoport<sup>4</sup> repeated Gershenson's experiments and he too could not observe any increase in s.l.l. rate. Muller<sup>5</sup> tried both thymonucleic and yeast nucleic acids and did observe some phenotypic abnormalities but again no increase in s.l.l. To quote Muller: "It is hardly conceivable that a variety of types of visible mutations could be induced *en masse* by any agent without there being some appreciable effect on the lethal mutation frequency also". He was therefore of the opinion that there is no indication of either visible or lethal mutations induced by this treatment. On general considerations, one would not expect any harmful or mutagenic effect of the natural DNA, which forms an important part of the food consumed by every living organism. However, the possibility of such mutagenic effect of certain DNA's for certain organisms under given conditions could not be absolutely ruled out.

Other considerations which led me to this investigation were the process of sterilization of food by employing strong doses of penetrating radiations. We know that radiations not only destroy the micro-organisms responsible for the decay processes, they produce certain definite, though not very well understood, changes in the food as well.

The proteins, the sugars and other carbohydrates will undergo changes as a result of direct and indirect action of the radiations. The most important, however, are the nucleoproteins and particularly the deoxyribonucleic acid (DNA) present in every cell nucleus. The DNA, as we know, is a polymer of high molecular weight, consisting of a chain of nucleotides, each nucleotide in turn consisting of a base, a sugar and phosphoric acid. Irradiation of DNA causes strand separation (rupture of the hydrogen bonds) and degradation or depolymerization (rupture of the main chain), in addition to other radiochemical changes. It is assumed that the irradiation products of DNA, denoted here as 'DNA<sub>m</sub>' (without specifying its exact physical and chemical nature at present) when taken orally, might pass through the intestinal wall and circulate in the body. These circulating bits of DNA<sub>m</sub>, on entering the cell-nuclei (by analogy with the action of the viruses) of the germ-cell line or other somatic cells, get incorporated somehow in the genetic apparatus, thus giving new hereditary characteristics to the affected cells; that is to say, inducing mutations. Mutations of the somatic cells might lead to cancerous growths or other abnormalities. Mutations in the germ cells will lead to hereditary changes in the progeny.

It was, therefore, tempting to investigate the radio-mimetic effect of DNA<sub>m</sub>, particularly because DNA is a part of the food, and of every cell-nucleus, of living organisms and is all the time under the influence of ionizing radiations (cosmic-rays and natural and artificial radioactivity, etc.) necessary to induce the desired structural changes. Further, unlike other mutagenic and carcinogenic substances (TEM, nitrogen-mustards, etc.) which are artificially produced and generally speaking artificially introduced into the organisms, and which are highly toxic and foreign to the body, DNA<sub>m</sub> might arise in the body of the organism (DNA + radiations) or may enter the body with food. The possibility of injecting DNA<sub>m</sub> into the organisms is not ruled out either. To my knowledge no investigations showing the mutagenic action of irradiated DNA (DNA<sub>m</sub>) have yet been reported for either *Drosophila melanogaster* or for any other organisms.

The experimental procedure employed to test the above hypothesis was the following. The DNA, in dry powder



form, was irradiated with 100,000 r. (180 keV, 20 m.amp with 2 mm Al filter) of X-rays at the rate of 625 r./min and 450 mg of this was added to about 25 g of the normal culture medium when warm, and mixed thoroughly by stirring with a glass rod. Adult *Drosophila melanogaster* flies (0-4 days old) of the 'Oregon-R' strain were allowed to feed on this medium and lay eggs therein for 10-12 days and were then discarded. The males emerging from the larvae that had had this treated food were tested for the presence of the s.l.l. by employing Muller's *CIB*-technique as shown below:

♂	♀
P: $l_m/Y$	$X\ CIB/v$
F <sub>1</sub> : $v/Y$	$X\ CIB/l_m$
F <sub>2</sub> : $l_m/Y +$	$CIB/v$
$CIB/Y +$	$l_m/v$

where  $v$  stands for vermilion and  $l_m$  denotes the lethal factor induced in the X-chromosome of the male under test through the action of DNA<sub>m</sub>. Each  $l_m/Y$  test male (0-2 days old) was mated with 2-3  $CIB/v$  females and a number of such cultures started. As the number of test males was large, some multi-male cultures were started as well. In F<sub>1</sub> the  $CIB/l_m$  females were selected and mated singly to  $v/Y$  brothers (8-10 such cultures per test-male). In F<sub>2</sub> these cultures were checked for the presence of males. The cultures containing at least 30 females and no males were taken to contain the lethal factor  $l_m$ . As we were not looking for the visible mutations, not all the cultures were checked thoroughly. However, the cultures containing flies with conspicuous features could not escape unnoticed. A check of these cultures led to the detection of a number of visible mutations of the wings, eyes, etc., in addition to a number of flies with gross morphological abnormalities.

In order to eliminate any possibility of the non-irradiated DNA being mutagenic or having any mutagenic contaminations, a parallel experiment was performed with non-irradiated DNA. The same amount (450 mg) of non-irradiated DNA was added to the culture medium as before and 0-4 days old 'Oregon-R' flies allowed to feed on it. The rest of the procedure was exactly the same. The experiments were performed at a temperature of 22-24°C.

Out of 635 DNA<sub>m</sub> cultures checked, 36 were without males, giving an s.l.l. rate of about 5.7 per cent as compared with 486 DNA-cultures where none was without males (s.l.l. rate 0.0 per cent). In addition, no conspicuous visible mutations or flies with morphological abnormalities were detected.

These results definitely establish the mutagenic effect of the irradiated DNA (DNA<sub>m</sub>) and the non-mutagenic nature of the normal DNA in the present case. Taking the spontaneous mutation rate to be 0.17 per cent, (ref. 6) for the sake of comparison, the present experiment shows a 32-fold increase over the spontaneous rate; this would correspond to a direct exposure of the spermatocytes to about 2,000 r. of X-rays.

It may not be inappropriate to mention the results obtained by Swaminathan *et al.*<sup>7</sup> by feeding *Drosophila melanogaster* on irradiated 'complex medium'. These results might be explained in the light of the effect observed by us; however, it may be remarked that irradiation of the complex medium results in much more complicated changes of the medium. Stable or meta-stable mutagenic compounds (H<sub>2</sub>O<sub>2</sub>, aldehydes, etc.) may be produced as a result of direct and indirect action of the radiations on the components of the employed medium (water, sugar and other carbohydrates, proteins and DNA, etc.), and one could not attribute the mutagenic effect to any definite mechanism or to any definite mutagens produced as a result of irradiation. In our case, however, it is definitely the DNA<sub>m</sub> and this alone that is acting as mutagen.

Due to the general importance of the part played by DNA and the ionizing radiations in the evolutionary,

biochemical and genetical processes it is thought worthwhile to publish this preliminary report. Further, if our conclusions are substantiated, one might infer that the sterilization of food by using high doses of ionizing radiations is not desirable. In addition, it may be mentioned here that in DNA<sub>m</sub> we have a natural mutagenic substance with all the implied potentialities. Other experiments to verify these results are in progress and will be reported in detail elsewhere.

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### Giant Chromosomes in Protozoa

So far as we know polytene chromosomes have been previously observed only as typical structures in the salivary gland cells of Diptera, and in certain plant cells. By using orcein we have been able to demonstrate such structures (Fig. 1) in the macronuclear anlage of *Stylonychia muscorum* Kahl (a hypotrichous ciliate). According to our preliminary observations, there are in this species 50-60 giant chromosomes which show a great diversity in both size and banding. They range from 15μ to 55μ in length, and from 0.8μ to 2.5μ in width.

We hope to improve the method in order to explore such chromosomes in other ciliate species, principally *Paramecium aurelia*, *P. bursaria* and *Euplotes* sp., which have been more widely investigated in terms of their genetics and macronuclear behaviour.

If giant chromosomes are generally characteristic of ciliates, they will provide a more exact basis for the



Fig. 1. Some giant chromosomes from the macronuclear anlage of *Stylonychia muscorum*



understanding of the nature and behaviour of the macro-nucleus and a new approach to the genetics and taxonomy of this group of Protozoa.

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### Genetic Control of the Caffeine Content of Coffee

CAFFEINE is one of the main constituents of the coffee bean. In spite of the existence of a limited but expanding market for decaffeinated coffee, most people still seem to prefer the normal product with caffeine. So important is the caffeine in coffee that Chevalier<sup>1</sup> separated all 18 coffee species of Madagascar and Mascarene Islands into a special section *Mascarocoffea*, on the basis of the presence, or absence, of caffeine. On the other hand, the *Eucoffea* section, which includes the cultivated coffee species, comprises only species with caffeine.

Caffeine content in coffee has been extensively considered by various authors<sup>2</sup> and found to vary between and within species. A series of chemical analyses has also been run in Campinas with seed samples of plants belonging to the main cultivars and varieties of *Coffea arabica*. Reasonable variations were found, particularly among the 'Mundo Novo' strains, indicating the possibility of isolating coffee selections with different levels of this alkaloid<sup>3</sup>.

Preliminary information from another series of *Coffea arabica*<sup>4</sup> obtained in Campinas has indicated that 'Laurina' has about half as much caffeine as 'Typica'. The 'Laurina' variety possibly originated in the Reunion Islands (Mascarene Islands) or was introduced to this island many years ago from Ethiopia or from the Yemen<sup>1,5</sup>. The plants have a tight growth, small leaves and pear-shaped fruits containing two small pointed seeds. The same type of seeds is found in some other non-cultivated diploid species belonging to different sections.

Genetic analysis of plants having the 'Laurina' characteristics revealed that this phenotype is controlled by a recessive pair of alleles *lr lr*, with a strong pleiotropic effect. Other varieties, except 'Mokka', are homozygous for the normal allele *Lr*. The 'Mokka' variety as described by Cramer<sup>6</sup> differs from the standard 'Typica' by the presence of both *mo* and *lr* alleles in homozygous condition, and coffee trees with the genetic constitution *LrLrmo mo* are quite similar to 'Mokka', differing only in the shape and height of the plants. This form has been called 'Kona' in some coffee collections.

In order to determine if the *lr* allele was responsible for the drastic reduction in the caffeine content, an experimental trial was made (randomized block with four replications) with samples taken from nine varieties and cultivars shown in Table 1. The 'Typica' variety was used as a standard. The *L.S.D.* at one per cent level indicated that only the Mokka-Maragogipe recombinant had a higher caffeine content than 'Typica'. The three varieties having *lr lr* alleles have a very low amount of caffeine. The Duncan test, applied to the data of this experiment, permitted the grouping of the varieties in five classes, as shown in Table 1. Among the entries having the *lr lr* alleles, the form 'Laurina' (*tt lrlr MoMo mgmg XcXc*) had a significantly lower caffeine content than the other group formed by the recombinants *tt lrlr momo mgmg XcXc* and *tt lrlr MoMo MgMg XcXc*. Samples of 'Bourbon Amarelo' (*tt LrLr MoMo mgmg xcxc*) and 'Bourbon Vermelho' (*tt LrLr MoMo mgmg XcXc*) form the third and fourth groups, while the other four varieties have a high caffeine content.

This information indicates that the presence of the *lr lr* alleles is responsible for a drastic reduction in the

Table 1. AVERAGE WEIGHT OF CAFFEINE EXPRESSED IN MG PER 10 G OF GREEN COFFEE BEAN, FROM SAMPLES TAKEN FROM CAMPINAS COLLECTION IN 1963, AND GROUPED ACCORDING TO THE DUNCAN TEST

Group	Variety	Genetic constitution	Caffeine (mg/10 g)
1	Laurina	<i>tt lrlr MoMo mgmg XcXc</i>	60.5
2	Mokka	<i>tt lrlr momo mgmg XcXc</i>	77.8
	Laurina-Maragogipe	<i>tt (?) lrlr MoMo MgMg XcXc</i>	79.2
3	Bourbon Amarelo	<i>tt LrLr MoMo mgmg xcxc</i>	109.9
4	Bourbon Vermelho	<i>tt LrLr MoMo mgmg XcXc</i>	113.7
	Typica	<i>TT LrLr MoMo mgmg XcXc</i>	122.7
5	Kona	<i>TT (?) LrLr momo mgmg XcXc</i>	123.1
	Maragogipe	<i>TT LrLr MoMo MgMg XcXc</i>	123.9
	Mokka-Maragogipe	<i>TT (?) LrLr momo MgMg XcXc</i>	127.9

*L.S.D.* = 3.58 mg

*C.V.* = 1.74 per cent

caffeine content and that the *xanthocarpa* (*xc*) and *bourbon* (*t*) factors also have a slight tendency to diminish the caffeine. The *maragogipe* (*Mg*) and the *mokka* (*mo*) alleles, conversely, seem to increase the caffeine content.

At present there is no information about which steps in the chain of reactions leading to caffeine formation and accumulation in the coffee bean are affected by the *lr* and other alleles mentioned.

We thank Dr. Sherman J. Leonard for advice.

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<sup>1</sup> Chevalier, A., *Les caféiers du globe*, 3 (Paul Le Chevalier, Paris, 1947).

<sup>2</sup> Wellman, F. L., *Coffee* (Leonard Hill (Books), Ltd., London, 1961).

<sup>3</sup> Tango, J. S., and Teixeira, C. G., *Bol. Suptda Serv. Café, S. Paulo*, **36**, 6 (1961).

<sup>4</sup> Tango, J. S., *Bragantia* (in the press).

<sup>5</sup> Carvalho, A., *Bol. Suptda Serv. Café, S. Paulo*, **20**, 1138 (1945); **21**, 6, 69, 127, 173 (1946).

<sup>6</sup> Cramer, P. J. S., *Gegevens oer de Variabiliteit van de in Nederlandsch-Indië Verbouwd Koffie-soorten* (G. Kolff and Co., Batavia, 1913).

### SOIL SCIENCE

#### Circulation of Water in Soil under a Temperature Gradient

SEVERAL workers (for example Gurr *et al.*<sup>1</sup>, Hutcheon<sup>2</sup>) have suggested that, when a temperature gradient is applied to a uniform closed soil column, a circulatory system is set up, and that any eventual steady moisture distribution is actually a dynamic balance of opposing fluxes, predominantly vapour from hot to cold, and predominantly liquid from cold to hot. In this communication we show that, at equilibrium, a static system cannot exist, and confirm experimentally the existence of a circulatory system.

In a porous material:

$$\ln h = g\Phi/RT = g(\psi + \pi)/RT \quad (1)$$

where  $h$  = relative humidity,  $g$  = acceleration due to gravity ( $\text{cm/s}^2$ ),  $R$  = gas constant for water vapour ( $\text{erg/g deg}$ ),  $T$  = temperature ( $\text{deg K}$ ), and  $\Phi$  is the total water potential ( $\text{cm water}$ ) comprising matric ( $\psi$ ) and osmotic ( $\pi$ ) components.

Also, for water vapour, over a small temperature-range:

$$\ln \rho_s = \alpha + \beta T' \quad (2)$$

where  $\rho_s$  ( $\text{g/cm}^3$ ) is the saturated vapour density at  $T'$  K, and  $\alpha$  and  $\beta$  are positive constants.

At any point, the vapour density  $\rho = h\rho_s$ , so differentiating and using (1) and (2), the vapour density change along a column subjected to a temperature gradient must satisfy:

$$\frac{1}{\rho} \delta \rho = \frac{g}{RT} \left( \delta \psi - \frac{\psi}{T} \delta T \right) + \frac{g}{RT} \left( \delta \pi - \frac{\pi}{T} \delta T \right) + \beta \delta T \quad (3)$$

For static equilibrium, the fluxes of liquid, vapour and solute must all be zero simultaneously, that is,  $\delta \psi$ ,  $\delta \rho$  and  $\delta \pi$  must all be zero, and therefore:

$$0 = \delta T (\beta - g\Phi/RT^2)$$

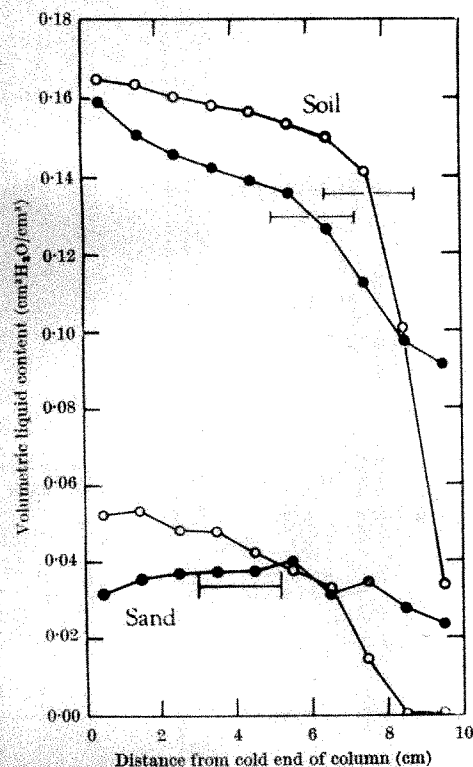


Fig. 1. Final liquid distributions. Volumetric liquid content plotted against distance from the cold end of the soil columns.  $\circ$ , non-saline;  $\bullet$ , saline;  $\text{—}$ , initial mean value (horizontal line) and standard deviation (vertical bar)

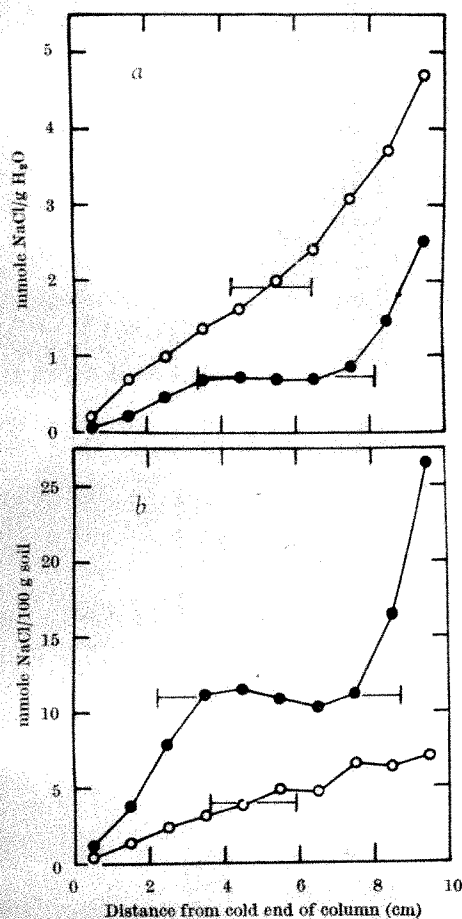


Fig. 2. Final salt distributions: (a) ratio mmole NaCl/g  $\text{H}_2\text{O}$ , and (b) ratio mmole NaCl/100 g soil as functions of distance from cold end of columns.  $\circ$ , sand;  $\bullet$ , soil

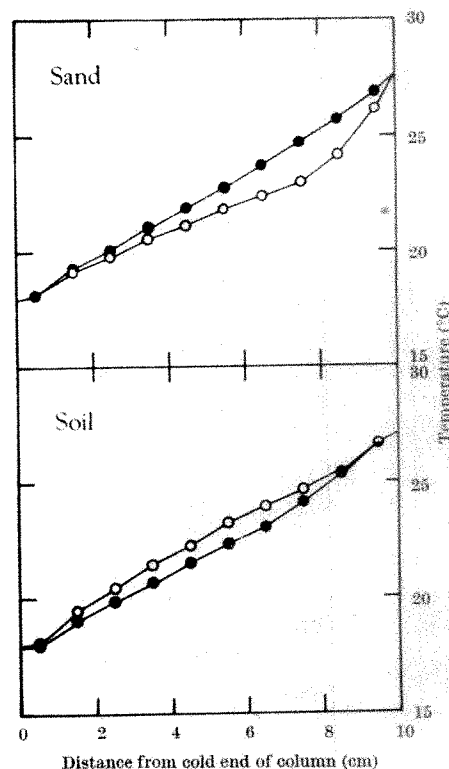


Fig. 3. Final temperature distributions:  $\circ$ , non-saline;  $\bullet$ , saline

which is impossible because  $\Phi$  is always negative or zero. Thus simultaneous static equilibrium of temperature, vapour, liquid and solutes cannot be achieved.

Columns, 10 cm length and 3.8 cm diameter, built from 1-cm sections of 'Perspex' tube, were uniformly packed with soil, saturated, drained at  $\psi = -100$  cm, and sealed with a vacuum sealing compound. The columns, well insulated, were screwed to thick brass plates in intimate contact with constant-temperature baths at 17.6 and 27.8°C.

Two inert porous media were used: (1) a fine white sand, of density 2.65 g/cm<sup>3</sup> and porosity 0.39; (2) ignited 0.5–1 mm aggregates of local top-soil, of density 2.54 g/cm<sup>3</sup> and porosity 0.66. In each experiment there were four replicate columns, two each with water and strong sodium chloride solution. Temperatures were measured with copper-constantan thermocouples inserted at 1 cm intervals.

After 12 days the columns were sectioned, the liquid distribution found by oven-drying, and the salt distribution estimated conductometrically. The initial uniformity of salt and liquid distributions within the columns was tested in the same way on other replicates not subjected to a temperature gradient.

Fig. 1 shows the final liquid distributions, and Fig. 2 the final salt distributions; 2 (a) the ratio mmole NaCl/g water, and 2 (b) the ratio mmole NaCl/100 g soil. Each point is the mean of duplicates, and the mean value and initial deviation for the column are indicated by each line. Temperature distributions in the saline and non-saline columns differed (Fig. 3): the results for sand are consistent with thermal conductivity differences caused by liquid content differences.

For both materials there was, as expected, net water movement from hot to cold, the movement being greater in the absence of salt, possibly because the salt acted as a sink for water vapour at the hot end. In the sand there was little net transfer of water in the presence of salt.

Fig. 2 is of more interest. When a temperature gradient is applied along a column in which water and salt are initially uniformly distributed, two kinds of transfer are



possible. If water moves only as vapour, the ratio mmole salt/g soil will remain constant, but the ratio mmole salt/g water will increase in regions where evaporation occurs and decrease in regions of condensation. Conversely, if water moves only as liquid, the ratio mmole salt/g water will remain constant, but that of mmole salt/g soil will increase in the regions to which the liquid flows and decrease in those from which it flows. The results of Fig. 2 are thus consistent with a vapour flux moving from hot to cold, and a return liquid flux from cold to hot—a circulatory system.

During this work, one of us (R. D. J.) was an O.E.C.D. Fellow.

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<sup>1</sup> Gurr, C. G., Marshall, T. J., and Hutton, J. T., *Soil Sci.*, **74**, 335 (1952).

<sup>2</sup> Hutcheon, W. L., *Highway Res. Board*, **SR40**, 113 (1958).

### Changes in Phosphate Potential on Re-wetting Air-dry Soil

WHEN soil is suspended in dilute calcium chloride solution the calcium dihydrogen phosphate potential ( $pH_2PO_4 + \frac{1}{2}pCa$ ) varies with the soil:solution ratio<sup>1</sup> and with the period for which the soil is in suspension<sup>2</sup>. Air-drying and re-wetting also affect the phosphate potential<sup>3</sup>. Laboratory measurements lacking precision will help little in predicting phosphate availability in field soils.

In Rhodesia arable top soils are always dry after the almost rainless winter, so only the changes on re-wetting were examined here. Using a soil:solution ratio of 1:2, a sandy loam derived from micaceous schist was equilibrated in 0.01 M  $CaCl_2$  for 1 h at 21° C after the following treatments: shaken by wrist-action in stoppered bottles (soil:solution = 1:2), or incubated at field capacity (15 per cent moisture) at either 21° or 35° C, each treatment being continued for different periods up to 12 days, with and without adding 120  $\mu$ mole  $KH_2PO_4$ /100 g soil. Changes due to microbial action during the final 1 h equilibration were probably negligible<sup>4</sup>. pH was measured in the suspensions, and the concentrations of P and Ca + Mg (expressed as Ca) in the filtrates; activities were calculated by the Debye-Hückel equation (using Aslyng's<sup>5</sup> correction for  $pH_2PO_4$ ). Total mineral nitrogen was determined in similarly treated samples.

Fig. 1 shows that in all treatments  $pH_2PO_4 + \frac{1}{2}pCa$  initially increased with time, that is, soluble phosphate decreased. On moistening soil to field capacity the phosphate potential approached a constant value after 4–5 days, but in suspension it changed more, over a longer period. This may be due to microbial action<sup>6</sup>, but the mineral nitrogen content changed more at field capacity than in suspension (Table 1); incubation at 35° gave more

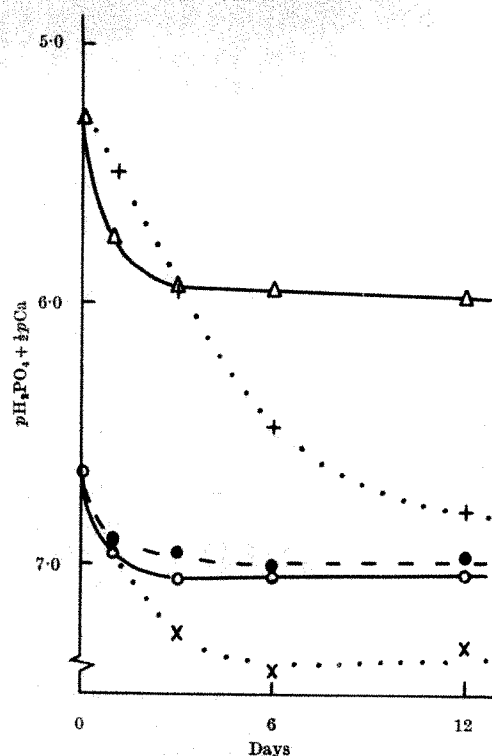


Fig. 1. Changes in phosphate potential with time of incubation.  $\Delta$ , +P (field capacity, 21°); +P (suspension, 21°);  $\bullet$ , -P (field capacity, 35°);  $\circ$ , -P (field capacity, 21°);  $\times$ , -P (suspension, 21°)

mineral nitrogen than at 21° C, but had little effect on the phosphate potential. If the effects of microbial action differ between soils in suspension and at field capacity, the latter is closer to conditions in field soils.

Larsen and Widdowson<sup>1</sup> suggested that increasing soil:solution ratios decreased pH and the phosphate potential in soil suspensions because more  $CO_2$  was produced. Presumably more  $CO_2$  is produced after longer periods in suspension, but in the present work pH and phosphate potential (Table 1 and Fig. 1) both increased with longer suspension, more so than with aerobic incubation at field capacity.

Another sandy soil was incubated at field capacity for 7 days at 21° C, then equilibrated in 0.01 M  $CaCl_2$  for 1 h (21° C), using 10, 38, 138 or 470 g soil/l. Table 2 shows that pH and phosphate potential decreased with increasing soil:solution ratio, as reported by Larsen and Widdowson<sup>1</sup>, although changes in  $CO_2$ -pressure must have been negligible<sup>3</sup>. The effects of soil:solution ratio were almost certainly due to the soil being changed more from its original state in attaining equilibrium at smaller soil:solution ratios.

Where there are distinct dry and rainy seasons, so that field soils are naturally air-dried before cropping, moistening samples to field capacity at room temperature for at least 4 days (7 days is convenient for routine measurements) before equilibrating for 1 h in suspension gives steady values of the phosphate potential (or of phosphate concentration, if this is preferred<sup>5</sup>). These may enable the relationships between soil solution measurements and plant composition to be tested more reliably than by methods previously proposed.

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Table 1. CHANGES IN pH AND MINERAL NITROGEN CONTENT OF SOIL WITH TIME OF INCUBATION

Days of treatment	Field capacity		1:2 Suspension	
	pH	Min N (p.p.m.)	pH	Min N (p.p.m.)
0	5.55	15 (15)	5.55	15
1	5.60	18 (32)	5.61	14
3	5.85	31 (43)	6.04	20
6	5.87	38 (48)	6.41	18
12	5.88	44 (50)	6.43	—

Figures in parentheses obtained at 35° C, all others at 21°.

Table 2. EFFECT OF SOIL:SOLUTION RATIO ON pH AND  $pH_2PO_4 + \frac{1}{2}pCa$

g soil/l.	$pH_2PO_4 + \frac{1}{2}pCa$	pH
10	8.21	5.30
38	7.56	5.10
138	7.08	4.90
470	6.90	4.80

<sup>1</sup> Larsen, S., and Widdowson, A. E., *Nature*, **203**, 942 (1964).

<sup>2</sup> White, R. E., and Beckett, P. H. T., *Plant and Soil*, **20**, 1 (1964).

<sup>3</sup> White, R. E., *Plant and Soil*, **20**, 184 (1964).

<sup>4</sup> Aslyng, H. C., Ph.D. thesis, London Univ. (1950).

<sup>5</sup> Wild, A., *Nature*, **203**, 326 (1964).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, January 18

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Dr. W. E. Knowles Middleton: "The Nature of Clouds".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. J. F. Lovett: "Analysis of Lindane and Its Formulations".

UNIVERSITY OF LONDON (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. von Wettstein (University of Copenhagen): "Macromolecular Physiology of Plastids".\*

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 7 p.m.—Dr. A. E. A. Werner: "The Scientific Examination of Paintings and Antiquities" (Ninth Annual Ladies Evening).

## Tuesday, January 19

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. A. A. Fulton and Mr. L. H. Dickerson: "Design and Construction Features of Hydro-Electric Dams Built in Scotland Since 1945".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Performance of Hill-Climbing Systems—The Gradient Estimation Problem".

QUEEN ELIZABETH COLLEGE (University of London) (at Campden Hill Road, London, W.8), at 5.30 p.m.—Prof. J. Edelman: "The Development of Experimental Botany" (Inaugural Lecture).

ROYAL AERONAUTICAL SOCIETY (joint meeting with the Institution of Electrical Engineers, at 4 Hamilton Place, London, W.1), at 6 p.m.—Discussion Meeting on "The Development of Dependable Fire Warning Systems in Aircraft" opened by Mr. J. R. Stevens.

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION AND EDUCATION AND TRAINING GROUP (at the Chamber of Commerce, 75 Harborne Road, Edgbaston, Birmingham 15), at 6.30 p.m.—Discussion on "The Motor Industry: Future Requirements for Education and Training".

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at the Coachmakers Arms, 88 Marylebone Lane, London, W.1), at 6.30 p.m.—Forum—Mr. J. H. Ellis: "Equipment for Finishing and Decorating Plastics: Hot Foil Stamping Machinery". Mr. J. Colterrell: "Silk Screening Equipment". Mr. A. Coates: "Deflashing of Mouldings".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Slough College Scientific Society, at Slough College, William Street, Slough), at 7.30 p.m.—Mr. T. Green: "Colour".

## Wednesday, January 20

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—The Rev. A. Q. Morton: "The Authorship of the Pauline Epistles—a Scientific Approach".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. W. D. Humpage and Mr. B. Stott: "Effect of Auto-Reclosing Circuit-Breakers on Transient Stability in Extra-High-Voltage Transmission Systems".

UNIVERSITY OF LONDON (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. von Wettstein (University of Copenhagen): "On the Genetic Control of Chloroplast Structures and Function".\*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Dr. B. K. Gazey and Dr. J. C. Morris: "Acoustic Communication Underwater".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. H. L. Willett: "New Safety Techniques in Mining" (Cadman Memorial Lecture).

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. R. T. Lovelock: "A Central Recording Station for a Climatic Laboratory".

INSTITUTE OF INFORMATION SCIENTISTS (at State House, High Holborn, London, W.C.1), at 6.15 p.m.—Mr. E. Martindale: "Technical Information Services and D.S.I.R.".\*

INSTITUTION OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Mr. D. J. Veasey: "L.T.E. Victoria Line Extension".

OIL AND COLOUR CHEMISTS' ASSOCIATION, STUDENT GROUP (at the London College of Printing, Elephant and Castle, London, S.E.1), at 7 p.m.—Meeting on "Working Properties of Ink—Rheology and Tack".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 7.30 p.m.—Dr. David K. Brooks: "Water and Electroclyte Metabolism".

ASSOCIATION OF THE WILLIAM PENGELLY CAVE RESEARCH CENTRE in conjunction with Imperial College Caving Club (in the Physics Department, Imperial College, Prince Consort Road, London, S.W.7), at 8 p.m.—Mr. David Attenborough and Mr. George Black: "Caves in Danger".\*

## Thursday, January 21

UNIVERSITY COLLEGE, LONDON (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. W. J. D. Jones: "Growing Whiskers—the Development of High Strength Materials".\*

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 4 p.m.—Discussion on "Keeping the Engineer Up-to-Date and Finding Information".

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. Pierre Oy: "Sampling of Ores and Metallurgical Products During Continuous Transport"; Dr. V. L. Bosazza: "The Formation of Malachite and Chrysocolla from Chalcopryrite in Rocks of the Bushman Mine Series, Northern Bechuanaland Protectorate".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Meeting on "Electron Microscopy in Relation to Taxonomy in Unicellular Algae" with contributions by Mrs. Katharine Harris, Mr. R. Ross and Prof. Irene Mantou, F.R.S.

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. H. T. Croft: "9 Points and 7 Points in 3 Dimensions".

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, Westminster, London, W.1), at 5.30 p.m.—Informal Discussion on "Hydro-Geology and Its Part in the Hydrological Cycle" introduced by Dr. S. Buchan.

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Discussion Meeting on "The Current Procedure for Advising the Government on Aviation Policy" opened by Sir George Gardner.

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. M. J. Dunblaton and Mr. P. F. Sherwood: "The Use of Lime and Mixtures of Lime and Pulverized Fuel Ash for Soil Stabilization".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. L. J. Bruce-Chwatt: "Malaria Research for Malaria Eradication".

## Friday, January 22

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E./I.E.R.E. MEDICAL ELECTRONICS GROUP (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Discussion on "The Direct Recording of Biological Signals".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "Network Analysis".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION, CAMBRIDGE SUB-SECTION (joint meeting with the Cambridge University Chemical Society, at the University Chemical Laboratory, Lensfield Road, Cambridge), at 8.30 p.m.—Dr. T. M. Sugden, F.R.S.: "Some Aspects of the Chemistry of Flames".

## Saturday, January 23

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Prof. W. F. Grimes: "The Temple of Mithras in Walbrook".\*

## Monday, January 25

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E./I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 10.30 a.m.—Colloquium on "Logic Circuits".

UNIVERSITY COLLEGE, LONDON (in the Botany Theatre, Gower Street, London, W.C.1), at 5 p.m.—Dr. P. N. Magee: "The Biochemical Pathology of Intracellular Alkylation Reactions".\*

UNIVERSITY OF LONDON (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. H. Hubel (Harvard Medical School): "The Eye, The Brain and Perception" (Further lectures on January 27 and 29).\*

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Problem of Obtaining Training Places for Six-Month Sandwich Course Students".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. J. Hardwood: "Flame Retardant Processes".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

COMPUTING ASSISTANT (with a good honours degree in mathematics or in a pure or applied science, and preferably some experience with large scale computing machinery) IN THE ELECTRONIC COMPUTING LABORATORY—The Registrar, The University, Leeds, 2 (January 22).

ASSISTANT LECTURER or LECTURER (preferably with an interest in ethics and perception) IN THE DEPARTMENT OF PHILOSOPHY—The Secretary, University College of Swansea, Singleton Park, Swansea (January 23).

DEMONSTRATOR IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Liverpool, 3. Quoting Ref. CV/391 (January 23).

LECTURER or ASSISTANT LECTURER IN SOCIOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (January 25).

LECTURER (with a first degree in physics, engineering or mathematics and appropriate postgraduate experience) IN APPLIED PHYSICAL SCIENCE IN THE DEPARTMENT OF PHYSICS—The Registrar (Room 22, O.R.B.), The University, Reading (January 25).

LECTURERS (with field work experience, preferably in the United Kingdom as well as overseas) IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY—The Secretary, The University, Edinburgh (January 28).

LECTURERS or ASSISTANT LECTURERS (2) IN SOCIOLOGY—The Registrar, The University, Hull (January 29).

CHAIR OF APPLIED MATHEMATICS—The Registrar, University College of Swansea, Singleton Park, Swansea (January 30).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Registrar, University College of Swansea, Singleton Park, Swansea (January 30).

LECTURER or ASSISTANT LECTURER IN INORGANIC CHEMISTRY; and a LECTURER or ASSISTANT LECTURER IN PHYSICAL CHEMISTRY—The Secretary, The University, Aberdeen (January 30).

LECTURER or ASSISTANT LECTURER IN PURE MATHEMATICS—The Secretary, University College of Swansea, Singleton Park, Swansea (January 30).  
LECTURER or ASSISTANT LECTURER (suitably qualified candidate in either experimental or theoretical physics) IN PHYSICS—The Registrar, University College of Swansea, Singleton Park, Swansea (January 30).

LECTURER (preferably with experience in neurophysiology) IN THE DEPARTMENT OF PHYSIOLOGY—The Secretary, The University, Aberdeen (January 30).

LECTURER (with a good honours geography degree and preferably a Doctorate in the field of physical geography emphasizing climatology) IN GEOGRAPHY—The Secretary, The University, Edinburgh (January 30).

ASSOCIATE or ASSISTANT PROFESSOR (preferably with qualifications and experience in modern mathematics and procedures) OF MATHEMATICS—The Secretary, University of Guyana, P.O. Box 841, Georgetown, British Guiana (January 31).

CHAIR OF GEOGRAPHY at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 31).

LECTURER or ASSISTANT LECTURER IN PHYSIOLOGY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (January 31).

RESEARCH ASSISTANT (with a good honours degree, preferably in physics or chemical engineering, and a special interest in particle mechanics, fluid dynamics or rheology) for a Nuffield Foundation project dealing with particle/fluid flow in model systems—The Registrar, The University, Nottingham (January 31).

LECTURER or ASSISTANT LECTURER (with an honours degree in a biological subject or in physiology) IN BIOLOGICAL OCEANOGRAPHY; and a LECTURER or ASSISTANT LECTURER (with an honours degree in mathematics, electronics or a physical science) IN PHYSICAL OCEANOGRAPHY—The Deputy Secretary, The University, Southampton (February 1).

LECTURER IN PURE MATHEMATICS—The Registrar and Secretary, Old Shire Hall, University of Durham (February 5).

DIRECTOR OF THE UNIVERSITY OF SHEFFIELD INSTITUTE OF EDUCATION—The Registrar, The University, Sheffield, 10 (February 6).

LECTURER, ASSISTANT LECTURER or TERMINABLE LECTURER (medically qualified or with a good honours degree in chemistry or biochemistry) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY—The Secretary, The University, Aberdeen (February 6).

WEST RIDING CHAIR OF MICROBIOLOGY—The Registrar, The University, Sheffield (February 6).

LECTURER and an ASSISTANT LECTURER (with a good honours degree in physiology or biochemistry, or a medical qualification) IN THE DEPARTMENT OF PHYSIOLOGY—The Registrar, The University, Manchester, 13 (February 10).

SENIOR LECTURER or LECTURER IN AGRICULTURAL BOTANY at Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (February 10).

LECTURER IN PHYSICS at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, February 15).

ASSISTANT LECTURERS IN PHYSICS—The Secretary, The Queen's University, Belfast, Northern Ireland (February 20).

SENIOR LECTURER or LECTURER (with an interest in plant taxonomy, morphology or physiology) IN BOTANY at Ahmadu Bello University, Northern Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (February 20).

EXPERIMENTAL OFFICER (with a degree, Graduateship of the Royal Institute of Chemistry or of the Institute of Physics, or at least a Higher National Certificate) IN MASS SPECTROSCOPY IN THE DEPARTMENT OF CHEMISTRY, to maintain and operate a high resolution mass spectrometer as a service to the department, and in due course help in the interpretation of the spectra obtained—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (February 21).

SENIOR DEMONSTRATOR IN PHYSICAL CHEMISTRY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (February 21).

LECTURER IN GEOLOGY at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, February 22).

VIROLOGIST IN THE DIVISION OF IMMUNOLOGICAL PRODUCTS CONTROL at Hampstead—The Director, National Institute for Medical Research, Mill Hill, London, N.W.7 (March 20).

LIBRARY ASSISTANT (young science graduate) IN THE NEW BIOCHEMISTRY LIBRARY—The Secretary, Biochemistry Department, Imperial College of Science and Technology, London, S.W.7.

MICROBIOLOGIST (national of the United Kingdom or the Republic of Ireland, with a B.Sc. and experience in either microbiology or mycology) in Nigeria, to carry out research on microbiology of stored food products in the south of Nigeria—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC.213/130/61.

PHYSICS or ELECTRONICS ENGINEER (with a first- or second-class honours degree) IN THE PHYSICS DEPARTMENT to assist in the development of techniques associated with the automatic analysis and display of data from clinical scintillation scanners—The Deputy Secretary, St. George's Hospital, Hyde Park Corner, London, S.W.1.

water supplies by the suppression of adverse facts and research, by misleading statements and by exaggerated claims.) Pp. 36. (Thorpe End, Almondbury, Huddersfield: Mrs. W. M. Sykes, Hon. Secretary, National Pure Water Association, 1964.) 2s. 6d. [2511]

Tobacco Research Council. Research Papers, No. 8: Report on a Study of Environmental Factors Associated with Lung Cancer and Bronchitis Mortality in Areas of North East England. By A. J. Wicken and Dr. S. F. Buck. Pp. ii+33. (London: Tobacco Research Council, 1964.) [2511]

Annual Report on the Meteorological Office for the year 1 January to 31 December 1963. Pp. viii+77+8 plates. (Met.O. 756.) (London: H.M. Stationery Office, 1964.) 6s. 6d. net. [2511]

Central Youth Employment Executive. Choice of Careers, No. 103: Art and Design. Second edition. Pp. 55. (London: H.M. Stationery Office, 1964.) 2s. net. [2511]

Oundle School Natural History Society. Annual Report, 1963. Pp. 18. (Oundle, Peterborough: Oundle School Natural History Society, 1964.) [2511]

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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 105: Diabase-Gabbro Sills and Related Rocks of Banks and Victoria Islands, Arctic Archipelago. By R. L. Christie. Pp. 13 (5 plates). 75 cents. Bulletin 116: Lower Jurassic and Bajocian Ammonoid Faunas of Northwestern British Columbia and Southern Yukon. By Hans Fretold. Pp. 31+8 plates. 2 dollars. Paper 63-39: Reconnaissance of the Ordovician and Silurian Rocks of Northern Yukon Territory. By B. S. Norford. Pp. 139. 75 cents. Paper 64-11: Illustrations of Canadian Fossils—Lower Cretaceous Marine Index Fossils of the Sedimentary Basins of Western and Arctic Canada. By J. A. Jezelsky. Pp. 101 (36 plates). 75 cents. Paper 64-12: Surficial Geology, Beauveville Map-Area, Quebec. By N. R. Gadd. Pp. 3+map. 35 cents. Paper 64-22: Mineral Industry of District of Mackenzie, 1963. By E. A. Schiller and E. H. Hornbrook. Pp. 43. 75 cents. Paper 64-36: The Mineral Industry of Yukon Territory and Southwestern District of Mackenzie, Northwest Territories, 1963. By L. H. Green and C. I. Godwin. Pp. vi+94. 75 cents. Paper 64-41: Frequency Rejection Filter for Use on DC Resistivity Surveys. By L. S. Collett and B. H. Ahrens. Pp. 10. 35 cents. Bulletin 109: Devonian Miospores from the Ghost River Formation, Alberta. By D. C. McGregor. Pp. 31+2 plates. 1.30 dollars. (Ottawa: Queen's Printer, 1964.) [311]

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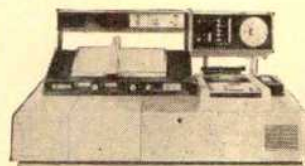
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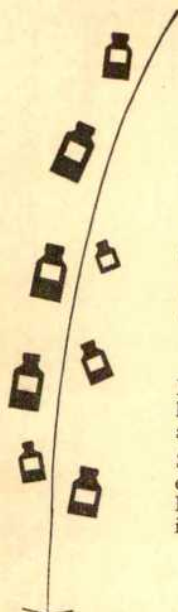
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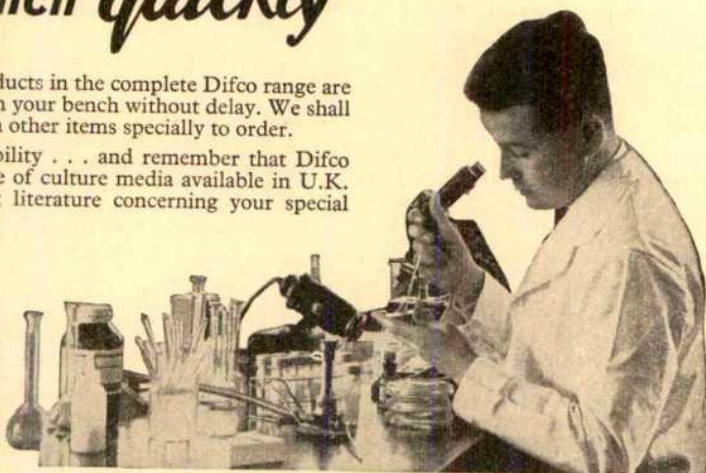
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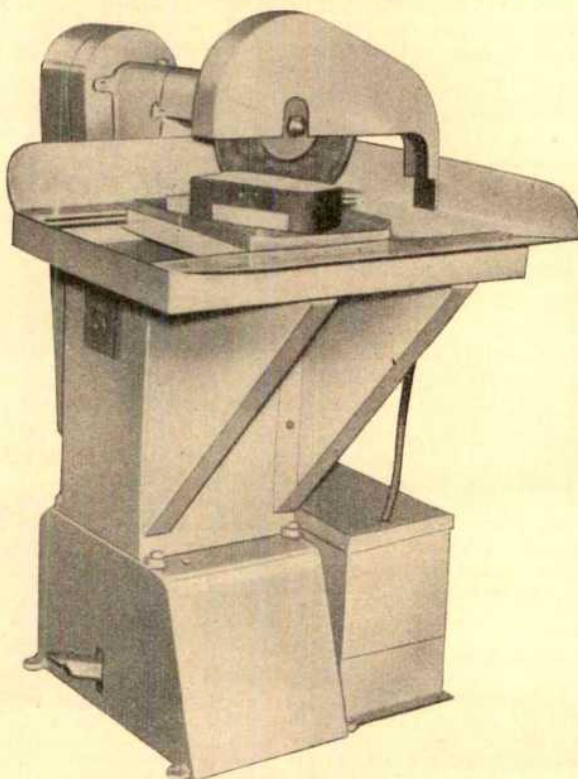
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## A MATTER OF SCIENTIFIC PRIORITIES

THE seventeenth annual report of the Advisory Council on Scientific Policy covers the year 1963-64\*. It is the last which will appear over the signature of Lord Todd as chairman and also the last to be submitted from the Advisory Council in its present form. In character and in calibre it fully matches the outstanding report submitted for the year 1962-63 (*Nature*, 200, 1133; 1963). The brief review of some major changes in the general environment of science during the life of the Advisory Council and of some achievements of the Council further demonstrate that its successor and the new arrangements will be hard pressed to match, let alone improve on, the efforts of the Council. Moreover, the topics selected for special comment in this report not only illustrate the soundness of the Advisory Council's judgment but also direct attention to one or two matters which have received insufficient attention in the new arrangements, at least up to the present time.

At its establishment the Council faced four main issues and, with the exception of the first, all face the Minister of Technology and the Secretary of State for Education and Science and their advisers to-day. Even the first, in a different form, now faces those and other Ministers, and indeed has been given as a specific reason for establishing the Ministry of Technology. The problems uncovered by the Advisory Council in seeking to recommend the appropriate form that Britain's research effort should take to achieve a maximum increase in national productivity during the decade 1947-57 are still largely with us, nor could it be held that the place of science in Government or the operation of Britain's scientific organization has yet been determined. For all the increase in the number of scientific advisers in the civil departments, the question of the mechanism of scientific advice and the location of research stations is unresolved. The Trend Committee was undecided and the decisions so far taken by the present Government tend to be inconsistent and show no clear pattern.

Likewise, the fourth major task of the original Advisory Council, that of continuous enquiry into the long-term development of Britain's scientific manpower and scientific resources, is a major preoccupation of any Government. While the Committee on Scientific Manpower, which was set up by the Advisory Council, is to continue, insufficient thought appears as yet to have been given to the implications in this task of the establishment of separate Ministries for Technology and for Education and Science. Only a brief section in the present report deals with higher education and scientific manpower, but that brief section stresses two points which have been neglected. The first is the question of postgraduate training and research, and in this context the Council's Committee on Scientific Manpower is at present surveying existing postgraduate courses in technology. The second is the matter of the supply of technicians, a survey of which is being combined with the triennial survey of scientific and technological manpower, to be made by the same Committee in 1965.

\* Annual Report of the Advisory Council on Scientific Policy, 1963-1964. Pp. vi+48. (Cmd. 2538.) (London: H.M.S.O., 1964.) 4s. net. See also p. 347 of this issue of *Nature*.

A related question is that of the status of technology, which is discussed at some length in the light of the findings of a Committee set up in 1963 under the Office of the Minister for Science to consider what might be done to create a climate of opinion more favourable to technology, and so to improve the quality of those entering on technological education. The findings and recommendations of this interdepartmental Committee are appended to the Council's report, and in general the Council agrees with the Committee's conclusions. It does not think, however, that the superior prestige attached in Britain to science as compared with technology is simply a matter of academic prestige. There also appears to be a widely held public attitude that a career in industry is socially unattractive, and the Committee may have over-estimated the significance of the fact that university entrants have not been taught technology at school, and that potential technologists are taught at school by scientists.

Nevertheless, the Council accepts the Committee's recommendation that all possible encouragement should be given to developing contacts between science teachers, the engineering profession and industry; that careers advisers should be approached by means of conferences and literature, and that careers literature for pupils should stress the national importance and intellectual interest of technology as a career; and that the introduction into the teaching of physics and mathematics of experiments illustrating the application of basic physical principles in technology should be further encouraged. The Advisory Council, however, insists that schools must not be diverted from their primary function of providing potential technologists, no less than others, with a broad foundation of knowledge as a basis for their subsequent careers. Again, while agreeing with the Committee on the value of sandwich courses, the Advisory Council thinks that conversion of existing university first-degree courses into sandwich courses is unlikely to improve either the quality of entrants to schools of technology or overall recruitment. While previous industrial experience should not be an absolute requirement for entry to university schools of technology, such experience is valuable, and such students, as well as prospective students of science, should be encouraged to acquire it. Strong support is also given to the Committee's proposals for strengthening the links between industry and the teaching staffs of universities and colleges of technology, including increased use, as part-time teachers, of men holding posts in industry.

The Advisory Council stresses particularly the opportunities for more flexibility in courses which the present expansion of higher education offers. Moreover, it notes with approval that the Committee recommends flexible arrangements to allow students to change their course of study from science to technology, if necessary, with extension of maintenance grant. The desirability of such flexibility has already been emphasized by the University Grants Committee. While, however, the Advisory Council recognizes a need for improved training of graduates by industry, it does not think that



the more progressive firms are likely to share the inter-departmental Committee's doubts, based on views expressed by some employers, as to whether technologists from the universities will meet the needs of industry as well as those who have qualified by part-time study while working in industry. Again, attention is directed to the desirability of clarifying the confusion which often exists in the public mind between technologists, on one hand, and technicians and members of the engineering craft unions on the other. It welcomes the Committee's recommendation, which is understood to have been implemented, to set up a working group to co-ordinate efforts of Government Departments in a strong and continuing public relations drive favourable to technology.

These various facets of the manpower situation all demonstrate the unsoundness of a rigid demarcation between technology and science, though they do not necessarily invalidate the recent reorganization, provided that really effective means of interdepartmental co-ordination are established. This is equally true of a further related problem to which the Advisory Council directs fresh attention and which is discussed at some length in a section of the report—that of scientific documentation, or ensuring the effective dissemination of the results of scientific and technical effort over as wide a field as possible. Here, the establishment of the new National Lending Library for Science and Technology, largely as the outcome of the work and recommendation of the Advisory Council's Scientific Library and Technical Information Committee, is seen as the chief result of the Council's effort in this field. Its essential counterpart, however, is a National Reference Library of Science and Invention, and the Council's final report includes a timely reminder that this vital project, in spite of some progress, is still uncompleted.

The present report does not enlarge on that situation, and the urgency of the matter could well have been stressed. Nor is this the only feature of the whole field that is far from satisfactory. In consequence of a special review undertaken by the Department of Scientific and Industrial Research at the request of the Minister for Science, the Advisory Council decided to obtain the views of a representative sample of scientists in a survey designed to show what kind of information working scientists find most useful and how they obtain it. This survey, limited in the first instance to chemists and physicists, was undertaken, under the general guidance of Prof. B. H. Flowers, by the Department of Scientific and Industrial Research, with the help of the Chemical Society, the Royal Institute of Chemistry, the Institute of Physics and the Physical Society.

The results of this survey, which identified, if only qualitatively, more or less general views which should facilitate action and the formulation of policy, are summarized in an appendix to the report. While minor differences appear between the methods of chemists and physicists in recording and seeking information, the proposals for improving the available services and the use made of them which emerge from the report are far more interesting and important. Published papers are still regarded as a most useful source of information, but a strong desire for changes and improvements is manifest. More published reviews of all types are needed but with a greater emphasis on those of the introductory, rather than the intermediate or advanced, type. There appears to be no present need for abstract periodicals to increase their coverage, but further investigation of the relative

functions of abstracts and titles seems desirable, and a need was evident for comprehensive abstracts periodicals covering particular disciplines. Some scientists obviously lacked expertise in the use of guides to the literature, and at some stage in their education scientists should be given formal training in the use of scientific literature. Similarly, the Advisory Council stresses that, if university libraries are to be used to the full, more university librarians must be employed with a scientific training and with a broad understanding of the needs of the scientists consulting them.

If such dynamic service is to be achieved, however, there are corollaries which are not examined in the survey or by the Advisory Council itself. Scientists will not be attracted to such a career unless reasonable prospects are provided. This rarely obtains at present, and even less in some of the other types of library which form an essential element in any adequate national library and information system. It is to be hoped that this will be adequately emphasized when, in due course, the Committee on Libraries of the University Grants Committee reports. Meanwhile, the position and the need have been ably analysed by Mr. J. Thompson in an article, "University Libraries and Higher Education", contributed to the *Library Association Record* for November 1964. They could well, however, also receive attention from the new organization for scientific and technical information recommended by a working party set up by the Department of Scientific and Industrial Research at the invitation of the Advisory Council.

This Working Party arose out of proposals made to the Advisory Council by the Council for Scientific and Industrial Research. The Working Party was set up to consider and amplify these proposals and suggested that the new organization would be most effective, at least initially, if it were supported directly by a Government department. It recommended accordingly that all Government scientific and technical information work of a general character should be brought under a single administrative unit in the Ministry of Education and Science, and guided by an independent committee appointed by the Secretary of State. The main functions of this new organization are envisaged as including provision of advice and support in the field of scientific and technical information over the whole range of the natural sciences, their relevant technologies and related aspects of the social sciences, including advice on the participation of the United Kingdom in the work of international agencies in this field. Included also would be the promotion of research and development in scientific communications, classification, storage, retrieval and translation of information, and operation of information services for scientists, technologists and others. While it will also be responsible for existing services, such as the National Lending Library for Science and Technology and grant-aid to the Association of Special Libraries and Information Bureaux (Aslib), as well as for co-ordinating existing and future activities in this field, there has been no reference to the National Central Library or the National Reference Library of Science and Invention, nor to the place of the university libraries, those of the colleges of technology or the contribution of the regional libraries and other public libraries.

All of these are now within the responsibility of the same Minister, and will have an essential part to play if the expenditure of the new organization is to be effective and efficient, whether of the anticipated order of £1.5



million or higher. The Advisory Council is satisfied that there is a need for a new organization on the lines recommended, and stresses again the need for the closest liaison between the new organization and the National Reference Library for Science and Invention. The implications are not discussed, and, however welcome is the emphasis laid in this report on this question of a national policy and organization in library and information work, it does not remove the misgivings which have been aroused by the neglect of such problems in the reorganization of civil science generally.

The Advisory Council notes in passing that it has been required to give some attention to scientific aid to emergent countries in the Commonwealth, particularly in Africa and to Britain's overseas scientific relations in general. It records without comment the demise of the Overseas Research Council, but it points out that Britain's participation in international scientific adventures is bound to grow in importance: the particular topic of high-energy physics and the national programme of scientific research is discussed in a separate section of the report, in which the Council accepts the proposals of the Council of the European Organization for Nuclear Research to develop a new machine of the order of 300 GeV and storage rings to increase the potentialities of the existing 28-GeV machine as scientifically desirable. It recommends that these proposals for developing high-energy physics in the United Kingdom should be supported by the Government, provided the requirements of other fields of science on the scale indicated are also satisfied.

That impinges on the main and most enduring issue which has exercised the Council during its seventeen years of existence; the adequacy of any new arrangements must largely be judged by the competence with which it deals with this question of the scale and balance of Britain's national effort in civil science. Expenditure in this field is estimated at more than ten times the resources available when the Advisory Council was constituted, and demands of which the Advisory Council is already aware could double the present figure in the next five years. The Advisory Council believes that this level of expenditure may be necessary if we are to continue to compete successfully in those fields of science in which we are now operating, and that such a scale would enable the present output of scientists to deploy their abilities fully and to build the essential background to our technological effort in industry as well as provide the trained manpower which industry requires to meet the increasing strength of overseas competition.

Nevertheless, the choice of priorities remains, and the Advisory Council, in reminding us that some choice between expensive fields, such as space technology and high-energy physics, is inevitable, admits that it has never had the powers to deal with this problem. There is also a shrewd reminder of the difficulty of foresight. The Advisory Council itself is of the opinion that Britain is devoting too little of her gross national product to the advancement and exploitation of science, though, like Mr. C. F. Carter and Prof. B. R. Williams in their paper, "Government Scientific Policy and the Growth of the British Economy" (*The Manchester School*; September 1964), it recognizes the difficulties and complexities involved in any such decision.

That, as Mr. Carter and Prof. Williams also recognize, is related to the question of innovation in industry, and to that subject the Advisory Council devotes the longest

section of its report. The Advisory Council has sought to discover whether, in the light of scientific knowledge now available, large-scale investment in an expanded programme of power generation in the electrical industry would be desirable, using funds comparable in size to those required for the development of research in nuclear physics. The recent debate in the House of Lords on the electricity, gas and nuclear energy industries indicated that considerable scope exists for further enquiry in this field, and the discussions which the Advisory Council had with the Ministry of Power and the Central Electricity Generating Board immediately raised points of much wider interest in relation to technological innovation generally.

It is in this wider context that the Advisory Council submitted its conclusions last August to the Secretary of State for Education and Science. Its report points out that, since innovation is the result of applying existing knowledge to the satisfaction of human needs, an increase in innovation is likely to follow a powerful and sustained research effort in any field of knowledge, though the relation is not simple. Technical innovation, however, springs from the combination of an inventive human kind, or an organization willing to venture to risk capital on it, and accordingly, except where there are other motives than financial or economic gain, the enterprise must have good prospect of showing a substantial commercial profit. The circumstances which promote or impede innovation in industries science based or technologically advanced differ from those which affect innovation in traditional industries, or in modern industries which produce consumer goods on a massive scale. While the discussion of this subject differs considerably from that of Prof. I. D. Rattee in his lecture on "Discovery and Invention" (*Nature*, 204, 1017; 1964), in that the Advisory Council is much less ruthless towards the inefficient and backward, the conclusions are broadly similar.

The Advisory Council concluded that very advanced research and development of long-term national importance is required in an increasing number of fields, but this is beyond the capacity of many industries as now organized in Britain, and it instances the generation of electricity. Here it suggests that the possibility of operating through consortia should be studied, with the Government participating as a partner in the enterprise. In the traditional industries, it considers that the position is unlikely to improve without more research or much more attention to the utilization of available knowledge. It would encourage 'innovation' by development contracts, and the Council is confident that here, and in those industries engaged in mass production of consumer goods, research associations should find great scope.

The closer integration of traditional industries with the sciences on which they rest depends above all on the wider employment of scientists and technologists in these industries, and to that end the Advisory Council suggests that Britain should aim at closer relations and deeper understanding between those industries and the universities. However ready the universities are to co-operate and to seek to understand the problems of such industries, it is difficult to visualize an adequate response from the industrial side before such permeation with scientific staff occurs on a sufficient scale: extension of the policy of part-time professors and lecturers from industries presupposes the existence of experienced scientific staff in industry. This is more likely to be a long-term than a short-term result of a wider public understanding

of the importance and status of technology, the promotion of which has already been discussed.

In any consideration of innovation in industry, however, problems of organization loom large, and the Advisory Council stresses the prime importance of management. In this connexion it is pointed out that this is a matter not merely of having scientists or technologists in the highest administrative posts, but equally of dispersing people with scientific and technological qualifications throughout the industrial organization. Such a state of affairs could enable minor technical problems to be solved quickly and major technical ones recognized and referred to the research department for handling by those qualified to do so. This again is part of the unsolved problem of stimulating research and development in the small or backward firm, and to that end this investigation by the Advisory Council does not contribute anything new. It suggests, however, that immediate attention be directed to a thorough study of the means of communication between the innovator and those responsible for determining policy. It also urges the need for examination of the social factors affecting innovation, such as case studies of the social consequences of a major innovation in one of Britain's mature industries.

In this its last report the Advisory Council on Scientific Policy has left its successor—or successors—with some definite lines of enquiry which should be continued or initiated. The report should remove any false ideas that rapid solutions are likely to result from any changes in organization alone: success is more likely to flow from persistent effort in many fields, above all, from effort unhampered by preconceived ideas of any kind. There may well be some concern as to whether the present changes, for a time at least, will impede progress and cause some overlapping or dispersion of effort. This would appear distinctly possible in the arrangements regarding manpower and the responsibility for technology, as well as for information services and libraries. Whatever the future may hold, the Advisory Council on Scientific Policy is manifestly entitled to the nation's gratitude for seventeen years of outstanding service to the nation and to science. Its record is unlikely to be surpassed, and may not be attained, unless its successors are accorded an authority and resources which the Advisory Council itself has never enjoyed.

## VARIATION PRINCIPLES IN COLLISION THEORIES

### Variational Principles in the Theory of Collisions

By Yu. N. Demkov. Translated from the Russian by N. Kemmer. Pp. x + 157. (London and New York: Pergamon Press, 1963.) 42s. net.

VARIATIONAL principles have played an important part in quantum mechanics almost from the very beginning. However, the first applications were confined to the discussion and approximate determination of the energies of bound states of nuclear, atomic and molecular systems. It was not until the 1940's that ways were found for applying variational principles to scattering problems. Since then a variational outlook on the treatment of collision phenomena has been developed which has not only provided new methods for approximate calculation of collision cross-sections but has also led to deeper and clearer theoretical understanding of non-stationary quantum phenomena.

Demkov has presented a very clear account of the way in which variational principles have been derived in collision theory. The first chapter discusses the formulation of the variational principles of Hulthén and of Kohn for both elastic and inelastic collisions. In the next chapter the connexions between these and other variational principles and also perturbation methods are discussed. Applications to the elastic collisions of electrons with hydrogen atoms are given in this chapter to illustrate the use of the principles for actual evaluation of approximate cross-sections. Chapter 3 deals with more formal questions of the symmetry properties of the functionals, the principles of detailed balance and the unitarity of the scattering operator, in terms of variational principles. Finally, in Chapter 4, variational methods are used to obtain results analogous to the virial theorem in classical mechanics.

The author's presentation is very clear and free from unnecessary formalism, while the translation is excellent. It is most instructive reading for any student of quantum mechanics and should certainly be examined carefully by anyone working on atomic collision problems. The only cause for regret is that the price of the book is so high.

HARRIE MASSEY

## ELECTRONIC SPECTRA AND ORGANIC COMPOUNDS

### Interpretation of the Ultraviolet Spectra of Natural Products

By Prof. A. I. Scott. (International Series of Monographs on Organic Chemistry, Vol. 7.) Pp. x + 443. (London and New York: Pergamon Press, 1964.) 84s. net.

INCREASING use of infra-red, nuclear magnetic resonance and mass spectra for the elucidation of the structures of organic compounds may have given the impression that the earlier-developed method of electronic spectra was no longer being used. Prof. Scott's book is a reminder that ultra-violet and visible spectra continue to play a valuable part among the tools of the organic chemist, especially the chemist concerned with natural products. The simultaneous use of all these techniques is advocated to reduce experimentation to a minimum, particularly where only minute amounts of labile material or compounds that are difficult to separate are being handled.

More information will be found in *Interpretation of the Ultraviolet Spectra of Natural Products* than is suggested by its title, because the first part deals systematically with simple chromophores, albeit from an empirical and phenomenological point of view. After an introduction there are chapters on single chromophores, conjugated chromophores, C-aromatic compounds, O-, S-, and N-hetero-aromatic compounds, each of which is liberally illustrated by examples. There is a short chapter by Dr. C. J. W. Brooks on miscellaneous applications with particular mention of analytical estimations and colour reactions. This leads on to the longest chapter, 85 pages, on the investigation of molecular structure, in which selected classes of natural products are discussed in greater detail. The final chapter takes a range of specific examples of natural products of quite complicated structures, including terramycin, shellolic acid, some alkaloids, mould metabolites, streptimidone, limonin and plumieride, and shows how the electronic spectra together with other spectroscopic and chemical evidence have allowed the structures to be elucidated. The book concludes with an appendix on steroids consisting mainly of 54 pages of tabulated spectra.

Some of the less desirable features of the book deserve comment. The index is inadequate, having only approximately 1,000 entries for 443 pages. Many readers will seek

spectroscopic information on specific compounds and not find an entry even though the compound may be discussed in the text. While there are copious references to the original literature, it is noteworthy that fewer than 9 per cent of the citations refer to the post-1959 period, and almost the entire number of these to 1960 and 1961. There are a number of blemishes in the production, carelessly printed formulae and minor typographical errors, which are more irritating than confusing. In some sections the author's arguments would have been more clear if he had been more concise and if the tables had been more compact and placed closer to the relevant discussion. Some of the structures are accompanied by wave-lengths but not by intensities, which have to be sought for in the text or in the tables. In some cases intensities are not quoted at all, for example, for three of the four bands of acridine, and for riboflavin. A more serious comment is that some of the numerical values quoted do not agree well with values that are given in the literature.

Prof. Scott is to be congratulated on having gathered together so much information and imposed an order on it. Undoubtedly all natural product chemists and many others as well will want to have ready access to this book.

C. J. TIMMONS

## A COURSE IN ENDOCRINOLOGY

### Experimental Endocrinology

A Sourcebook of Basic Techniques. By M. X. Zarrow, J. M. Yochim and J. L. McCarthy. With a chapter on Invertebrate Hormones by R. C. Sanborn. Pp. xvi + 519. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 107s. 6d.

IN the curriculum of some North American universities endocrinology receives special consideration, but in Britain it is taught largely in the context of other main subjects such as zoology, physiology and medicine, and formal instruction for the advanced student is uncommon. A course in endocrinology that was first taught in the laboratory of Prof. F. L. Hisaw in 1946 forms the basis of a new book, *Experimental Endocrinology. A Sourcebook of Basic Techniques*. The scope of this book is clearly stated in the preface by Prof. Zarrow: to provide material for "an advanced course or for use by the individual student in a project course". More than a hundred experiments are described, ranging from the classical methods of the 1930's to those developed more recently (up to about 1962), emphasizing the widespread derivation of techniques from disciplines that include anatomy and physiology, biochemistry and pharmacology. It is both text-book and laboratory manual, and the authors claim that about 85 per cent of the experiments they describe have worked successfully "at the student level".

An earlier definition of a hormone as "a specific chemical entity that is secreted into the blood by a well-defined group of cells and is transported via the vascular system to a site distant from its point of origin, where its action takes place", is considered to be no longer adequate. It is now expressed in more flexible terms to include those "physiological organic substances" secreted from a "relatively restricted area of the organism" that diffuse or are transported to sites of action where their effect is one of adjustment or integration. Although this raises problems with respect to certain substances, it goes some way to describe others such as the posterior pituitary hormones vasopressin and oxytocin.

Classified according to their chemical structure, the hormones are divided into three main groups: first, steroids related to cholesterol, such as oestrogens and adrenocorticoids; secondly, amino-acids or their deriva-

tives, including catechol amines and the thyroid hormones; and thirdly, polypeptides and proteins such as relaxin and insulin, corticotrophin and parathormone. The main members of these groups are considered in thirteen chapters in which about one-half of the text is devoted to the source and nature of the hormone, its biological effects and mode of action, and its biosynthesis and metabolism. This is followed by a separate section dealing with the basis of suitable assays, biological and chemical. In chapters on oestrogens, androgens, thyroid hormones, and, to a lesser extent, gonadotrophins, the question of anti-hormones is considered. The text covers numerous aspects: physical properties of somatotrophin and parathormone, amino-acid sequences of polypeptide and protein hormones, the secretion of catechol amines, the action of pancreatic hormones in metabolic pathways, the interrelationships of gonadotrophins in the female mammal, and many others. In the final chapter on invertebrate hormones, R. C. Sanborn discusses the regulation of moulting, growth and reproduction in the Insecta and the Crustacea as well as activity rhythms and pheromones, to give a few examples.

Important contributions have been made by experiments that involve ablation of endocrine glands, replacement therapy and the isolation and estimation of hormones. In the second half of every chapter the authors illustrate the use of these techniques in experimental endocrinology. There is a valuable series of experiments that includes ablation of glands, described with usually helpful diagrams (hypophysectomy in rat and chicken, parathyroidectomy in pigeon, adrenalectomy in rat), techniques of transplantation and parabiosis, biological assays and experiments using replacement therapy, and chemical procedures for the extraction and estimation of hormones involving chromatography, fluorimetry, spectrophotometry and radioisotope methods. The series is not exhaustive, and inconsistencies occur—for example, the absence of a chemical determination for progesterone when more complex methods are introduced in other sections—but many laboratories will find here much useful information if it is understood that the book is intended primarily for course-work and not as a definitive account of experiments in endocrinology.

The text is usually concise, comprehensive and well-documented, the authors presenting much of their material in tabular form which, although readily assimilable, tends to repeat information already given in the text. Similarly, there is a repetition of biosynthetic pathways in some chapters on steroids that could have been avoided by a composite and more informative diagram as used to describe the metabolism of adrenocorticoids. Several inconsistencies were noted, especially in the presentation of references to original sources, and for a book of this price, in which the print and lay-out are generally good, it is to be regretted that the illustrations frequently lack clarity and contrast.

It is hoped that the authors will be stimulated to produce future editions of this book as further techniques, suitable for teaching courses, become available.

R. B. HEAP

## REVIEW OF CATALYSIS

### Advances in Catalysis and Related Subjects

Vol. 14. Edited by D. D. Eley, Herman Pines and Paul B. Weisz. Pp. xii + 522. (New York and London: Academic Press, 1963.) 114s. 6d.

VOLUME 14 of *Advances in Catalysis and Related Subjects* presents again the now familiar but still admirable annual medley of review articles on adsorption and catalysis, together with a welcome intrusion from a cognate region of biochemistry.



In two very helpful accounts of techniques, it is gratifying to find proper credit given to early workers: much recent writing seems to imply that the present-day solutions emerged fully-fledged within the past decade. Leftin and Hobson in "Application of Spectrophotometry to the Study of Catalyst Systems" (83 pp. and references 4 pp.) deal with optical absorption spectroscopy, applied to the investigation of some chemisorbed complexes on metals, oxides and salts; coverage is complete but acid catalysts, especially silica-aluminas, receive most attention as befits the interests of these authors and the pattern of previous reviews. Erlich's "Modern Methods in Surface Kinetics" (168 pp. and references 4 pp.) provides, in characteristic style, a thorough survey of flash desorption, field emission microscopy and ultrahigh vacuum technique (both methods and typical results) with a timely section on practical details. Evidently these powerful tools, together with low-energy electron-diffraction, will soon be commonplace in research on clean metal surfaces. But when will the bridge between surface physics and surface chemistry be consolidated?

There are three contributions to the mainstream of catalysis, each in different vein. Mars, Scholten and Zwietering, in "The Catalytic Decomposition of Formic Acid" (75 pp. and references 4 pp.), survey present research and points of view concerning this model-reaction, about which discussion has sometimes reached polemic-force; dehydrogenation and dehydration over metals and oxides are discussed in detail and related to the water-gas shift reaction and the decomposition of bulk formates. The system is well suited to tests of the intermediate compound theory of catalysis, but the authors are rightly guarded in their appraisal of recent generalizations. Catalysis in preparative organic chemistry is represented by the valuable, descriptive paper entitled "Hydrogenation of Pyridines and Quinolines" (45 pp. and references 5 pp.) by Freifelder, which collects results obtained on the selective hydrogenation of these awkward, self-poisoning heterocycles: one feels here the lack of a short section on mechanism designed to crystallize the topic. Margolis covers a wide and important field somewhat discursively in the chapter entitled "Catalytic Oxidation of Hydrocarbons" (68 pp. and references 5 pp.). The trend of Russian research emerges strongly, but strange phraseology obscures some passages, and vital points, such as the evidence for various forms of chemisorbed oxygen, are treated too lightly. There is a nice counterpoint between electron-transfer and chemical complex theories, but one is left wondering whether sensitive surface-potential methods are central to the investigation of polyfunctional surfaces.

Calvin approaches the fundamental biochemical photocatalytic problem, in "Quantum Conversion in Chemoplasts" (31 pp. and references 3 pp.), with the aid of photoconductive organic systems suggested by present organic semiconductor research, and draws some interesting parallels: it is exciting to see how the gap between the quick and the dead narrows.

In all, a well-composed medley—but surely the editor cannot still maintain that such diverse catalytic chemistry comprises a discipline "in its own right".

D. A. DOWDEN

## POPULAR PRESENTATION OF MEDICAL RESEARCH

### Our Most Interesting Diseases

By Harold Burn. Pp. 180. (London: George Allen and Unwin Ltd., 1964.) 25s.

TO give to the lay public a description of the growing points of medical research to-day is an important and urgent task. The difficulty that the writer has to overcome

is one of communication: it is essential to exercise economy in the use of technical terms and to explain adequately and in simple language those that are used. Once again Prof. H. Burn has set himself this task and once again he has been successful. This book deserves to find many readers.

The topics that Prof. Burn has selected are all of great importance to those who live in a modern society. It is interesting that in his catalogue of our most interesting diseases the microbial infections are not listed. That they are omitted is a development that we owe to relatively recent successes in chemotherapy. The diseases that this book deals with are mainly those of late or middle age. Cancer, vascular and heart disease, gastric and duodenal ulcer, bronchitis—these are topics that we find discussed.

In a book of this kind the reader cannot expect to find final or authoritative answers. The description of modern research is a tale of trial and error. The author's individual attitude must determine also how he selects and weighs the material discussed. What matters is that what is selected is adequately described, and that in each chapter the story has cohesion and adds up to give a clear picture of the present state of our knowledge. There are a number of instances where the reader would like to know if all the experts are in agreement with Prof. Burn's evaluation of the evidence; but it is one of the great merits of this book that we are given the evidence and that we are allowed to arrive at our own conclusions.

Prof. Burn does not see medical science as a body of knowledge that is unrelated to our lives. The influence of environment, of social conditions and habits on disease and health is discussed. Almost inevitably this leads in the final chapter of the book to a guide for healthier living. We are told to eat less, to drink less and to smoke less and not to give up physical exercise. These precepts are given with moderation and common sense; and this gives the author's injunctions both authority and authenticity.

H. BLASCHKO

## THEORY AND PRACTICE OF BIOLOGICAL CONTROL

### Biological Control of Insect Pests and Weeds

Edited by Paul DeBach. Pp. xxiv + 844. (London: Chapman and Hall, Ltd., 1964.) 120s.

C. P. CLAUSEN once remarked that insects are their own worst enemies. Their other enemies include pathogenic micro-organisms, while their other victims include plants. These ideas are well illustrated by numerous successes in the biological control of pests and weeds described in this book. Many of the successes have been in California. This is attributable partly to local ecological features, but also in great measure to a number of enthusiastic exponents of the method, notably to the leadership of the late Prof. Harry S. Smith, to whom the book is dedicated. Its 16 authors are all present or past staff members of the University of California, all specialists on the topics with which they deal, some of them pre-eminent authorities. The outcome is a most impressive and comprehensive treatise, in which the advantages of multiple authorship have been fully exploited; thanks to careful planning and editing, the disadvantages have, on the whole, been overcome. The subject is clearly and logically subdivided in a scheme fully set out in a contents list of 15 pages.

In the first of the eight sections, P. DeBach deals with the scope of biological control and R. L. Doutt with its historical development. In Section 2, the first 72 pages are devoted to an outline of the development of population ecology, and to a discussion of ideas of natural control

and the role of density-dependent processes, by C. B. Huffaker and P. S. Messenger. Then Doutt and DeBach discuss some much debated questions dealing with biological control—on population equilibria; the effects of attacks by more than one parasite in individual hosts, and on parasites by hyperparasites; the relative value of parasites and predators; the sequence theory; the island theory; the influence of different types of environment on the chances of success, and so on. They have skilfully fitted into 24 pages the essential facts and views, and their own conclusions, apparently without serious omissions. In Section 3, Doutt and K. S. Hagen deal with the biology of natural enemies (100 pages), and E. I. Schlinger and Doutt discuss systematics in relation to biological control, beginning with theory and concluding with keys to families of entomophagous insects.

The fourth section of 144 pages is in effect a detailed practical handbook by six of the authors on the methods of collection, maintenance, release and recovery of entomophagous insects, illustrated with photographs of insectaries and equipment.

In the fifth section, of 83 pages, five authors describe methods of enhancing the effectiveness of parasites and predators by such measures as the introduction of supplementary hosts or food and the modification of the environment, and discuss the integration of chemical and biological control. In Section 6 (113 pages), E. A. Steinhaus deals with microbial diseases of insects, Y. Tanada with their epizootiology, and M. E. Martignoni and I. M. Hall with their mass production and use in control. In Section 7, of 40 pages, Huffaker outlines the fundamentals of the biological control of weeds, and J. K. Holloway describes the success in various countries of measures to control 13 major weeds by the introduction of insects. In a concluding section of similar length, DeBach lists over 200 cases of biological control (of insect pests) rated as completely, substantially or as partly successful, and discusses present trends and future possibilities.

There is a bibliography of more than 2,500 titles, extending to mid-1961, and an index to species. The book is compact and well produced; the numerous illustrations are on the whole effective, although a few of the half-tone pictures are too small and some of the line drawings are poorly reproduced.

Hitherto the standard work in English has been H. L. Sweetman's *The Principles of Biological Control* (revised 1958). The plans of the two books are somewhat similar, but Sweetman's is much shorter (and may still be preferred as a text for students). *Biological Control of Insects and Weeds* is more comprehensive, more authoritative and more up to date, and presents the subject more adequately in its theoretical setting. It will no doubt be the definitive work for many years to come.

M. E. SOLOMON

## SCIENTIFIC AND PHILOSOPHICAL UNCERTAINTY

### Scientific Uncertainty, and Information

By Prof. Leon Brillouin. Pp. xiv+164. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 52s.

OUR knowledge of the world is inevitably imprecise and uncertain. In his latest book, Prof. Brillouin stresses, illustrates, and draws rather far-reaching consequences from this fact. His stress on the importance and inevitableness of imprecision is very welcome. Experimental error has too often been discounted as a tiresome but trivial excrescence on the neat deductive structure of science. This has led, on one hand, to needless alarm at the appearance of theories incorporating imprecision and, on the other, to misguided attacks on the

notion that scientific explanation is deductive. The second part of the book, "Uncertainty in Classical Mechanics", should help to dispel the former complaint. There is a valuable discussion and extension of Poincaré's theorem. Several examples are given of instability, and especially of discontinuities of prediction arising from infinitesimal changes in initial conditions. In all this the author gives due credit to Poincaré; he might have acknowledged his debt to Duhem's *The Aim and Structure of Physical Theory*, which also discusses the "mathematics of approximation" in some detail.

The first part, "Information and Uncertainty in Science", which strays into philosophy, is not nearly so good. Here Duhem not merely forestalls (by nearly fifty years) but also far exceeds. It is a great pity that eminent scientists will not inform themselves about philosophy before pronouncing on it (and, of course, vice versa). The author's scientific insights are offset by a philosophical confusion which greatly diminishes the value of what might have been a major work in the philosophy of science. To start with, positivism is confused with solipsism: "A narrow positivism intended to limit each individual to the knowledge of his own sensations: other human beings around him appeared to him only as ghosts" (p. 47). This is just part of the more serious confusion about the existence of an external world: "The scientist should never confuse the actual outside world with his *self-invented physical world model*" (p. 51) ... "The modern scientist must absolutely renounce the idea of a real objective world" (p. 52). Must the 'modern scientist' become a 'narrow positivist'? If there is no 'actual outside' (= 'real objective'?) world, how does the scientist's confusion arise? On p. 50, Planck is represented as saying: "This world is not directly accessible to us". Three lines below, this is referred to with the remark: "what is the use of speaking of an inaccessible world?"—which is scarcely a faithful reproduction.

A corollary of the author's confusion about reality is his equivocal attitude to fact and theory. In the introduction, the assertion: "We select experimental results that appear to us as logically connected together, and we ignore many facts that do not fit into our 'logic'", is immediately followed by the scarcely compatible assertion: "... a theory may be discarded, while facts remain ... and the connexions (between the facts) will be maintained in a different theory". In what sense do facts 'remain' if we are free (to an extent) to ignore those which the new theory does not explain? This is a serious problem, but oscillating between contradictory answers is no way to deal with it. This uncertainty pervades the whole book: warnings of the temporary, relative, corrigible nature of theories are interspersed with passages in which consequences of quantum and information theories are presented as facts about the world.

One particular consequence is made a great deal too much of, namely the "interaction between observer and the observed element" (p. 52). In the first place, the author seems to suppose that pointing out that "the interaction is finite" refutes what "was currently assumed, that this interaction could be made very small and negligible" (p. 52). In the second place, he confuses this with the impossibility of making precise predictions. But it does not follow that because an observer interacts with what he observes he cannot know the effect of his observation and hence compute both what the future will be and what it would have been if he had not made the observation. Given some present-day theory, it may follow; but I take the author to be making a quite general point.

Of the other topics dealt with, there is space only to record further confusion about causality, determinism and the arrow of time, and an appalling looseness in applying the concepts of information and probability.

D. H. MELLOR

**Animal Ancestors**

By Sonia Cole. Drawings and Reconstructions by M. Maitland Howard. Pp. 78. (London: Phoenix House, Ltd.; New York: E. P. Dutton and Co., Inc., 1964.) 13s. 6d. net.

WHEN some striking palaeontological find is made, the non-specialist student has until now had great difficulty in finding any information other than in works of a very technical and scholarly nature, or in the often rather inaccurate and very generalized popular science books. Mrs. Cole is to be congratulated in her production of a clear, concise and thoroughly readable work which fills this gap and makes mammalian palaeontology a living science which can be compared with our own personal knowledge of the world we live in to-day. Miss Maitland Howard's excellent drawings are an essential part of this book; the differentiation, by colour, of known fact from enlightened supposition is particularly clear.

Mammalian evolution is a very wide subject and extremely difficult to condense into a book which is easily read, light to handle and reasonable in price; it is inevitable that much has to be left out. No two palaeontologists would agree on the choice of priorities, or even on the much debated branches of family trees, which in *Animal Ancestors* have to be very generalized and so unfortunately lose some of their value. The relationship between hyraxes and elephants is perhaps too strongly emphasized; the mention of undescribed remains from the Upper Miocene of Kenya which could be a connecting link between anthracotheres and hippos as having "actually been proved" seems rather premature.

There is a full and comprehensive index, so often omitted in works of this nature; the addition of a list of books for further reading would be of benefit to those readers wishing to continue studying the subject which Mrs. Cole and Miss Maitland Howard have introduced with such clarity. I feel sure *Animal Ancestors* will have a wide appeal and will quickly find its way on to the shelves of public libraries and educational institutions as well as private collections.

S. C. CORYNDON

**Monograph on Radio Waves and Circuits**

Proceedings of Commission VI on Radio Waves and Circuits during the XIIIth General Assembly of URSI, London, September 1960. Edited by Samuel Silver. Pp. vii + 377. (Amsterdam, London and New York: Elsevier Publishing Company, 1963.) 150s.

THIS book is primarily a collection of papers describing new work, prepared by acknowledged experts for discussion at meetings of specialists in Commission VI of the International Scientific Radio Union. Even in one small volume the range of subjects touched on is wide: electromagnetic wave boundary problems, propagation of waves in statistically inhomogeneous media, information theory and coding problems, aeriels and data-processing for radio astronomy, and solid-state circuits. The diversity of subjects covered by Commission VI is explained by the editor in terms of the realization of the need for a co-ordinating unit within the International Scientific Radio Union which could deal with the many expanding new areas of research and the new developments in applied mathematics; it would bring together engineers, physicists and mathematicians on problems which were previously regarded as purely ancillary by the other Commissions, the interests of which were turning more to the geophysical and cosmological import of radio propagation research. Commission VI was formed with this object in mind.

Most of the papers in the book describe original work on a particular aspect of a subject, although one or two are in the nature of reviews, and useful references are given. As with all material in the forefront of knowledge, the papers provoked discussion at the meetings,

and this discussion is reported in the book; some is of philosophical interest, as, for example, that on surface waves.

It should be emphasized again that this publication is primarily a collection of the papers which were presented at Commission VI, and does not purport to be a text-book. It will be of interest to specialists and those wishing to sense developments taking place in new fields. At the price of £7 10s. this is not, perhaps, a book which the average reader would expect to buy, but he might, nevertheless, expect to find it on the shelves of a good specialist library.

R. W. MEADOWS

**Tables of Natural and Common Logarithms to 110 Decimals**

By W. E. Mansell. (Royal Society Mathematical Tables, Vol. 8.) Pp. xviii + 95. (Cambridge: At the University Press, 1964. Published for the Royal Society.) 40s. net.

THESE tables originated in a part of the extensive computations made, without machine, by William Ernest Mansell (1877-1953), an accountant, who in his will made provision for their publication. They have been edited by Dr. A. J. Thompson, with assistance in checking from several persons and institutions. They give both logarithms to base  $e$  (pp. 1-47) and logarithms to base 10 (pp. 49-95), to 110 decimals, of all integers up to 1,000 and also of  $1+m.10^{-n}$ , where  $m=1(1)9$ ,  $n=5(1)12$ . An introduction explaining construction, checking and use of the tables ends (p. xviii) with a noteworthy table of mathematical constants to 110 decimals.

There is no difficulty in justifying a certain amount of tabulation of important functions to, say, 20 or 25 decimals. Dr. Thompson's own monumental 20-decimal *Logarithmetica Britannica* must have struck many numerical analysts as particularly well judged. To justify tabulation to more than 100 decimals is always more difficult. The editor acknowledges that logarithms to so many decimals are infrequently required. One feels that Mansell must have been a compulsive calculator who followed, with no more equipment and with similar *éclat*, in the footsteps of those great computers Abraham Sharp (1653-1742) and Isaac Wolfram (c. 1725-c. 1787). It is impossible not to admire a work so definitive of its kind, and it is pleasant to think that these logarithms, known for some time to have been computed, have now been fortunate in finding skilful editing and publication. Any computing enthusiast who purchases this volume will acquire, in a moderate compass, a powerful tool to which he will probably turn more often than he at first anticipates. It goes without saying that the volume should be available in specialized libraries of numerical analysis.

ALAN FLETCHER

**Chymia**

Annual Studies in the History of Chemistry, Vol. 9. Editor-in-Chief: Henry M. Leicester. Pp. 221. (Philadelphia: University of Pennsylvania Press; London: Oxford University Press, 1964.) 40s. net.

VOLUME 9 of *Chymia* contains more articles than usual and covers a range of topics. There are obituaries, with portraits of Eva V. Armstrong and Clara de Milt, both contributors to the history of chemistry. There are three papers dealing with, or connected with, Sir Humphry Davy, one of these concerning the work of Grotthuss on gaseous combustion. Three papers on chemistry and technology in the East, and discussions of chemical laboratories in Russia, indicators, Sir B. C. Brodie and his calculus of chemical operations, elements and nucleosynthesis in the nineteenth century, Boyle on the degradation of gold, mining education in South America, and the water controversy, make up the rest of this interesting collection.

J. R. PARTINGTON



## RADIO PULSES FROM EXTENSIVE COSMIC-RAY AIR SHOWERS

By DR. J. V. JELLEY and J. H. FRUIN  
Atomic Energy Research Establishment, Harwell

PROF. N. A. PORTER and T. C. WEEKES  
University College, Dublin

AND

PROF. F. G. SMITH and R. A. PORTER  
University of Manchester, Nuffield Radio Astronomy Laboratories, Jodrell Bank

VARIOUS proposals have been considered for the detection of large cosmic-ray air showers by radio techniques. Neither the original proposal of pulse radar<sup>1</sup> nor the passive detection of Čerenkov radiation at microwave frequencies<sup>2</sup> now appears to be feasible. A re-assessment of the possibilities of Čerenkov radiation at longer wave-lengths has, however, been stimulated by the recent suggestion<sup>3</sup> that a considerable enhancement of the intensity may be expected in the radiation from an electron-photon cascade. This is due to a fractional negative charge excess  $\varepsilon$  arising from the annihilation of positrons in flight. For a shower with a total of  $N$  particles, the radiation intensity at low frequencies is proportional to  $(\varepsilon N)^2 \nu \delta\nu$  within a bandwidth  $\delta\nu$  at a frequency  $\nu$ , and the enhancement factor is  $\varepsilon^2 N$  over that from  $N$  particles radiating incoherently. In a typical shower of primary energy  $E_0 \sim 10^{15}$  eV, for which  $N \sim 10^8$  and the electron and positron energies are of order  $10^8$  eV, we expect  $\varepsilon \sim 0.1$ , and  $\varepsilon^2 N \sim 10^4$ .

If we consider a shower front as a disk of thickness  $t$  and area  $S$  incident vertically close to an aerial array of area  $A$ , two conditions must be satisfied to achieve the maximum mutual coherence: (i) the wave-length  $\lambda$  must exceed  $t$ , and (ii) the radiation from all parts of the shower front must arrive in the same relative phase at all elements of  $A$ . In such a shower  $t \sim 2$  m (ref. 4) and the effective radius of the shower is  $\sim 50$  m (ref. 5), so that these conditions are reasonably satisfied for  $\lambda \geq 4$  m and for altitudes  $\geq 3$  km.

The present experiment was conducted on the basis of favourable estimates of the expected yield from this enhanced Čerenkov radiation. Cosmic-ray showers in the energy region  $10^{15}$ – $10^{16}$  eV were detected at a rate of  $\sim 3.2$  h<sup>-1</sup> with a simple array of three Geiger-Müller counter trays  $G$  (Fig. 1) operated in coincidence (resolving time 5  $\mu$ sec). These detectors also included smaller counters which operated a hodoscope; the display on this instrument gave some indication of the total energy and position of the shower. To eliminate spurious events, the entire shower recording system, including the Geiger counters, was battery operated. The 20- $\mu$ sec time-bases of a double-beam recording oscillograph were triggered by shower coincidences. As a control the oscillograph was triggered artificially every half-hour.

The choice of wave-length and bandwidth was dictated mainly by available

frequencies locally free from radio and television signals. It was decided to operate at  $\nu = 44$  Mc/s ( $\lambda = 6.8$  m) and  $\delta\nu = 4$  Mc/s. The receiving aerial  $A$  consisted of a wide-band broadside array of 72 full-wave dipoles oriented east-west, and supported  $\sim \lambda/8$  above the ground; these were connected by open-line feeders to a transistorized pre-amplifier  $P$  situated at the centre of the array. The 44-Mc/s signal passed through a delay unit  $\Delta t$  (of delay  $\sim 5$   $\mu$ s) to the receiver, consisting of tuned radio-frequency stages followed by a square-law

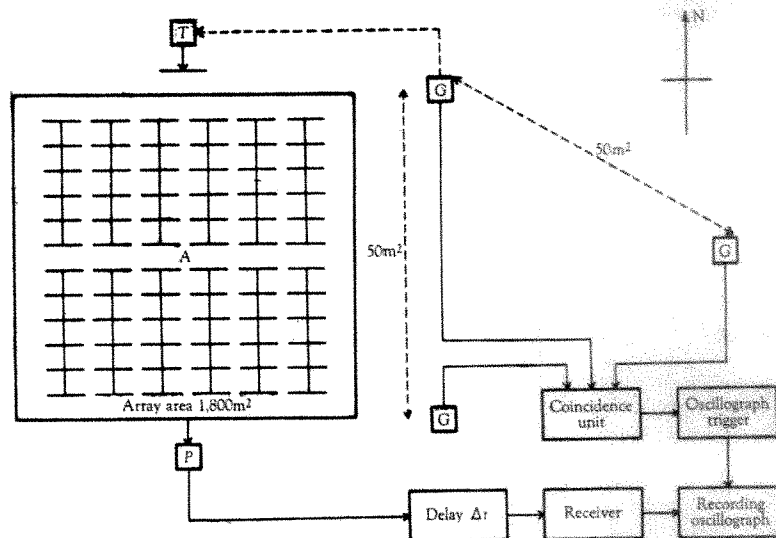


Fig. 1. Arrangement of the receiving apparatus. Coincident pulses from the Geiger counters  $G$  trigger an oscillograph which displays the output of the receiver delayed by  $\Delta t$ .  $P$  is a transistor pre-amplifier at the aerial array,  $A$ . The transmitter  $T$  can be triggered by a single counter  $G$ .

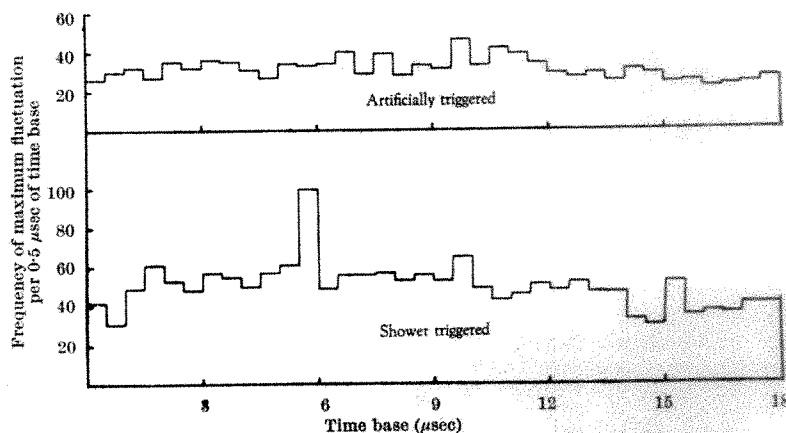


Fig. 2. Histograms showing frequency of occurrence of the fluctuation of largest amplitude along the time-base, between 0 and 18  $\mu$ sec. The upper histogram is for 1,117 randomly triggered recordings, and the lower histogram is for 1,794 shower-triggered recordings.

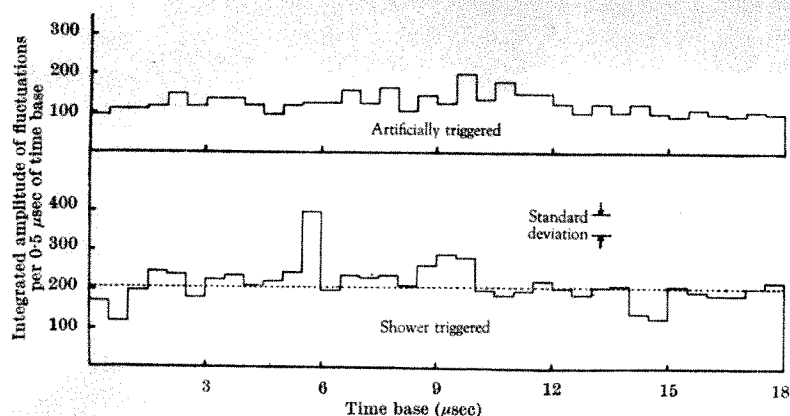


Fig. 3. Histograms showing integrated amplitudes of fluctuations for the same recordings as in Fig. 2

detector, and was then applied to the deflexion circuits of one beam of the oscillograph, the second beam carrying a 4-Mc/s sine-wave for calibration purposes. All the low-level stages of the receiver were run from an independent battery supply, and the total power was monitored on a chart recorder. As required, a small 44-Mc/s pulsed transmitter *T*, connected to a dipole *D*, could be driven from one of the counters. The sensitivity of the receiver was limited by cosmic noise, at a system noise temperature varying between 7,000° K and 14,000° K according to sidereal time.

The following results were obtained. During the recording of 1,799 showers five pulses have been observed which were bandwidth-limited in duration and which had amplitudes of 8, 10, 10, 11 and > 45 (off scale) times the root mean square noise level, corresponding to received energies of 4, 5, 3, 3 and >  $20 \times 10^{-12}$  ergs. These all had delays of between 5.4 and 6.0 μsec with respect to the start of the time-base. No bandwidth-limited pulses were observed in the intervals 0–5 or 6–20 μsec on any of the 1,799 shower recordings, and no such pulses were observed on any portion of the 20-μsec time-bases for the 1,117 artificially triggered recordings.

A statistical analysis of the recordings was made to search for smaller events. Fig. 2 shows histograms both for 1,794 shower-triggered and for 1,117 artificially triggered recordings, in which the ordinates represent

frequency of occurrence of the fluctuation of greatest amplitude along the time-base, between 0 and 18 μsec. Fig. 3 shows a similar histogram in which the ordinates represent the integrated amplitudes of the fluctuations. Excluding the five large pulses mentioned earlier, the main peaks in both histograms occur in the interval 5.5 to 6.0 μsec. In Fig. 3 the peak of the histogram is four times the standard deviation of the fluctuations. The pulses were observed to have a time-delay identical with that from the test transmitter when it was driven from a single counter, after allowing for the delay in firing the counters themselves. This latter delay, which averages 0.75 μsec, and which was determined in a separate experiment, is sufficient to allow us to eliminate completely the possibility that

the observed radio pulses originated in the electronics of the Geiger-Müller counter circuits.

These observations lead us to conclude that short radio pulses of duration < 0.25 μsec have been observed from extensive air showers. Further details of the work will be presented in due course, and experiments are to continue.

While the energy received in these pulses is consistent with the hypothesis of enhanced Čerenkov radiation, it appears also that pulses of comparable amplitude may be expected to arise from the separation of charges in the Earth's magnetic field. This latter radiation would be linearly polarized east-west, affording some possibility of distinguishing between these two mechanisms. We have also considered bremsstrahlung and transition radiation as possible origins of the pulses, but we believe these to be of secondary importance. We also believe that direct effects of induction by the shower particles in close proximity to the aerial elements are negligible.

We thank Sir Bernard Lovell for his enthusiasm and encouragement throughout this work, and also for making available the wide facilities at Jodrell Bank.

<sup>1</sup> Blackett, P. M. S., and Lovell, A. C. B., *Proc. Roy. Soc.*, **177**, 183 (1941).

<sup>2</sup> Jelley, J. V., *Nuovo Cimento*, Supp. 2, **8**, 583 (1958).

<sup>3</sup> Askaryan, G. A., *Soviet Physics, J.E.T.P.*, **14**, 441 (1962).

<sup>4</sup> Bassi, P., Clark, G. W., and Rossi, B., *Phys. Rev.*, **92**, 441 (1953).

<sup>5</sup> Greisen, K., *Ann. Rev. Nucl. Sci.*, **10**, 63 (1960).

## A THEORY OF MACROMOLECULAR AND CELLULAR ORIGINS

By PROF. SIDNEY W. FOX

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AT this point in the history of science, descriptions of life can be treated more rigorously and with less controversy than can comprehensive definitions of life. Two outstanding aspects of the description have been its bewildering variety and the overwhelming complexity of its units.

The first quality of variety is that which clearly confronted Charles Darwin, A. R. Wallace, other naturalists, all scientists, and indeed all thinking people more than a century ago. A general dismay engendered in attempting to understand such variety was dispelled by resort to the evolutionary principles which rely on progression from simplicity to complexity.

In this century, we are confronted with the problem which concerns the processes which must have been a prelude to the primitive organism from which all species,

according to Darwin, descended. From present vantage points the complexities of the incompletely understood molecules of protein or nucleic acid can easily be overwhelming, as can the chemical and structural complexity of the simplest cell that we might visualize. The heart of the problem has been aptly stated at one level by Harold Blum<sup>1</sup> as follows:

"... I do not see, for example, how proteins could have leapt suddenly into being. . . .

"The riddle seems to be: *How, when no life existed, did substances come into being which today are absolutely essential to living systems yet which can only be formed by those systems?* [italics Blum's]. It seems begging the question to suggest that the first protein molecules were formed by some more primitive 'non-protein living system', for it still remains to define and account for the origin of that system."

We can, again, hope to learn by honouring, however, in heuristic experiments the evolutionary principles of the stepwise development of complexity from simplicity and of the internal self-limitation of such development<sup>2</sup>. This philosophy is truly naturalistic in that it emulates the empirical approach which the evolutionist is required to impute to the primitive environment.

Although much of the knowledge of biochemical origins has been controversial, fairly general agreement now exists for the analytical outline of the problem of the origin of the first terrestrial organism (Table 1).

Table 1. SUBORDINATE PROBLEMS OF THE ORIGIN OF THE FIRST ORGANISM

- (1) Production and proliferation of organic micromolecules from the simplest compounds.
- (2) Spontaneous generation of macromolecules.
  - (a) Protein.
  - (b) Nucleic acid.
  - (c) Polysaccharide.
- (3) Self-organization of multimolecular systems (precellular forms).
  - (a) Membranous properties.
  - (b) Growth.
  - (c) Division.
- (4) Origin of metabolism.

This analysis is evolutionary in that it, in large measure, proceeds from simplicity to complexity. It also constitutes a gross outline of biochemistry. The sequence in the outline is based on a premise of the emergence of the first complex macromolecules prior to the origin of cells. Perhaps most interesting is the fact that almost entirely within the past decade an experimental model for each item in the outline has been brought forward. The problem is regarded by increasing numbers of theorists as solvable; the more favourable evaluations regard the problem in principle as more solved than unsolved.

The experimental demonstrations of the geological origin of biochemical substances indicate not necessarily what did happen, but what might have happened. The problem may now be viewed more as one that requires limiting of speculation by determining which model reactions examined in the laboratory are sequentially compatible, rather than as a problem of determining at least one way in which each key biochemical substance might have arisen spontaneously. Investigation of the variation in volcanic gases in eruption<sup>3</sup>, for example, helps to emphasize the tremendous variety of possible spontaneous chemical reactions in the history of the Earth.

The intellectual climate and technical understanding which stimulated and permitted the experiments that have been recorded owed much of their basis to the detailed proposals of Oparin<sup>4,5</sup>, Haldane<sup>6</sup>, Bernal<sup>7</sup>, Blum<sup>1</sup>, Calvin<sup>8</sup>, Urey<sup>9</sup>, Wald<sup>10</sup>, and others.

The first experiments expressly designed to explain the origin of organic compounds such as occur in living things were those of Calvin *et al.*<sup>8</sup>, who converted carbon dioxide and water to formaldehyde and formic acid in the cyclotron. A primitive atmosphere having some carbon dioxide is in accord with the models of Rubey<sup>11</sup>, Revelle<sup>12</sup> and Bernal<sup>7</sup>. Through the aldol condensation, one could visualize the formation of carbon-carbon bonds and a variety of organic compounds from formaldehyde. It is Calvin who deserves the credit for initiating the era of experimental chemical abiogenesis. Additional attention to experimental abiogenesis was stimulated by the Urey-Miller experiments, using electrical discharge in a 'primitive atmosphere'<sup>13</sup>. Miller obtained four amino-acids of the protein type and many other organic compounds<sup>14,15</sup>.

As Miller pointed out<sup>15</sup>, Löb<sup>16</sup> had earlier (1913) synthesized glycine by electric discharge in a mixture of carbon monoxide, ammonia and water. Many papers published in the era of early organic chemistry can, in fact, be re-interpreted in the more modern context of biochemical origins. Löb was actually close to such interpretation inasmuch as he proposed his synthesis as a model of reactions that could occur in Nature.

Apart from amino-acids, which could arise in many ways demonstrated in the laboratory<sup>17</sup>, other micro-

molecules which have been synthesized in a presumably geological fashion are the monosaccharides<sup>18,19</sup> (especially deoxyribose<sup>20</sup>), urea<sup>15</sup>, nitrogen bases<sup>21,22</sup> of nucleic acid, and the biochemically significant energy transfer substance, ATP (ref. 23). The production of guanine is noteworthy in that it arises during heating of amino-acids<sup>24</sup>, a process which also leads to salient macromolecules.

In view of the attention which has been devoted to abiotic syntheses of amino-acids, the finding that most of the amino-acids common to protein are produced simultaneously from methane, ammonia and water at 1,000° C, in the volcanic range of temperature, may be especially pertinent<sup>25</sup>.

Although amino-acids have resulted under many sets of conditions, indicating the possibility of their synthesis under primordial conditions, the synthesis from primitive gases has been investigated only recently. Dr. Harada has examined the conversion of ammonia, water and methane at volcanic temperatures to products hydrolysable to amino-acids<sup>25</sup>. He used vapour phase reactions of these gases through beds of silica gel, silica beach sand, a volcanic beach sand from Stromboli, and alumina gel. Except for the two kinds of silica, the results were quite different. Amino-acid formation was not observed at 800°, but it was evident at 900°. In each of many experiments, fourteen of the eighteen common amino-acids were identified. Two of the other four, histidine and tryptophan, will require special identification. Cystine and methionine could not be anticipated inasmuch as no form of sulphur was included in the reaction. This absence is part of the evidence that the amino-acids are not due to hydrolysates of microbial contaminants. No amino-acids of a non-protein variety have been found in such a reaction product. Examinations of this type have been indicated by the continuing development of the thermal theory<sup>26</sup>. At about the same time, the laboratories of Ponnampetuma and of Oró<sup>1</sup> had begun similar investigations<sup>27</sup>.

The interest in explaining the origin of amino-acids, manifest in the fact that so many possibilities of this sort have been published<sup>17</sup>, stems from the need for understanding the origin of protein. This emphasis comports with Blum's 1955 analysis of the overall problem of origins. The approach which has developed in our laboratory was based on a back-extrapolation. The extrapolation was from data accumulated during investigations of Darwinian evolution at the molecular level, with proteins<sup>28</sup>.

The systematics of proteins can be examined in a number of ways<sup>28</sup>. One of these is through the perspective of the quantitative contents of unfractionated proteins of organisms throughout phylogeny (Fig. 1). The chart shown<sup>29</sup> reaffirms in a quantitative way the principle of the 'unity of biochemistry'<sup>30</sup>.

The other emphasis derivable from this investigation is that of the relatively high proportion of two amino-acids, the dicarboxylic amino-acids, aspartic acid and glutamic acid. This fact suggested the possibility that the generation of primordial protein relied on a relatively large proportion of the dicarboxylic amino-acids, a dominance which might persist in the record of molecular evolution in cells.

Focusing on the origin of proteins at that stage in the development of the theory of abiogenesis differed from investigations of the origin of amino-acids and of other micromolecules. We had been influenced in our thinking by the chemists' typical assumption that, whereas simple compounds require simple syntheses, complex compounds, for example, proteins, require complex processes. What now seems to be a more logical premise is that Nature would not have been able to accommodate a process as intricate as, for example, a carbobenzoxy synthesis and would have used instead some internally self-organizing simple process such as an appropriate type of



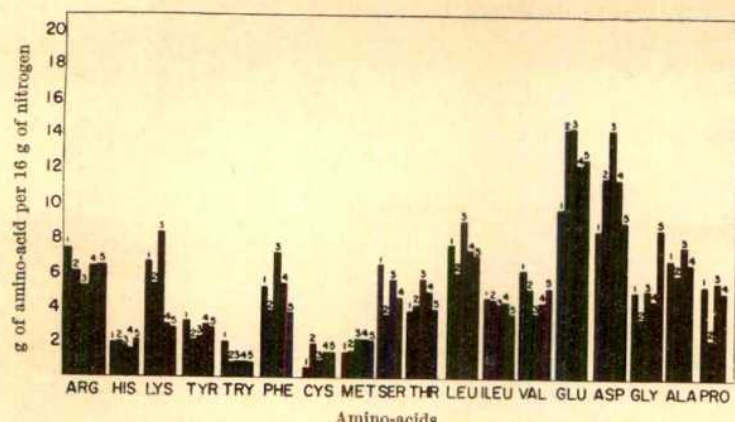


Fig. 1. Amino-acid compositions of unfractionated proteins of different organisms: (1) algae; (2) bacteria; (3) protozoa; (4) invertebrates; (5) mammals

polymerization. An additional reason for polymerizing amino-acids rather than analogues or derivatives is based on a recapitulationist premise<sup>28,31,32</sup>. This emphasis led to experiments different from those used as other models of formation of peptide bonds in the primitive world<sup>33,34</sup>.

The effort to use the presumed clue furnished by the moderately high aspartic acid and glutamic acid contents was based on heating in the dry state, for thermodynamic and other reasons<sup>35</sup>. When heat was used, a second scientific heresy was committed. The literature reveals that heating  $\alpha$ -amino-acids above the boiling-point of water typically yields diketopiperazines, amines and pyrolytic tars<sup>36</sup>. If one heats the amino-acids in the initially dry state and heeds the admonition from the evolutionary analyses, lightly coloured polymers having peptide bonds are, however, produced. These polymers can then be further purified by procedures which are standard for protein fractionation either by salting out from aqueous solution by ammonium sulphate<sup>37</sup> or on ion-exchange columns<sup>38</sup>. In order to obtain workable quantities of such polymers, at least one mole of dicarboxylic amino-acid per two moles of total neutral and basic amino-acid should be used.

An ultimate objective of these experiments was the simultaneous combination of all or nearly all the amino-acids common to contemporary protein. Comparative examinations suggest that the primordial

reactions must have embraced a maximum number of types of amino-acid. This inference is consistent with the fact that 'primitive' organisms contain all the contemporary amino-acids plus diaminopimelic acid<sup>39</sup>, and the genetic and biochemical reasoning which leads to the inference that a maximal variety of organic compounds was present in the primordial reaction<sup>40</sup>. Any method which would combine all the 18-20 common amino-acids could be expected easily to combine a smaller number. In addition, inclusion of all the eighteen amino-acids would provide a heteropolymer which, if it had a sufficient number of activities, might constitute (subject to definition) a synthetic general protein.

Many man-years of effort were, however, expended in systematic investigation of the thermal copolymerization of two or three amino-acids<sup>41,42</sup>. When the essential principle of thermal copolymerization was tested simultaneously on all the eighteen amino-acids common to protein<sup>43</sup>, it was found to apply to those circumstances also (Fig. 2). Some of each of the amino-acids was found in the polymer. This result was somewhat surprising against the background of knowledge that nine or ten of the amino-acids have reactive side-chains. The visualization of irreversible reactions of such side-chains was easy. However, thermal proteinoids which have undergone moderate purification can be hydrolysed by mineral acid and the contained amino-acids can be recovered quantitatively<sup>44</sup>. The conditions of the reaction, therefore, are not brutal, except for substantial destruction of serine and threonine; this is, however, largely controllable by use of a reducing mixture of hypophosphite and polyphosphoric acid<sup>45</sup>. The thermal copolymerizations and many of the analyses of the products have been repeated in other laboratories. These polymers were at the outset found to have, besides a complete qualitative roster of amino-acids, many of the properties of proteins. They are referred to as proteinoids, a term which originally indicated their similarity to proteins at the same time that it disavowed a claim of identity with contemporary protein. The possibility that the identity is great enough to allow regarding proteinoid as synthetic general protein is, however, in purview.

Typical reaction conditions are 170° C for 6 h. A 1:1:1-proteinoid, an example of one type of such polymer, is made from one part of aspartic acid, one part of glutamic acid, and one part of a mixture of the sixteen other amino-acids present in that part in equimolar proportions. As has been documented, temperatures of 170° C are terrestrially quite common<sup>46</sup>, yet the minimum temperature for polymerization within hours can be lowered to 65° by addition of appropriate phosphates to the reaction mixture. Yields of proteinoids, depending on conditions of reaction and degree of purification of product, typically range between 5 and 40 per cent. The higher yields are obtained with phosphates in the reaction mixture.

These conditions are closely similar to those reported from Schramm's laboratory and identical with ours for the polymerization of mononucleotides<sup>47</sup>. The fact that products as complex as proteinoids could be produced in a simple, and therefore geologically plausible, way suggested that polymers resembling polynucleotides might also be produced by simple heating. The characterization of thermal polynucleotides is, however, as yet, far less complete than that of the proteinoids.

In order to understand the essential processes which occur when amino-acids are heated, some of the special chemistry deserves examination.

The neutral amino-acids tend to undergo a head-and-tail condensation of two molecules to give a *cyclodipeptide* or diketopiperazine:

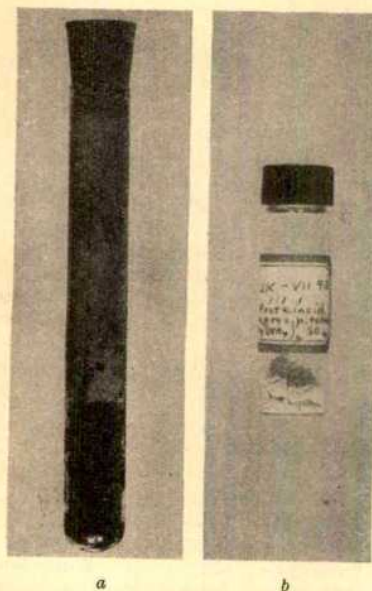
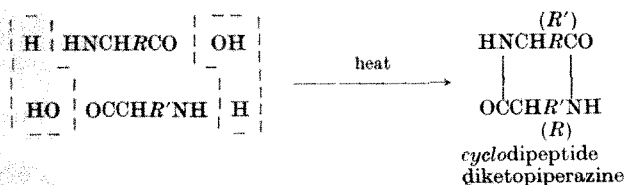


Fig. 2. a, Amino-acids heated above the boiling-point of water; b, ditto, with sufficient aspartic acid and glutamic acid, followed by purification by salting out



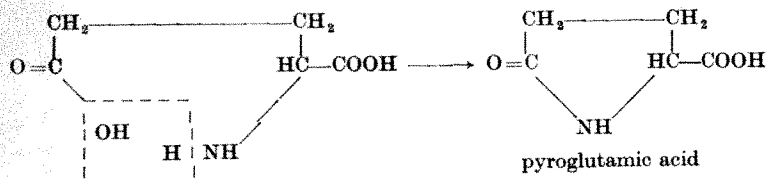


They also are decarboxylated to leave amines:

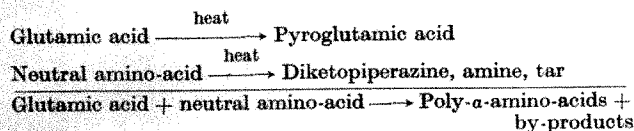


and more profound decomposition results in the formation of tar, as already noted.

Glutamic acid, however, undergoes an internal condensation in the single molecule to yield a lactam, pyroglutamic acid ( $\alpha$ -pyrrolidonecarboxylic acid).

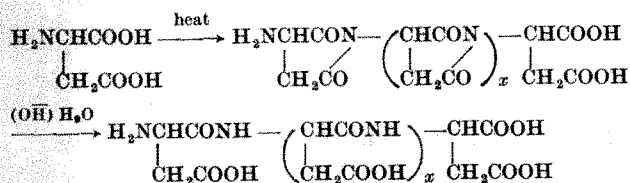


When glutamic acid and a neutral amino-acid are heated simultaneously, they behave together in a way that is different from their individual behaviours.

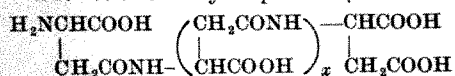


The concerted heating yields unwanted products also, but it also yields genuine peptide-type poly- $\alpha$ -amino-acids, often as the major product with little discoloration or contamination by tar.

Aspartic acid participates in copolymerization in a manner which is different from that of glutamic acid. Aspartic acid yields a polymer which is a polyimide:

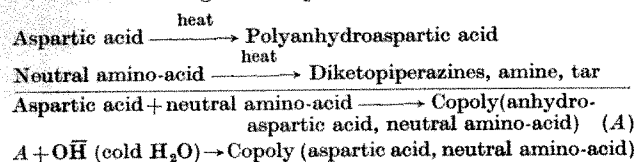


The residues may in part be  $\beta$ -linked as indicated:



for either or both the interior and N-terminal residues.

The polymerization of aspartic acid also permits bringing into polymeric combination amino-acids which would themselves undergo decomposition.



Most of the aspartic acid residues are in the imide form in (A). The hydrolysed A is the true peptide type of polyamino-acid instead of the imide type.

The basic amino-acids do not decompose as do neutral amino-acids; they also undergo thermal copolymerization. As stated before, all the amino-acids, including both dicarboxylic amino-acids and the three basic amino-acids, and the thirteen other  $\alpha$ -amino-acids common to protein, can be simultaneously copolymerized thermally. When the purified thermal proteinoid is

hydrolysed, quantitative recovery of the amino-acids which it contains is recorded, and one must conclude that irreversible reaction of functional side-chains cannot have occurred in a major portion of the raw polymer.

The basic amino-acids, especially lysine, are moreover capable of substituting for the acidic amino-acids in thermal polymerization<sup>48</sup>. Lysine can also be used for simultaneous thermal copolymerization of all the amino-acids to yield a basic lysine proteinoid<sup>49</sup>.

These results emphasize that the effect of simultaneous heating of amino-acids could not be predicted from homopolymerization or attempted homopolymerization. This difference between homopolymerization and copolymerization is consistent with the general experience of the polymer chemist. The polymer chemist, however, has not dealt with reactions involving eighteen monomers simultaneously. Accordingly, no precedent existed for the results of the present investigation. One may on this basis more easily understand a frequently observed first reaction of many chemists that such polymerizations can scarcely be reproducible or controllable, and that the polymers produced must be random in Nature.

Fig. 3 helps to answer this question. In Fig. 3 are seen three chromatograms, from the automatic amino-acid analyser, of three separate syntheses of proteinoids. These are three 2 : 2 : 1-proteinoids which differ in their synthesis and purification in one respect only. In b, the proportion of phenylalanine was the usual figure. In the experiment of a, all phenylalanine was omitted. In the experiment of c, the proportion of phenylalanine in the reaction mixture was 3.5 times that in b. Comparison of a, b and c reveals that the proportion of each amino-acid other than that of phenylalanine is quite constant. The thermal condensations of amino-acids are thus highly reproducible, as observed in many ways in other experiments. The proportion of phenylalanine is higher in the product from the reaction mixture containing a higher proportion of phenylalanine. Other experiments have shown that the proportion of an individual amino-acid in an acid proteinoid is regularly relatable to its proportion in the reaction mixture. The thermal polymerizations are thus quite controllable as well as reproducible.

The pair of proteinoids which differ by the absence of one amino-acid offer opportunities for investigation in protein nutrition. Some of the early nutritional biochemists such as Abderhalden and Mendel sought proteins lacking individual amino-acids so that they might examine the effect of individual amino-acids in protein nutrition. In this search they were doomed to failure because of the absence of such proteins in Nature. Instead, much of what we know we owe to W. C. Rose, who obtained the desired type of information by feeding first rats, then humans, with mixtures of pure amino-acids with systematic omissions. As Rose pointed out, such answers are imperfect in that caloric imbalances attend the feeding of amino-acids instead of protein<sup>50</sup>. In true protein nutrition, furthermore, amino-acids are gradually released from peptide bonds. In this respect the thermal proteinoids can function as synthetic proteins from which single amino-acids can systematically be omitted qualitatively and quantitatively. The question of nutritional availability of amino-acids from thermal polyamino-acids has been affirmatively answered for bacteria<sup>51</sup>, and for rats by Krampitz and Knappen<sup>51</sup>.

The question of molecular weights has been examined by end-group assay and by some analyses in the ultracentrifuge. Mean molecular weights of 3,000–10,000 have been recorded<sup>57</sup> for the acid proteinoids. These values may be compared with 6,000 for a small protein molecule, insulin. Since insulin has two end-groups, the figure for comparison is 3,000. Most proteins are considerably larger than 6,000, however; the molecular weights of the

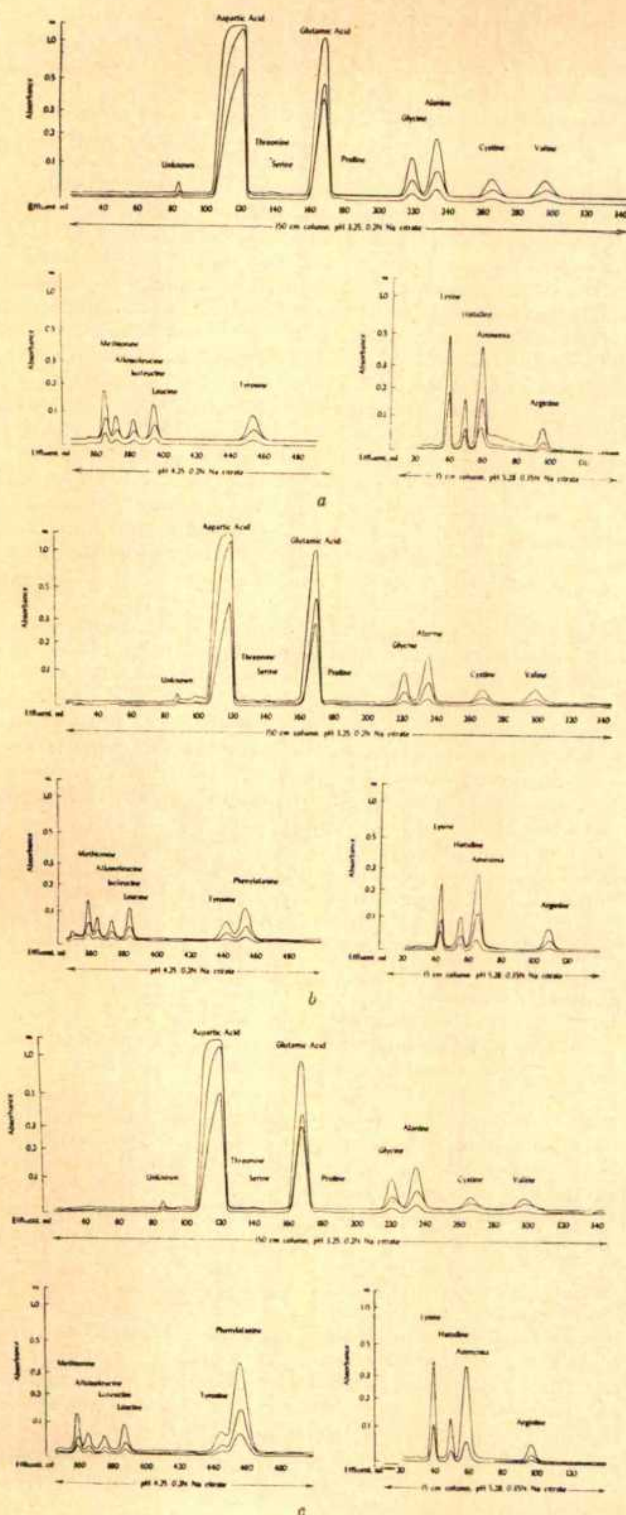


Fig. 3. Chromatograms of hydrolysates of proteinoids. *a*, 2:2:1-proteinoid reaction mixture lacked phenylalanine; *b*, standard 2:2:1-proteinoid; *c*, same as *b* with 3-5 times as much phenylalanine in the reaction mixture

proteinoids are thus in the lower end of the range of molecular weights of proteins. Cross-linking within the proteinoids is limited<sup>49</sup>.

A rigorous comparison of proteinoids with proteins cannot be carried out, because, like life itself, proteins are not rigorously defined nor rigorously definable. The variable nature of proteins precludes the possibility of a rigorous definition of those materials. Two descriptive definitions of proteins as a group can, however, be

cited<sup>52,53</sup>. The requirements of these definitions are met by the proteinoids as a class. The first of these requirements are the qualitative content of amino-acids and the molecular weights. In determining to what degree the complexities of protein molecules are mimicked by thermal proteinoids, many characteristics have been examined. These are outlined in Table 2. Missing from proteinoids as yet are the properties of antigenicity and helicity. They have not been assiduously sought in the proteinoids, however, nor are they universal properties of proteins. These properties are also found in synthetic polyamino-acids produced from Leuchs anhydrides<sup>54</sup>.

Table 2. PROPERTIES AND INTERPRETATIONS ASSOCIATED WITH THERMAL POLYAMINO-ACIDS AND PROTEINS

Composition, qualitative
Molecular weight range
Colour tests
Inclusion of non-amino-acid groups
Solubility ranges
Salting-in and salting-out properties
Precipitability by protein reagents
Composition, quantitative
Intramolecular bonds
Peptide (imide)
Bluret
I.R. maxima (1,550, 1,650, 3,080, 3,300 cm <sup>-1</sup> ) (1,720, 1,780 cm <sup>-1</sup> for imide)
Release of amino-acids by HCl hydrolysis
Proteolysability
Disulphide
By oxidizability to cysteic acid
Non-random arrangements of amino-acid residues
Terminal residues and total composition
Sequences in fragments
Susceptibility to proteolytic enzymes
Nutritive quality
Catalytic activity
Morphogenicity

The property of non-randomness is investigated most meaningfully in an unfractionated polymer. While we are working towards determining an entire sequence, this kind of work needs to be done on homogeneous preparations. The sequence of residues in a homogeneous preparation cannot provide information on variation in sequence or composition such as had been determined in the experiments just described. The fact of non-random arrangements (repeatedly indicated by comparisons of total compositions, N-terminal and C-terminal compositions) betokens a measure of order in the synthesis. This order must be determined by the amino-acids themselves since almost no other material is present. Such a finding is of evolutionary significance when viewed against the

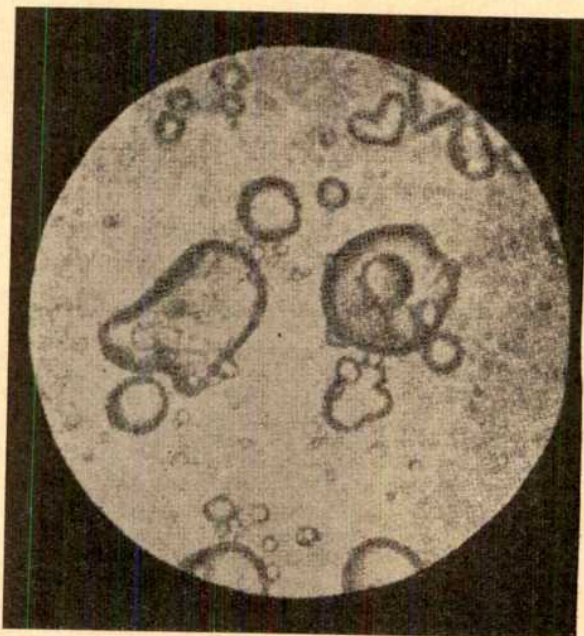


Fig. 4. Coacervate droplets from gelatin, gum arabic and ribonucleic acid (after Oparin). ( $\times 240$ )



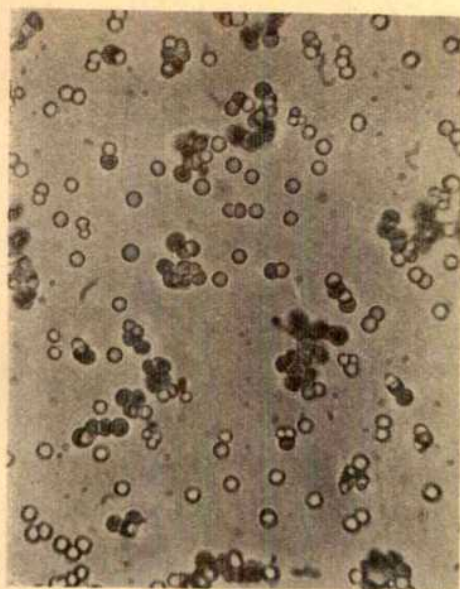


Fig. 5. Proteinoid microspheres prepared by allowing hot solution of proteinoid to cool. These units are approximately  $2\mu$  in diameter

background of recognizable order in organisms. The thermal proteinoids, as a model of abiotic polymer, demonstrate how the beginning of order could have developed at a molecular level in a pre-biological period. Since no genetic nucleic acid is present in the reaction, the degree of ordering that is found is ascribed to the internal influences of the amino-acids themselves. Such an effect can be understood in terms of the specific shapes and charge distribution of each of the eighteen types of amino-acid. The concept that order could have been partly specified by the primitive protein itself is substantiated by investigations of Pattee<sup>55</sup>, based on computer theory. The principle of internal self-direction is fundamental to general evolutionary theory<sup>56</sup>.

The conceptual possibility that pre-biological protein molecules might have arisen before pre-biological nucleic acids can be more seriously entertained in the light of the demonstration of internal self-control of sequence. Three properties which, in pre-vital protein, would be of first significance to organisms that might evolve therefrom are: service as proteinaceous food, catalytic activity and any tendency to form microstructures having boundaries with membranous properties. The first of these activities, in proteinoids, has been mentioned.

Catalytic activity in proteinoids was first observed for the unnatural substrate, *p*-nitrophenyl acetate<sup>49</sup>. Rohlfs<sup>56</sup> has shown that the relatively high activity observed is due to the simultaneous presence in the proteinoid of histidine residues and of aspartimide linkages. As the imide linkage is progressively opened (by mild treatment), the catalytic activity is also progressively decreased. The imide type of polyaspartic acid, without histidine, is devoid of activity.

A second type of catalytic activity which has been observed is found in proteinoids which have been suitably reacted with zinc. Inorganic salts of zinc are known to catalyze the hydrolysis of ATP at 80° (ref. 57). When zinc hydroxide gel is reacted with acid proteinoid, the resultant zinc-proteinoid is active in the hydrolysis of ATP at 40°. This effect, in conjunction with organized units, is later described more fully. Activity by combination with zinc can be viewed as an experimental model of a first step in the conceptual evolution of a metal-enzyme<sup>58</sup>.

A third type of catalytic activity is that which has been shown to enhance the decomposition of glucose to gluconic acid, carbon dioxide, and other products<sup>59</sup>.

Although this is a weak activity, no more than weak activity was needed in the first proteins. Furthermore, among the numerous activities now being catalogued for the proteinoids, some are considerably stronger. The essential feature is the occurrence in macromolecules of significant catalytic activity such as might launch a primordial, biochemically active, cell.

To this point, one can in any event visualize, on a basis of sequentially compatible reactions demonstrated in the laboratory, the thermal conversion of amino-acids to a primitive kind of protein.

In a coherent theory of origins, the manner in which primitive protein might modulate to primitive cells is one of the next questions.

The kind of experiment to which the biochemist, A. I. Oparin, has devoted major effort is that of the multi-molecular systems, or, more interpretatively, models of precellular organization. Oparin is properly credited with promulgating a natural origin of biochemical systems through organic chemistry<sup>4,5</sup> at a time when naturalistic explanations were not available. Equal credit is deserved by Oparin for his emphasis on the need to understand self-organizing phenomena yielding the first cells. George Wald<sup>10</sup>, and others, have also added explicitly to the theoretical underpinnings.

Oparin's experiments have used as a model of precellular organization the coacervate droplet introduced by the colloid chemist, Bungenberg de Jong<sup>60</sup>. Some of these are illustrated in Fig. 4. They can be seen to be microscopic, and not uniform. Oparin has concentrated enzymes and substrates within coacervate droplets. The presence of organization allows reactions to proceed in a concentrated fashion, apart from the environment. The value of packaged reactions in a unit which has separated from the pre-environment is that such a unit could then evolve metabolically along with the development of internally associated reactions.

Oparin has pointed out one defect of this model—the lack of stability. The coacervate droplet made from gelatin and gum arabic cannot withstand gentle centrifugation or some concentrations of salts without breaking down into two liquids. As Oparin has stated, the coacervate droplet would in an evolutionary way have to develop stability before it could function as a valid model of the late stages of precellular organization<sup>61</sup>. Another defect as a precellular model is the reliance for structural purposes on protein of recent origin such as gelatin.

From the thermal polyamino-acids, including thermal proteinoid, an alternative model emerges (Fig. 5). This model differs crucially from typical coacervate droplets and from other cell models<sup>62</sup>, in that the structural material is not a polymer from contemporary organisms. The material is instead a protein-like polymer which arose from simpler units, amino-acids, which can be formed and combined, as has been shown, by a kind of experimental geosynthesis. Another significant difference resides in the fact that the formed units have stability comparable to that of many contemporary cells. They withstand centrifugation in a clinical centrifuge. As first shown by Dr. Richard S. Young<sup>63</sup>, they are stable enough to be sectioned, allowing electron microscopy<sup>64</sup>, as represented in some of the photographs in this article.

The manner in which the proteinoid microspheres arise is yet simpler than that by which the polymer emerges. Water or various aqueous solutions are added to the hot polymer mixture, the hot clear solution is decanted and, following a few minutes of cooling, vast numbers of individual microspheres are seen to separate. It is possible also to use cold purified proteinoid by heating the suspension produced when water or aqueous solution is added to the solid. In some preparations, the propensity to form microspheres is so great that the units appear on mere contact of cold water with cold proteinoid.

The size is microscopic, those in Fig. 5 being slightly less than  $2\mu$  in diameter. This is then the size and shape



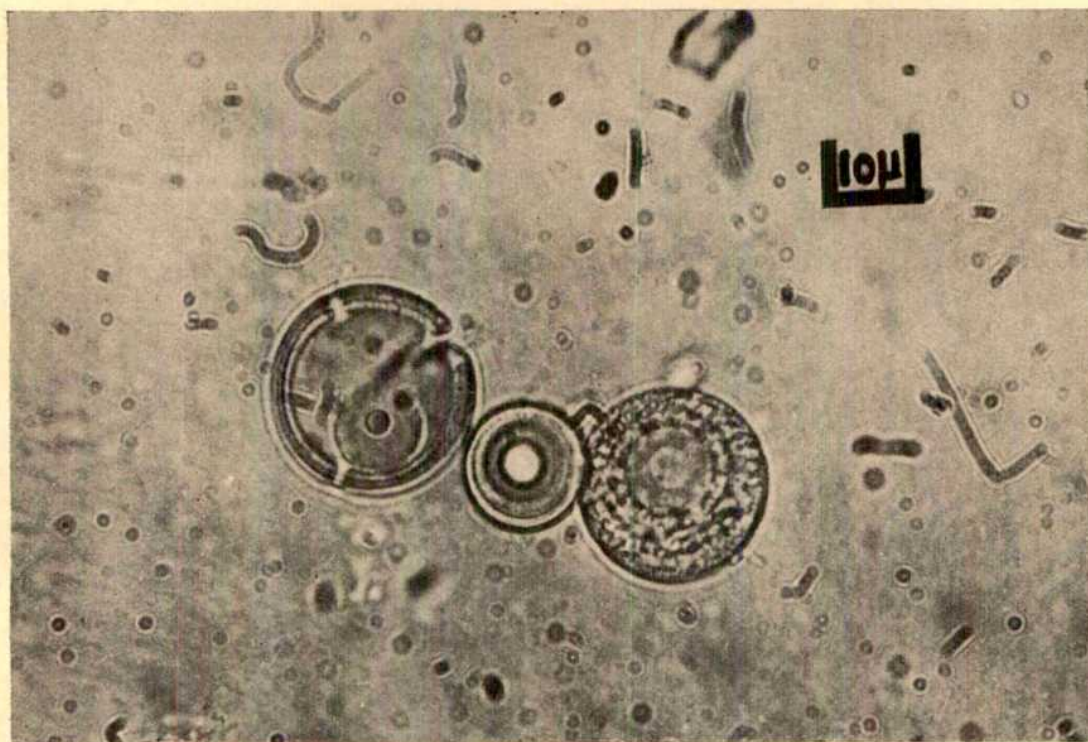


Fig. 6. Optical micrograph of microspheres. Fine details of structure may be noted

of the coccoid bacteria, which have been regarded by Kluyver and van Niel, and Lipmann<sup>65</sup>, as the most primitive of the bacteria.

As can be seen in Fig. 5, the spherules can be produced in a uniform size. This picture, however, demonstrates some of the variations in shape. The uniformity which is obtainable permits quantitative experiments. When transferred to solutions hypertonic or hypotonic to those in which they were made, the microspheres shrink or swell correspondingly<sup>66</sup>. This kind of behaviour is manifest at concentrations of salt much higher than those necessary for the demonstration of osmotic effects in true cells. What is seen, however, may be the basis for a kind of primitive osmometer<sup>67</sup>.

Another figure of interest is the simulated budding. In addition, 10 mg of proteinoid yields more than  $10^7$  microspheres. In the context of natural experiments, the appearance of this numerosity emphasizes, in addition to the number of experiments in vast numbers of individual locales, a very large number of experimental individuals per experiment. A single terrestrial experiment on a geological scale would be much larger than what can occur with 10 mg of thermal polymer.

Fig. 6 shows some larger microspheres, in which more anatomical detail is apparent than in Fig. 5. What looks like concentric shells, or a kind of double layer, may also be seen. One cannot be sure of this observation inasmuch as it is the result of optical microscopy. Apparent also are some filamentous particles. These are not lint or other result of unclean microscopy. They are associations of microspheres from a second population of smaller units, which are barely visible. These latter are quite uniform in size; their diameter is the same as that of the filaments. The filaments, on close examination, are seen to be segmented associations of the small microspheres. These structures, like the 'buds' and microspheres, are provocative in the

context of biomorphology. Only the microspheres have so far been extensively investigated.

Knowledge of the composition of the proteinoid has permitted using them as a model unit in investigation of cytochemical staining. In 1924, Stearn and Stearn attributed the Gram stain of bacteria to the acceptance of the stain by structural protein of the bacterial cell<sup>68</sup>. Because of the protein-like nature of the material composing the microspheres, the latter were tested for their acceptance of the Gram stain. The possibility that they could be used also for explaining the difference between Gram-negativeness and Gram-positiveness also suggested itself. Of the many theories of this difference, the recent concept that the difference depends on the proportion of basic biopolymer invited attention<sup>69</sup>.

The first finding was that, consistent with the stipulation of Stearn and Stearn, the typical microspheres of proteinoid accept the Gram stain; they are Gram-negative. To test the possibility that basic polymer might influence the sign of the stain, the lysine proteinoid

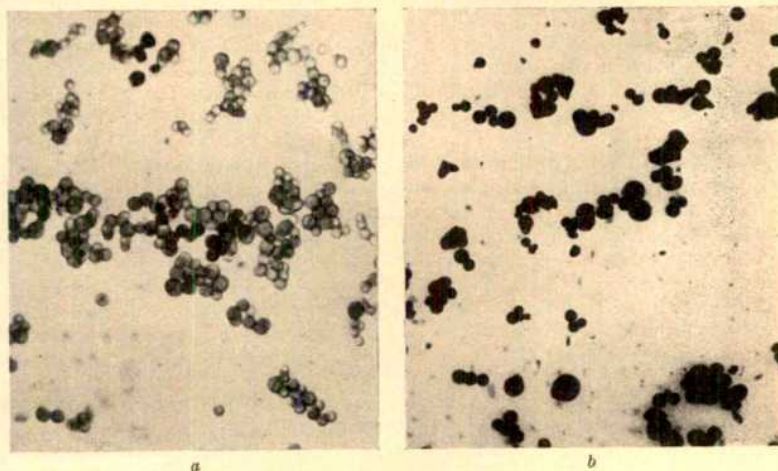
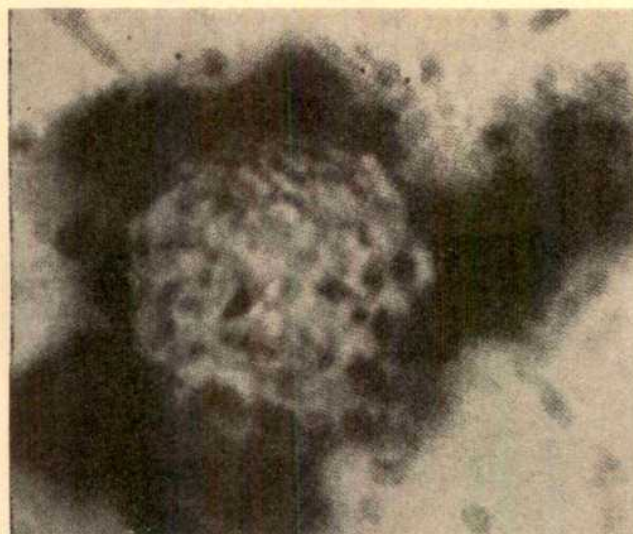
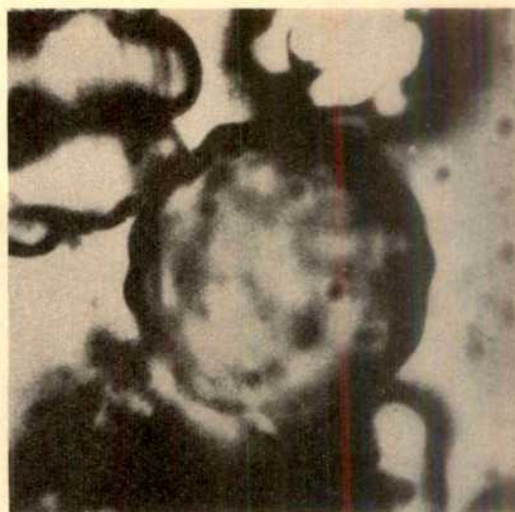


Fig. 7. a, Gram-negative microspheres; b, Gram-positive microspheres



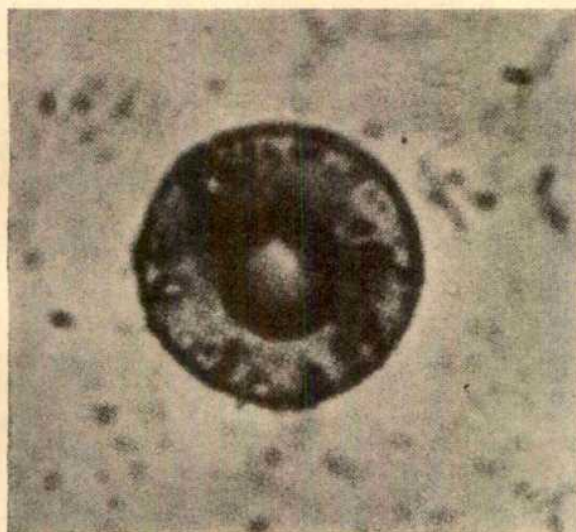


a

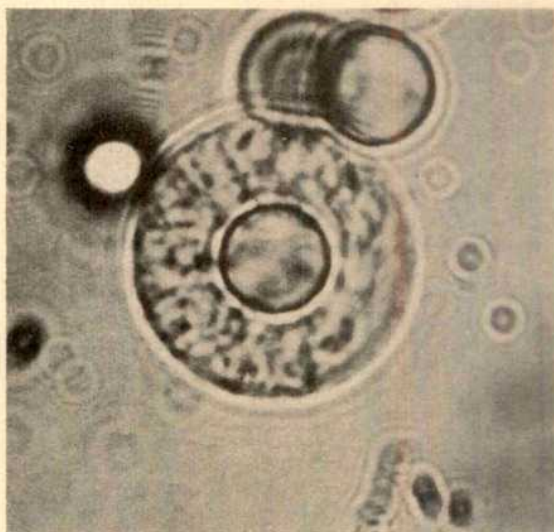


b

Fig. 8. *a*, 'Life-like' microparticle from Orgueil meteorite (Claus, G., Nagy, B., and Europa, D. L., *Ann. N.Y. Acad. Sci.*, **108**, 580; 1963); *b*, microparticle of thermal proteinoid from this laboratory

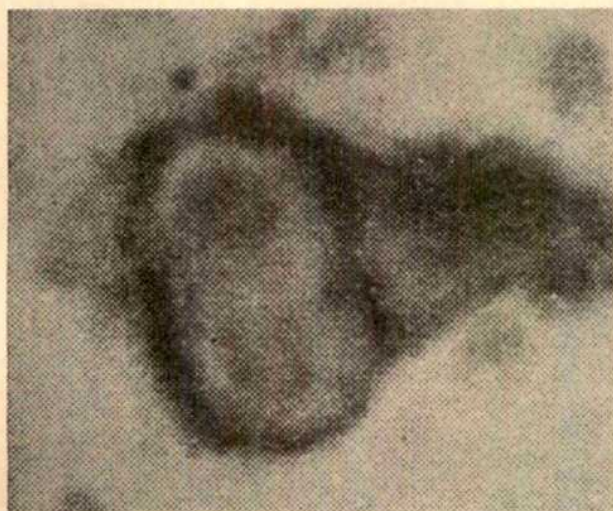


a

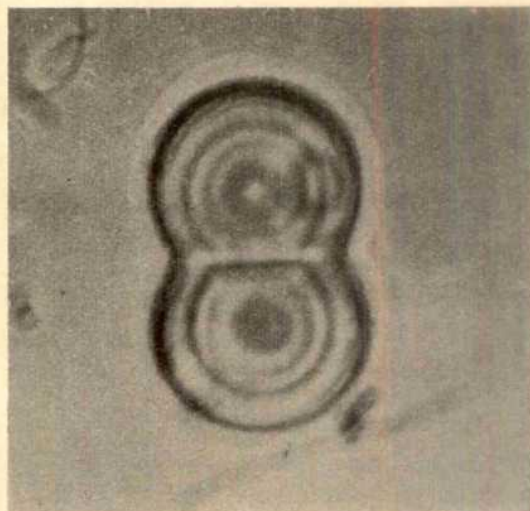


b

Fig. 9. *a*, As in Fig. 8*a*; *b*, as in Fig. 8*b*



a



b

Fig. 10. *a*, 'Life-like' form undergoing 'cell division', from Orgueil meteorite. (Claus, G., and Nagy, B., *Nature*, **192**, 594; 1961); *b*, as in Fig. 8*b*, pH has been raised moderately. All microparticles are roughly in same range of size



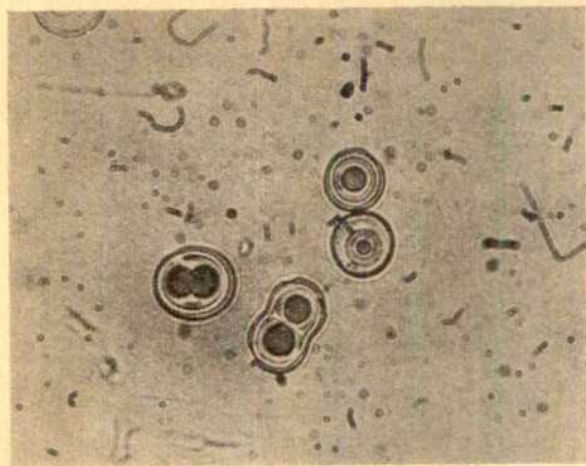


Fig. 11. Twinned proteinoid microspheres observed in suspension in which pH has been raised

mentioned earlier was used. Lysine proteinoid does not form microspheres in the manner that the acid proteinoid does. Mixture of lysine proteinoid and acid proteinoid containing less than 60 per cent of the former polymer will form microspheres which contain the latter. If the proportion of lysine proteinoid in the mixture exceeds 35 per cent, the microspheres stain Gram-positive (Fig. 7). Although this use of the model does not rule out other explanations, further consideration of the Gram stain must take such results into account, particularly since the results in the model are not subject to confusion by a number of other metabolites. The solubility of the Gram-negative microspheres in dilute alkali and the virtual insolubility of the Gram-positive type furthermore parallel quite exactly the solubilities of the Gram-negative and Gram-positive bacteria<sup>69</sup>, respectively.

The morphologies of the various micro-particles, inadvertently obtained, bear remarkable resemblances to almost all the meteoritic 'formed elements'<sup>70,71</sup> which have been published. Comparisons of three of these are shown in Figs. 8-10.

In Fig. 10 can be seen a formed element undergoing 'cell division' while it is being constricted at the middle, as pointed out by Claus and Nagy. On the right is a micro-particle from a laboratory experiment. The same features are brought out more clearly. In this case, the microspheres originally formed have been treated under the cover-glass with a few drops of buffer of pH 5.5-6.5, the original pH being approximately 3.0. Evidently some redistribution of charge is responsible for the re-arrangement of structure.

These comparisons pose the possibility that the 'formed elements' were at no time alive, rather that they were natural physico-chemical experiments which terminated before life emerged<sup>72</sup>. If so, they are of profound interest in a context of molecular evolution.

These similarities also are consistent with the inference that the laboratory experiments are closely akin to natural experiments. The thermal gradients and moisture which are present in the carbonaceous chondrites during their trips into the Earth's atmosphere<sup>73</sup> would provide the necessary conditions. Of related interest is

the inference by Kaplan *et al.*<sup>74</sup> that the types of carbon compounds which occur in organisms may be formed in meteorites.

One of the clearest pictures showing microspheres simulating cells in cleavage is that of Fig. 11. This picture has been mistaken often enough by biologists to be a field of dividing bacteria that the question has properly been asked if the view is not indeed one of contaminating bacteria in the process of division. That the particles in such views are synthetic is shown by the fact that they arise in periods of less than 2 h, and the fact that they can be redissolved by warming the microscope slide. In this picture may also be seen the small microspheres, and the filamentous associations thereof. A particularly provocative question in this case is the one of whether the particles which appear to have cleaved are the products of fusion or fission.

This question has been answered for some years as a process of fission. The first documented experiment is presented in the frames from a time-lapse sequence reproduced in Fig. 12. The change between the second and third frames in the mount clearly shows the formation of a septum and of two centres. This kind of cleavage simulates the type of division observed in the septate cocci<sup>75</sup>.

This time-lapse sequence also illustrates other phenomena. When the initial solution of proteinoid is heated for many minutes instead of the usual few seconds, the particles are not uniform; hence, the large microsphere near the centre of the frames. The sequence of photographs shows the progressive disappearance of the centre of the larger microsphere. In many experiments this centre has disappeared entirely but the boundary

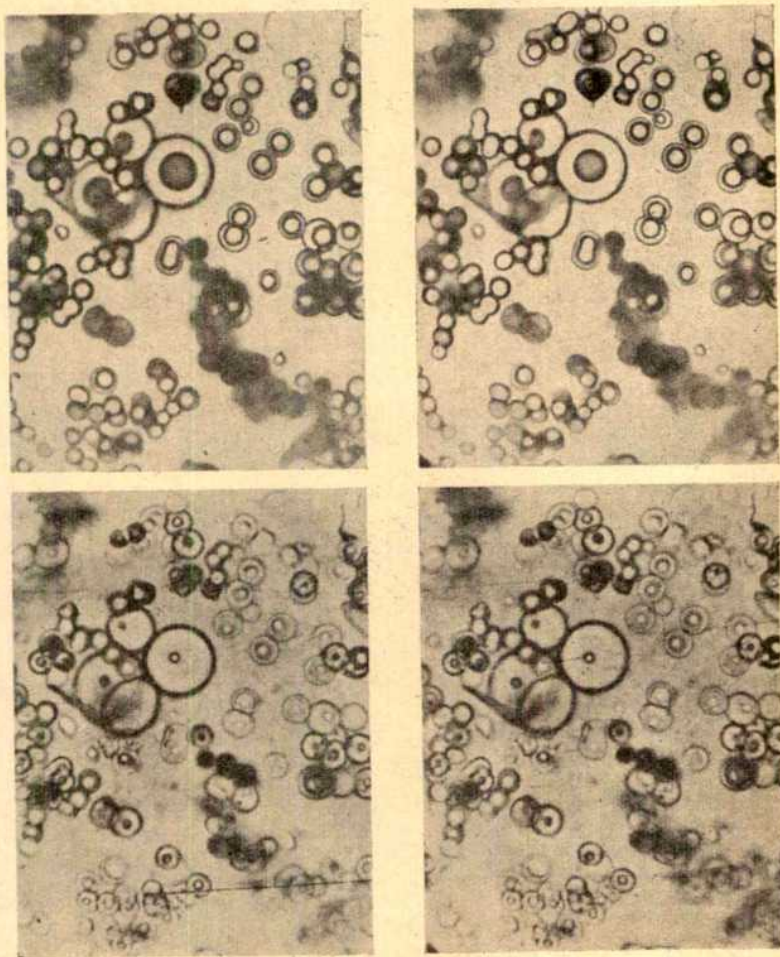


Fig. 12. Frames from a time-lapse series showing cleavage



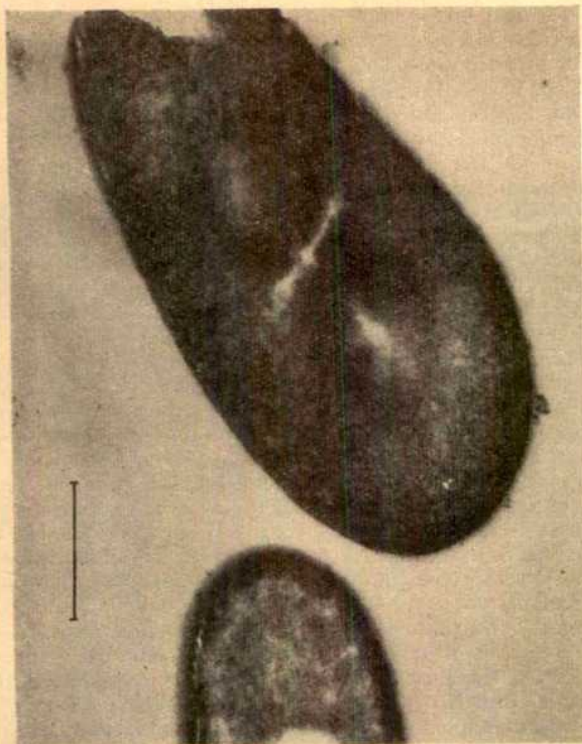


Fig. 13. Electron micrograph of proteinoid microsphere which has been stained with osmic acid, and embedded in a methacrylate block which was cured and sectioned

remains. Such a phenomenon poses a provocative chemical question of the difference between the visually unchanged boundary and the interior of the unit. Analyses have as yet shown no difference in amino-acid composition.

This behaviour is part of the evidence indicating that the kind of selectivity necessary for a primitive membrane is a property of these units<sup>75</sup>. The boundary appears to remain unchanged through the experiment, yet the interior, made up also of proteinoid, passes out through that boundary. The absence of material in the interior while the boundary persists is corroborated by some of the electron micrographs.

The first of the electron micrographs (Fig. 13) shows a single microsphere which has been stained with osmic acid, trapped in methacrylate which was cured, and sliced by microtome<sup>76</sup>. The sections in this treatment tend to be 800 Å thick. Although some bacteria reveal more structure than this to the electron microscope, some do not. A section of *Bacillus cereus* micrographed and reproduced in the treatise on *The Bacteria* is comparable<sup>77</sup>.

Fig. 14 shows five microspheres caught in a suspension<sup>76</sup>. In this case, the pH has been raised. Four of these illustrate various stages in the outward diffusion of the polymer from the interior. Several of them show very clearly a boundary.

Fig. 15 depicts more microspheres subjected to raised pH. Two fields are placed side by side here to show a progression in one phenomenon. This is the double layer, analogous to the double membrane of true cells<sup>78-83</sup>. This phenomenon had not been sought. If, however, a model of cellular origins had some validity, unsought features should occasionally appear. Recent thinking has emphasized the presence of phospholipid between the protein layers of a double membrane<sup>84</sup>. In this case, comparable chemical character might be attributed to the hydrocarbon side-chains of the amino-acid residues in the proteinoid. The double layer has been known to retain its structure after extraction of the lipid<sup>79</sup>, and recently Green *et al.*<sup>85</sup> have reported that the layering within mitochondria is undisturbed when the lipids are extracted from the mitochondria by aqueous acetone; they have

identified 'structural protein'. This kind of emphasis is consistent with the finding of double layering from proteinoid alone. These results and those with the Gram stain suggest that the emphasis in the introduction on experimenting with the conceptually simplest natural experiments can be extended. Such simple experiments can help to control investigations of complex phenomena in living cells when fewer than all the associated natural materials or processes in the living cell can be identified as pertinent.

Attempts to produce metabolizing microspheres have focused on introducing catalytically active cations, a conceptual possibility in view of the chemical structure of the proteinoids. Investigations have narrowed to zinc, which has been known to catalyse the hydrolysis of ATP in aqueous solution at 80° (ref. 57). Following many trials, zinc was introduced by reacting zinc hydroxide gel with proteinoid in suitable ratios in hot aqueous solution for 4 min. The hot clear solution deposits microspheres containing typically 1-3 per cent of zinc. The first two aqueous wash liquids show some activity, but the third through fifth washes reveal scarcely more activity than is found in the ATP spontaneous hydrolysis control, signifying the removal of zinc adhering to the microspheres (Fig. 16). The zinc-containing microspheres retain, however, substantial ATP-splitting activity. Cellular phosphatases often contain magnesium, but some of them contain zinc<sup>86</sup>. The level of activity in the microspheres is undoubtedly much weaker than that in a natural adenosine-triphosphatase. As Calvin has pointed out theoretically for iron-containing enzymes, weak activity would have been sufficient in the early stages of life<sup>87</sup>; evolution would have selected the more powerful enzymes that we find in contemporary cells.

With ATP-splitting activity localized in this fashion, visualization is possible of how a thermal synthesis of primitive protein might have modulated to the first steps towards a primitive ATP-dependent cellular synthesis of protein. Regardless of how much like the proteins the thermal proteinoids may be, the mechanisms of synthesis are energetically different. If thermal synthesis led to

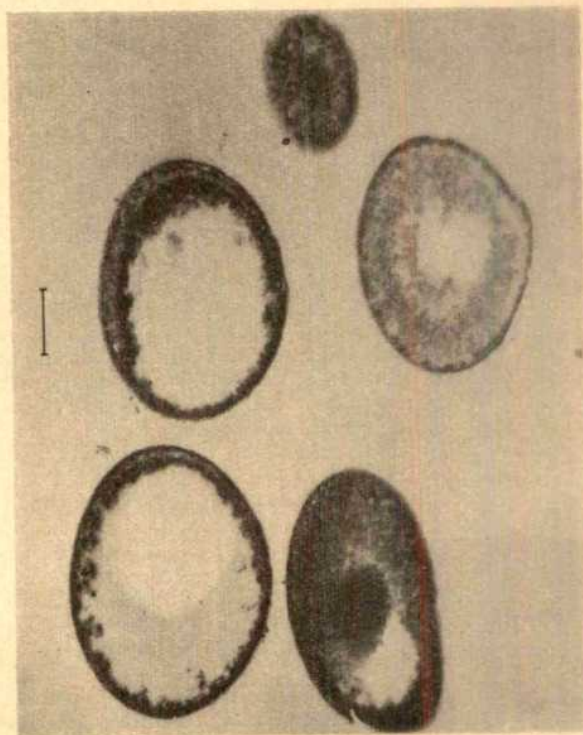


Fig. 14. Five proteinoid microspheres treated as for Fig. 13



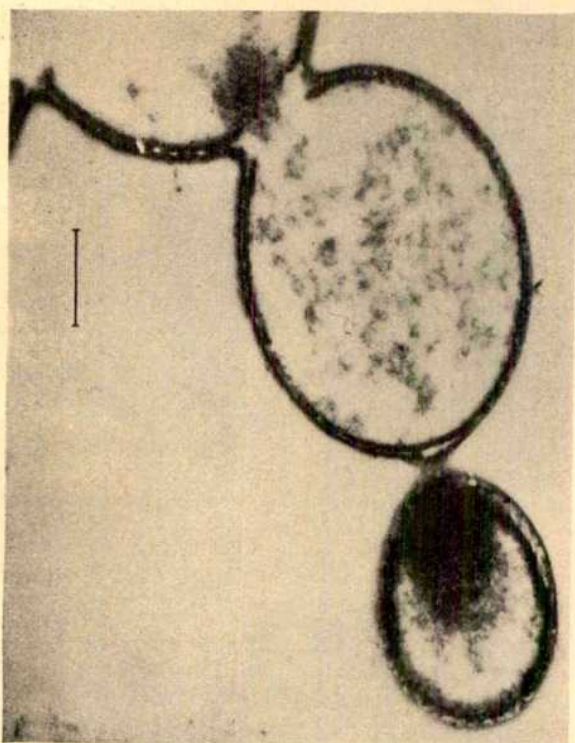


Fig. 15. Double layers in sections of proteinoid microspheres. This electron micrograph confirms optical evidence of Fig. 6

pre-protein, some modulation to a cellular type of synthesis was necessary.

The simulation of so many pre-biochemical steps by heat calls to mind the fact that the organic chemist in his laboratory usually finds heat to be most convenient for carrying out his reactions. Fundamentally, a principal reason is that many organic reactions are energized by volatilization of product water. The same explanation pertains to spontaneous geosynthesis. What then are suitable thermal locales on the Earth?

Many suitable locales for spontaneous thermal geosynthesis can be proposed at present<sup>46,47</sup> and geological theory suggests that this number may have been even greater in earlier times<sup>48</sup>. One can visualize that amino-acids might form directly from intermediates produced in vapour phase reaction in a volcanic region, according to the conditions specified by Dr. Harada's experiments. Enough water would have to enter the scene to promote hydrolysis of the amino-acid intermediates. Frequent hydrolysis and evaporation require no conceptual strain if one thinks of the frequency of rain in volcanic regions, as elsewhere. A deposit of amino-acids in a region of 100–200° would promptly yield pre-protein-like polymers to the extent that aspartic acid<sup>47</sup> and glutamic acid were present. A second possibility is that amino-acids might be formed in the atmosphere and then rain in the dry state on to hot volcanic areas. A third possibility is one of an oceanic soup of amino-acids of any concentration. When some of this would splash on to seaside lava beds (many volcanoes are near the sea) the water would evaporate very quickly if the amount splashed were small. The solid residue after evaporation would then consist of dry amino-acids. A fourth possibility is one in which the amino-acids in any of these ways, or in any other, came into contact with warm or hot phosphate beds<sup>49</sup>. Lesser temperatures could suffice for polymerization. The necessity of a proper balance of amino-acids continues to apply, however.

Any or all of these circumstances should have operated for the geosynthesis of pre-protein; probably other conditions were suitable as well. So far, other modes of

producing peptide bonds in the laboratory under quasi-geochemical conditions<sup>34,40</sup> have shown predominantly glycine in the polymer; glycine is one amino-acid which has long been known to undergo homopolymerization readily<sup>36</sup>. Reliance on recovery of small amounts of other amino-acids following hydrolysis does not support a statement that heteropeptides were formed, inasmuch as hydrolysable diketopiperazines form readily<sup>35,36</sup>. Quasi-geochemical methods of producing in water polyamino-acids with substantial proportions of many amino-acids which are surely bound as peptides may, however, yet be found. The first demonstration of how peptide bonds may have formed yields polymers remarkably like proteins (Table 2).

The geological conversion of such polymers to microspheres functioning as pre-cellular forms would be similarly simple and, according to the experiments, bound to occur in innumerable places on innumerable occasions. The requirements for the geosynthesis would merely be the intrusion of water and the inexorable tendency of such polymers to form cell-like units, as already demonstrated in the laboratory. Inasmuch as the sequence of reactions from primordial gas to microspheres under water is shown to occur in only hours, the organic material composing, or trapped in, the microspheres would be protected from destructive action of heat or other radiation by the overlying layers of water. When one recognizes the breadth of conditions under which the reactions occur and the breadth of terrestrial occurrence of these conditions, one must infer a truly tremendous number of natural experiments. Superposed on this picture is a huge number of microspheres in each experiment, with obviously some variation in individuals.

In biological evolution, selection is believed to be the major process<sup>51</sup>. The possibility of an internal limitation in the step of diversification preceding selection does not receive much serious attention<sup>51</sup>. An effect of this sort is, however, indicated for the molecular evolution which is modelled by the chemical experiments. When organic compounds are produced by imparting various types of energy to various mixtures of simple compounds of carbon, hydrogen, oxygen and nitrogen, the compounds formed tend to be predominantly amino-acids. This preference can be understood on the basis that amino-acids are inner salts; as inner salts they are relatively stable and are thus favoured<sup>57</sup>. Just as amino-acids are favoured over other organic compounds, certain amino-

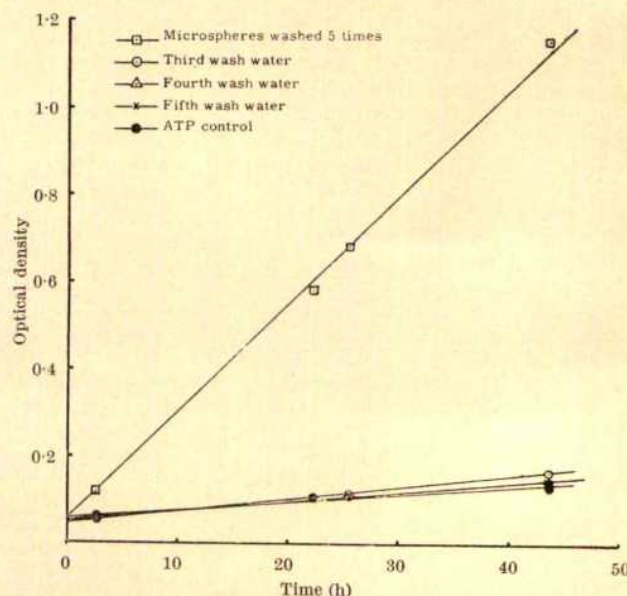


Fig. 16. ATP-splitting activity of washed zinc-containing microspheres from Fox, Joseph and Wiggert, *Origins of Prebiological Systems* (Academic Press, 1965)



acids result more easily and more often than others. Certain polyamino-acids would also be favoured over others for purely chemical reasons. If one believes that chemical processes operate in cells, we can anticipate that self-limiting diversification<sup>2</sup>, as well as selection, operates in bio-evolution. The picture, then, would be one of self-limitation in diversification spanning the pre-biological and the biological. In the biological era alone, selection would operate in addition.

The thermal model has developed from a pluralistic philosophy which uses contemporary biology for clues and as a framework of testing and uses experimental 'geosynthesis' primarily as a laboratory technique. A pluralistic philosophy of this sort has seemed necessary inasmuch as we cannot extrapolate back to a prebiotic system in a manner that is in the usual sense testable. This follows, of course, from our ignorance of what the prebiotic system or systems were. Complete knowledge of contemporary biochemical systems may prove to be useless, or even inhibitory or misleading for the purpose of understanding or synthesizing a prebiotic system. On the other hand, a sufficiently disciplined theory of pre-biological chemistry must prove eventually to be fundamental to biological chemistry.

Once research seems to have found its way on to the track which produces relatively simple prevital systems, further experiments can be directed at introducing in a stepwise manner additional characteristics of biocells in a naturalistic and empirical (albeit now guided by the experimental evolutionist) manner.

On the whole, research of this sort generates new questions faster than it does answers; but some answers are forthcoming. The possibility of understanding how materials as complex as proteins or nucleic acids might have arisen in a manner that is sufficiently simple to have occurred spontaneously on the primitive Earth is demonstrated by experiments.

The protein-cell-protein dilemma expressed by Blum and by others<sup>92,93</sup> is resolved by these experiments, as are other dilemmas. Protein of properties adequate to forming primitive protocells could have arisen without a living system to produce them; all that was needed was the necessary primordial gases forming amino-acids in suitable proportions, and heat. This kind of protein not only could form; our investigations in the field<sup>46</sup>, in the laboratory, and in the library indicate the occurrence of multitudinous geological possibilities. In this view, one can more easily visualize a large number of natural experiments of which one or more would happen to include the biochemical apparatus necessary for continuing replication. When that occurred, a first primitive cell would have emerged, and the entire parade of Darwinian evolution could begin.

A similar kind of dilemma is that posed by the need to visualize genes as arising before cells<sup>94,95</sup>. The demonstrated self-ordering properties of geosynthetically derivable macromolecules are such that we can now more easily visualize the origin of the cell before or simultaneous with the emergence of primordial nucleic acids<sup>75,96</sup>. We might refer to these synthetic polyamino-acids as morpho-molecules to emphasize the degree to which the visible structure of the particles is a manifestation of the nature of the molecules<sup>10,75,97</sup>.

The significance of any step in the thermal model is a function of the significance of the total model and *vice versa*. The larger theory is such as to permit us to visualize, through the discipline of experiments, how primordial gases could be spontaneously converted to amino-acids, these in turn to protein, and this latter to a membranous microparticle having many of the properties of cells, including the tendency to divide and to metabolize. If the pond is deferred, the visualization is then a detailed demonstration of Charles Darwin's now well-known statement<sup>67</sup>:

"It is often said that all the conditions for the first production of a living organism are now present, which

could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, and carbon present, that a proteine compound was chemically formed ready to undergo still more complex changes . . .".

Any remaining discontinuity between non-life and life should, according to present understanding, be regarded as not yet understood rather than as hopelessly incomprehensible. We may even adopt the position that, while we credit Darwin with that insight into pre-biological molecular evolution, we owe him more for presenting the principle of biological evolution, inasmuch as the later processes will in the long run prove to have been composed of more intricate and subtle sets of transformations.

This theory is derived from the efforts and critical discussions of numerous associates. The work was supported by grants from the U.S. National Aeronautics and Space Administration (NSG-173-62), the U.S. Public Health Service (C-3971), the National Science Foundation, the General Foods Corp., Eli Lilly and Co., and the Rockefeller Foundation.

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## CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS, SPAIN TWENTY-FIFTH ANNIVERSARY CELEBRATIONS

IN 1939, soon after the end of the Civil War, the Spanish Government set up the Consejo Superior de Investigaciones Cientificas for the purpose of fostering, directing and co-ordinating research in both the sciences and the humanities. During the week beginning on October 18, 1964, the Consejo celebrated its twenty-fifth anniversary by arranging a solemn opening session, scientific meetings, and certain social events; and it invited a number of foreign guests to attend.

The Consejo is divided into eight "patronatos", grouped together to form three main sections: the Division of Humanities, the Division of Sciences, and the Patronato of Scientific and Technological Research which deals with applied science. It is responsible for a large number of institutes, many, but by no means all, of which are in Madrid.

It is at first sight somewhat surprising that it was necessary to create a new organization, and one which, though under the Ministry of National Education, enjoys a considerable degree of independence, to foster the type of research which in most countries is regarded as appropriate to universities. Spanish tradition, however, in contrast, for example, with that of Germany, has hitherto regarded the universities primarily as places of professional training rather than as centres of learning and research; and if the necessary rapid progress were to be made some organization outside them was necessary. The progress made by the Consejo in its twenty-five years, the quality of the research which it is organizing, the influence which it is beginning to have on the outlook of the universities, and the impetus which it is giving to Spanish technological development all demonstrate the wisdom of the original decision to set it up and the competence with which its activities have been developed.

The celebrations began with a solemn inauguration on October 20 in the presence of the Head of State, at which

messages of congratulations from international and foreign organizations were delivered. Thereafter, except for unified social events, the proceedings were broken up into five colloquia. That organized by the Division of Humanities dealt with problems of Spanish art and history, and with linguistic questions, and had a very largely Spanish attendance. That organized by the Patronato of Scientific and Technological Research on the theme "Research and Industry" was mostly conducted in Spanish, but included papers by D. R. Vieweg (Germany) on staffing problems, by D. L. Jacqué (France) on programming of research, by D. R. R. Adams (United States) on the measurements of the profitability of research, and by D. E. Martindale (Great Britain) on scientific information. The eleven introductory papers led to much discussion, and some sessions were very lively: notably that in which the question was discussed whether university professors ought to be expected to do research.

The remaining three colloquia were organized by the Division of Sciences, and took on a more fully international character, though naturally with very considerable Spanish attendance and participation. Two were in the biological field, on current problems in biology and on the contributions of ecological and agricultural research in the world fight against hunger. The third dealt with the physical chemistry of processes on solid surfaces.

The colloquium on "Current Problems in Biology" covered a wide range of subjects; some of the contributors discussed in detail a small facet of a biological problem, and others attempted a wider conspectus of a particular field. The opening colloquium on the organization of biological investigation was largely directed to problems of organization and financing of biological research in Spain. The more general conferences were inaugurated by Dr. R. Stanier (United States), who dis-

Dr. Hoare has taken an active part in the administration of the University; he represented the Faculty of Science on the Court from 1950 until 1952, held office as Dean of the Faculty of Science from 1958 until 1961, and has served as an additional member of the Senate for the periods 1960-62 and 1964-65. He is a member of the Council's Advisory Committees on the Education and Training of Non-academic Staff and on the Welfare of Clerical, Technical and Maintenance Staffs. During his time at Leeds he has initiated investigations at very low temperatures and has built up the facilities for this work with particular reference to the properties of metals and alloys. He has published many papers on the thermal and magnetic properties of the transition elements and is the author of an undergraduate text-book on thermodynamics as well as part author and co-editor of a reference book on low-temperature techniques.

### Development of Nuclear Power in Britain

In a written answer in the House of Commons on December 1, the Minister of Power, Mr. F. Lee, said that the Government proposed to keep the further development of the British Nuclear Power programme under review as envisaged in the White Paper on the Second Nuclear Power Programme. The question of the type of reactor to be included would be considered when the results of the enquiry for tenders for the first station in the programme were available in 1965. In reply to a further question on December 8, Mr. Lee offered no further information, but the position of the electricity, gas and nuclear energy industries generally was raised in the House of Lords on December 8. Lord Hawke pointed out that capital costs had been coming down and were now about £40 per kW for conventional stations.

In reply for the Government, Lord Stonham said that the Government fully recognized that Britain's economy and planned expansion were dependent on the production of power and that the fulfilment of a policy to meet that demand efficiently and economically required investment on a massive and increasing scale. In 1963 the gas industry in Great Britain spent more than £90 million on capital account, and the nationalized electricity industry in England and Wales, including nuclear power stations, spent almost £490 million. The annual capital expenditure in the electricity industry was expected to increase substantially, and a figure of £673 million had been approved for the next financial year. Capital investment by the Gas Boards was not expected to be more than about £100 million a year, but it was impossible to give precise forecasts until the Government's present review of public expenditure and investment had been completed. It was certain, however, that the £770 million expected to be invested over the next year in gas and electricity would be insufficient if in the years ahead the growth of the economy were to be supported and domestic requirements met. The Generating Board's proposals should give a reserve margin of plant by about 1970 of some 17 per cent, and this would involve investment totalling £4,000 million over the next six years. Lord Stonham went on to point out that nuclear plants would play an increasingly important part, but the present nine stations were of the magnox type; the tenders which had been invited for the advanced gas-cooled reactor type could not be expected until well into the summer of 1965. Little hope could be held out for great expansion in regard to pump storage, although pump storage stations had a longer working life than conventional generating plants. Further advances in the technology of conventional generation might, however, make pump storage schemes more attractive, as would the growing availability of nuclear power when the capacity of nuclear power plants was sufficient to supply the minimum load on the system and surplus low-cost nuclear energy was available for night pumping. Lord Sherfield thought that the target of 5,000 MW of additional nuclear power by 1975 was on the low side,

although he agreed that there were difficult questions of priorities and national interest. He urged the importance of firm decisions, while Earl Ferris referred to the necessity of spending much more money on research into the possibility of laying underground cables more cheaply and more effectively, as was being done in other countries.

### Future of Teacher Training Colleges in England and Wales

In a statement in the House of Commons on December 11, the Secretary of State for Education and Science, Mr. M. Stewart, said the Government had now considered the recommendations of the Robbins Committee on the future of the teacher training colleges in England and Wales. The Government agreed that wider opportunities should be given to suitable teacher training college students to take a degree together with a professional teaching qualification by means of a four-year course. However, after considering the advice given by the University Grants Committee, the Government had concluded that the academic and administrative and financial aspects were separable and that fundamental changes should not, at the present time, be made in the administrative and financial structure of the teachers training system. It had therefore been decided that the colleges should continue to be administered by the existing maintaining body under the present system of overall supervision, but the present arrangements for the internal government of colleges would be reviewed forthwith by all those concerned, in the light of the recommendations of the Robbins Committee. It had been agreed that the training colleges could appropriately be renamed 'Colleges of Education'. Mr. Stewart believed that the colleges should have a proper measure of independence in academic matters, and he thought that it was particularly important that local authorities should attend to this.

### Overseas Development

In reply to questions in the House of Commons on December 15, the Minister of Overseas Development, Mrs. B. Castle, said that about 550 in the direct employment of her Department, including specialized project advisers, were expected to be serving overseas in 1965; some 140 could be attributed to functions of the Department of Technical Co-operation. The Commonwealth Development Corporation was being transferred to her Department, and when this was completed she would review the functions and resources of the Corporation in the light of the needs of the British aid programme as a whole. Arrangements already existed for the co-ordination of educational assistance under technical co-operation arrangements and through the British Council, and this co-ordination would be strengthened. The annual rate of appointment of qualified technical staff for Commonwealth Africa had been more than doubled since 1962 and a thorough review of recruitment policy generally had been commenced. Total assistance from Britain to the less-developed countries in 1963 was estimated at £227.8-£247.8 million compared with £273.9 million in 1961 and £218.3 million in 1962. Of the 1963 figure, bilateral economic aid was £138.9 million and private investment £70-90 million.

### Joint Study of Natural Gas Pipeline across the North Sea

SIR HENRY JONES, chairman of the Gas Council, stated in London on November 30: "The Gas Council and N.V. Nederlandse Aardolie Maatschappij (Gas Export) have agreed to initiate a joint study of the technical and economic aspects of a natural gas pipeline from the Netherlands to Great Britain across the North Sea. The study will enable work which has hitherto been performed separately to be continued in more detail. It does not indicate any commitment by either party to supply or to take gas at any future date".



## University of Saskatchewan Linear Accelerator Laboratory

THE University of Saskatchewan Linear Accelerator Laboratory was officially opened on November 6, 1964. More than one hundred European, American and Canadian scientists attended the ceremonies, and sessions of contributed and invited papers were held. This occasion marked the bringing into operation of a linear electron accelerator with 140-MeV maximum unloaded energy and a mean current of 200  $\mu$ amp at 100 MeV. The energy can be varied continuously from 5 MeV to its maximum value. Pulse durations from 5 nsec to 1  $\mu$ sec are available at a repetition rate of 0-1,400 pulses/sec. The energy spectrum at the output of the accelerator is about 2 per cent. The director of the Accelerator Laboratory is Prof. L. Katz.

## Biology at Euratom

A REPORT of the European Atomic Energy Community, entitled *Euratom's Biology Programme 1961-64: Report and Perspectives*, by R. K. Appleyard, describes the progress and growth of the Biology Division since its inception three years ago (Pp. 37. (EUR. 1884. e.) Brussels: European Atomic Energy Community—Euratom, 1964). The report is divided into three parts dealing briefly with administrative and political factors and more fully with the scientific programme of the Division. The basic policy of the Division is to encourage research by contractual arrangements with individual institutes or centres of research within the six member countries and to act as a centre for community effort within the field of radiobiology. Financial resources, and the uses to which they were put, are described in some detail, both within the text and by tables and diagrams in the appendixes. Direct research activities of the scientific staff of the Division were chiefly limited to investigations of environmental contamination and the movement of isotopes in ecological systems. Work done under contract was much more broadly based, however, and represented the greater part of the expenditure of the Division. One avowed aim of the support of outside work was to "encourage not only original ideas but new growing points of research activity and enhanced responsibilities among younger research workers". The latter intentions did not seem to be reflected in the contractual programmes listed in Appendix 3. The rate of accumulation of publications from the activities of the Division was also shown in Appendix 3. Although the total yield of publications was impressive, a list of selected literature would have been more instructive.

## Yale Arbovirus Research Unit

THE Rockefeller Foundation Virus Laboratories moved in December 1964 from New York to New Haven, Connecticut, where the virus research programme of the Foundation will be associated with the Department of Epidemiology and Public Health of Yale University as the Yale Arbovirus Research Unit. Dr. W. G. Downs, director of the Foundation's virus programme, and Dr. M. Theiler, former director of the Laboratories, have been appointed professors in the Yale University School of Medicine. Dr. L. Whitman, acting director of the Laboratories, Dr. Sonja M. Buckley, Dr. J. Casals-Ariet, Dr. Delphine H. Clarke, and Dr. R. W. Speir, of the staff of the Laboratories, will also have faculty affiliation. In New Haven the Laboratories will be located, in a new building recently completed for the Department of Epidemiology and Public Health, at 60 College Street.

## Journal of Applied Ecology

RECENT developments in the expanding field of ecology have stimulated the British Ecological Society to publish a third journal to supplement their two existing journals, the *Journal of Ecology* (started 1913) and the *Journal of*

*Animal Ecology* (started 1932) (*The Journal of Applied Ecology*, 1, No. 1, May 1964. Edited by A. H. Bunting and V. C. Wynne-Edwards. Pp. vi+218. Oxford: Blackwell Scientific Publications, 1964. Published twice a year, 70s. net. Annual subscription, post free, 120s., U.S. and Canada, 20 dollars). The first number contains sixteen papers and several book reviews. Six titles suggest something of the range of subjects covered: J. N. Black, "An Analysis of the Potential Production of Swards of Subterranean Clover (*Trifolium subterraneum* L.) at Adelaide, South Australia"; A. D. Ansell, K. F. Lander, J. Coughlan and F. A. Loosmore, "Studies on the Hard-shell Clam, *Venus mercenaria*, in British Waters"; Donald A. Spencer, "Porcupine Population Fluctuations in Past Centuries revealed by Dendrochronology"; F. H. W. Green, "A Map of Annual Average Potential Water Deficit in the British Isles"; G. Szeicz, J. L. Monteith and J. M. dos Santos, "Tube Solarimeter to Measure Radiation among Plants"; David Jenkins, Adam Watson and G. R. Miller, "Predation and Red Grouse Populations". The contributors, editors, publishers and printers are all to be congratulated on the high standard of presentation in this first number. Further numbers will help to clarify the exact scope of this new journal in relation to its older stablemates.

## The British Journal for the Philosophy of Science

THE purpose of the British Society for the Philosophy of Science is to study the logic, the method and the philosophy of science as well as those of the various special sciences, including the social sciences. The current number of the *Journal* (15, No. 59; November 1964) begins with three logical articles on the confirmation paradoxes by William M. Baumer, P. R. Wilson and Judith Schoenberg, respectively. An important section, "A New Approach to the Theory of Fundamental Processes", is by Mendel Sachs, of Boston University. It is remarkable how often to-day statements can be found in Mach's *The Science of Mechanics* which make good starting-points for modern physical theories. In a new attempt towards a unified theory in physics, Sachs quotes Mach: "However well fitted atomic theories may be to reproduce certain groups of facts, the physical inquirer who has laid to heart Newton's rules will only admit those theories as provisional helps, and will strive to attain, in a more natural way, a satisfactory substitute". The essential point of departure of the new approach is in the introduction of the following fundamental postulate: "The laws of Nature must be described in terms of field variables that may be associated only with elementary interactions". Sachs applies his theory to an interpretation of the Dirac field, the production and annihilation of particle-antiparticle pairs, Pauli's exclusion principle, electron spin, the Lamb shift in the spectrum of hydrogen and the hydrogen equation which has an additional term—a fundamental length. The new approach introduces the elementary interaction instead of the elementary particle as the fundamental entity of Nature. The three manifestations of elementary interactions in terms of the gravitational (Einstein), electromagnetic (Maxwell) and matter (Dirac) field descriptions can all be represented in terms of fundamental spinor and quaternion field variables. The quantitative predictions which result from this method are being studied at present.

## The British Association of Industrial Editors, Ltd.

THE periodic 'house magazine' is such an important instrument of communication between management and staff in our large industrial organizations to-day, and its appeal and popularity have become so firmly established, not only in the firms concerned but, by virtue of a wider circulation, in the minds of a larger public, both in the United Kingdom and overseas, that it is not surprising that there now exists an association of people primarily concerned with these industrial publications and with the

cussed "Structure and Functions of Prokaryotic Cells", Dr. A. Virtanen (Finland), who discussed "Milk Production without Proteins", and Dr. S. Ochoa (United States) on "Replication of Nucleic Acids in Viruses Containing R.N.A.". Of the remaining seventeen contributors—European, North American and South American—some dealt with plant endocrinology, the terminal oxidases of plants, acetyl co-enzyme A carboxylation reactions, and the implications of biological catalysis and of photosynthesis. There were three papers on neurophysiology, including one on the molecular aspects of curarization, two on antibiotics and three on experimental or population genetics. The session ended with rather a mixed collection of papers on human endocrinology, inflammation, the role of magnesium in mammalian life, and a new technique in electron microscopy.

Varied as the subjects were, there was no doubt, judging from the discussion and comments, that all of them in one way or another were of immediate interest both to the guests and to the Spanish biologists who were present.

The programme of the colloquium on the "Contributions of Ecological and Agricultural Research in the World Fight against Hunger" was made up of six sessions and a total of thirty-nine contributions. Spanish speakers predominated, but there were others from most Continental countries, Great Britain and America. A wide range of topics was discussed. Some were concerned with the broad principles of world production, others related to more specific problems of the Mediterranean region.

On a world basis, the importance of a more detailed knowledge of climatic conditions and vegetational, ecological and soil types was stressed, while the value of accurate maps for evaluation and comparison of the potential productivity of different regions was emphasized.

Another aspect was the place and role of food production in the national economy of developed and developing countries. For example, in many parts of the world water is the major factor limiting agricultural production, and careful consideration must be given to determining the best means of sharing the limited supplies between industrial, domestic and agricultural needs. Besides discussion of the water factor, there were papers concerned with the ultimate limits of productivity, nitrogen fixation and the most effective uses of fertilizers

and of the ever-increasing range of chemicals used in both agriculture and forestry.

In the colloquium on the "Physical Chemistry of Processes on Solid Surfaces" twenty-four papers were presented. The first, by P. J. W. Debye, on surface determination by X-ray scattering, set a high standard which was well maintained. The contributions ranged over the whole field of the relationship between surface structure and catalysis, including the use of radioisotopes in the examination of surface phenomena. Among those present were many of the leading authorities, and this ensured an unusually penetrating discussion of the many new ideas put forward. The colloquium, indeed, was an outstanding success, and it is good to know that the papers and discussions will be published in full.

The proceedings closed with a banquet on October 24 at which speeches of thanks and congratulations were made by visitors from Great Britain, Germany, Argentina and France. Reply was made by the Minister of National Education, Dr. Lora Tomayo, who presided at the banquet, and who was, until a few years ago, professor of organic chemistry in the Consejo's laboratories. His appointment as Minister was, indeed, a most interesting and welcome sign of the times, and an earnest of Spain's determination to put her scientific and technological development in the forefront of her programme.

Besides the banquet there was a full social programme which enabled visitors to meet one another and their Spanish colleagues and hosts under most agreeable conditions, and on the Sunday after the meetings an excursion was arranged to the Valle de los Caídos and the Escorial. All arrangements were excellently made, and our Spanish hosts were most generous.

Visitors were left with an impression of a great Spanish achievement. Not only has the Consejo firmly established itself over a wide field of learning and research, but also present plans envisage a rapid growth, particularly in buildings and facilities. At present, in many laboratories, the space available scarcely matches the excellence of the staff.

The work of the Consejo is likely to have a profound long-term influence on Spanish thought and organization. For this much of the credit must go to J. M. Albarceda. It is pleasant for us to reflect that he once spent two years in Britain as a Ramsay Memorial Fellow.

B. K. BLOUNT

## OBITUARIES

### Prof. J. M. Somerville

JACK MURIELLE SOMERVILLE, professor of physics in the University of New England, died suddenly on October 15, 1964. Though his silver hair made him look older, he was only fifty-one.

Born at Liverpool, New South Wales, in 1912 and educated at Wollongong High School and the University of Sydney, he began his career as a mathematician and on graduation won a Barker scholarship to Emmanuel College, Cambridge. When he returned to Australia in 1937, however, his main interest turned to physics and in 1938 he was appointed as one of the foundation members of the staff of the New England University College, where he was in charge of all teaching in both mathematics and physics. Thus began his long association with the present University of New England, an association interrupted for only four years during the Second World War when he returned to the University of Sydney as assistant director of radiophysics training in a war-time school to train operators and technicians in the then new science of radar. In 1954, when the College became autonomous as the University of New England, he was appointed as its foundation professor of physics and continued as head of the Department of Physics until his death.

Prof. Somerville's main research interests lay in the study of electric discharges in gases. His earlier work on discharges in magnetic fields earned him a D.Sc. from the University of Sydney in 1952, but he is chiefly known for his work on transient electric arcs and sparks which occupied the last fifteen years of his life. He liked to use a simple direct approach to physical problems whenever possible and developed a method for the study of spark channels, by which a current pulse, passing through the plasma of the channel, was made to leave an imprint on a thin insulating film covering the anode. From a study of these imprints the life history of a spark could be traced in detail over the first few microseconds of its existence. Later refinements of the work extended optical, spectroscopic and calorimetric methods to the study of these sparks.

Recognition of his work was wide and his circle of personal friends included many people working in related fields in the United Kingdom, Europe and America. He spent 1952–53 as Warren Research Fellow of the Royal Society at the University College of Swansea, and an extended trip in 1963 allowed him to visit many laboratories where work related to his interests was being carried on.

His influence on science in Australia was considerable and he was much sought after to serve on important committees both within and outside the University. He was a council member and past president of the Australian Institute of Nuclear Science and Engineering, a member of the committee at present drafting a new science syllabus for secondary schools in New South Wales and a member for many years of the Council of the University of New England.

Prof. Somerville's wise and kindly influence on his many students and colleagues will be long remembered, as will his contribution to the establishment of the University of New England.

N. H. FLETCHER

### Mr. C. C. L. Gregory

MR. GREGORY, who was killed in a street accident on November 24, 1964, will be remembered as the astronomer who set up the University of London Observatory at Mill Hill. However, he had many other interests and activities.

He was born on May 13, 1892, and developed an interest in astronomy in early life. He gained a B.A. honours degree at the University of Cambridge in 1915 and started research at the Imperial College of Science and Technology, London, where the Diploma of the Imperial College was conferred on him in 1917. From studying the ammonium spectrum in London, he went, in 1919, as chief assistant to the Helwan Observatory, Egypt, there

to make a major contribution to the photographic survey of nebulae with the 36-in. Reynolds reflector.

From 1921 he was lecturer in astronomy in the Mathematics Department of the University College, London. However, Mr. Gregory was a practically minded astronomer, and talk of a research observatory was soon opened between him and Prof. Filon. About 1924 a 24-in. reflecting telescope was found to be available. This was accepted from Mr. F. G. Wilson (County Westmeath) and erected in 1929 at Mill Hill. Mr. Gregory became the Wilson Observer. Under his direction the Observatory continued to expand—the large Radcliffe twin refractor was erected in 1939. He mixed day-time lecturing with Observatory management and night observing. He kept up a centre of undergraduate astronomical teaching in the University College at a time when other university observatories were turning preferentially to postgraduate studies.

Throughout his life he had a strong interest in parapsychology and metaphysics, and in 1950 he resigned from astronomy to turn completely to such subjects. In 1954 he was joint author with Anita Kohsen of a book entitled *Physical and Psychical Research (An Analysis of Belief)*. Later, Mr. Gregory and Miss Kohsen were married. Jointly they set up the Institute for the Study of Mental Images and brought out a journal entitled *Cosmos*. This journal was to terminate with its forty-first number, and by tragic coincidence Mr. Gregory's death occurred while No. 41 was being prepared.

C. W. ALLEN

## NEWS and VIEWS

### Biological Sciences in the College of Advanced Technology, Birmingham:

Prof. A. J. Matty

DR. A. J. MATTY, who is at present senior lecturer in comparative pharmacology in the University of St. Andrews, has been appointed professor of biological sciences and head of the new Department at the College of Advanced Technology, Birmingham, as from January 1. Dr. Matty, who is forty years old, was educated at the Central Grammar School, Birmingham, and at University College, Nottingham, where in 1948 he took a B.Sc. (Special) London External degree in zoology. In 1951 he obtained his Ph.D. at the University of Nottingham. Dr. Matty began his career as a demonstrator in zoology at the University of Nottingham, where he was later appointed lecturer. In 1959 he became lecturer at the University of St. Andrews, where, at the newly built Wellcome Laboratories of Pharmacology at the Gatty Marine Laboratory, he established teaching and research in comparative pharmacology. Dr. Matty's research has been concerned with comparative and cellular endocrinology, particularly on problems of thyroid function in lower animals. He was awarded one of the first Royal Society and Nuffield Foundation Commonwealth Bursaries in 1955 for research in Bermuda on the thyroid gland of *Scarus guamacia*. Other research work carried out by Dr. Matty has been concerned with the effect of thyroid hormones on membrane permeability, on the pituitaries of fishes, on cyclostome endocrinology, and on intestinal transport in mammals.

### Mathematics in the University of Keele:

Prof. A. P. Robertson

DR. A. P. ROBERTSON, whose appointment to the chair of mathematics in the University of Keele in succession to Prof. D. S. Jones was announced recently, was born in 1925. He pursued his undergraduate studies in the University of Glasgow, graduating M.A. with first-class honours in mathematics and natural philosophy early in 1946. Because of the requirements of the National

Service Act he was not able to leave Glasgow to continue his studies elsewhere and acted as an assistant lecturer in mathematics until October 1947, when he entered St. John's College, Cambridge, to read for the Mathematics Tripos. He gained a B.A. of the University of Cambridge in 1949 and Ph.D. in 1954 (being elected a Fellow of his own college in that year). Apart from a year in the University of Kansas, Dr. Robertson has been on the mathematics staff of the University of Glasgow since 1961, first as a lecturer and latterly as a senior lecturer. Dr. Robertson is well known for his original contributions to 'classical' analysis and functional analysis. This year saw the publication of the Cambridge Mathematical Tract *Topological Vector Spaces* written by Dr. Robertson and his wife; this has already been acclaimed as a most useful survey of an important part of functional analysis. His interest in reforms in the mathematical curriculum both at school and university, as well as his status as a research worker in an important branch of modern pure mathematics, fit him admirably for the chair he is now called to occupy.

### Physics in the University of Leeds: Prof. F. E. Hoare

DR. F. E. HOARE, reader in physics in the University of Leeds, has been appointed to an additional chair in the Department of Physics in the University. Dr. Hoare was educated at the Municipal Secondary School, Brighton, and the Imperial College of Science and Technology, London. He gained first-class honours in physics in the examinations for the degree of B.Sc. and Associateship of the Royal College of Science. He was awarded the degrees of M.Sc. in 1930 and Ph.D. in 1932 for work on radiation measurements. In 1928 Dr. Hoare was appointed assistant lecturer in physics at the then University College, Exeter, and was promoted to a lectureship in 1934. During the Second World War he was engaged on scientific work for the Admiralty. He joined the University of Leeds in 1946 as lecturer in physics and was promoted to a senior lectureship in 1952 and a readership in 1954.



interests of all those responsible for the increasingly high quality achieved in these productions. In *Communication* 64 (Pp. 56. London: The British Association of Industrial Editors, Ltd., 1964. 5s.), a review prepared by the British Association of Industrial Editors, there occurs this statement which clearly epitomizes the aims of this Association: "Communication can be the O.K. formula word, the fashionable word of industry, the vague, facile excuse word, the all-things-to-all-pseudos word. Properly considered it can be the key to understanding between individuals, groups and nations—the supreme aim of civilized society. Over a decade ago the President of the American Management Association said: 'The number one management problem to-day is communication'. The problem remains. This survey takes a realistic look at an ingredient vital to industrial and social progress". Among the articles in the present publication are "The Future of Mass Communications", by I. Colquhoun; "Men, Management and Morale in Efficient Business", wherein industrial relations and the importance of the systematic approach are reviewed by J. Garnett; "Walls between People", the eccentricities and idiosyncrasies which provide obstacles to communication, by G. C. B. Andrew; "The Meaning of B.A.I.E.", the growth of the Association from enthusiastic amateurs to professional status, detailed by G. Phillips; "British Editor: Industrial Species", a description of the present-day role of the industrial editor in Great Britain, by R. C. E. Dancer; "The Communicators"—being interviews with five leaders of industry on the role of communications; "Case Studies", some examples of success and failure in house journals analysed by E. H. Dodimead. This publication sets a standard not only in subject-matter and illustration, but also in style and presentation of the message it seeks to convey; if industrial editors responsible for house magazines, either established or at present only at planning stage, can emulate it, they will not go far wrong.

### Needs of the Scientific Writer

In a paper read at a Library Circle meeting of the Royal Institution in March 1964, and now published in the *Proceedings of the Institution* (No. 182, 40; 1964), Mr. J. G. Crowther describes with insight the dependence of the scientific writer on library facilities and the characteristics of the facilities such writers need. His warning that mere technical efficiency is insufficient is timely, but Mr. Crowther could well have mentioned also the need for an easy and rapid means of verifying references and quotations. Mr. Crowther refers appreciatively to the establishment of the National Lending Library for Science and Technology. His talk deserves attention from the Committee on Libraries of the University Grants Committee, as well as elsewhere, for it points to some gaps in the national library system which call for imaginative attention. The scientific writer has an important part to play in spreading a general understanding of science and technology and in raising productivity, and he cannot do so with full efficiency unless these library needs are met.

### New Zealand Forestry

THE annual report of the Director-General of Forests, New Zealand, for the year ended March 31, 1964, makes very interesting reading (Pp. 59. Wellington: Government Printer, 1964. 2s. 6d.). New Zealand's forest economy is largely dependent on her exotic coniferous forests, and now they have three times the output of the indigenous forests. More than this, the annual exports of forest products have risen in the past ten years from less than £1 million to nearly £11 million. This prosperity is to a great extent associated with the rapid rise of the pulp and paper industry, for more than three-quarters of the 1963 exports was due to newsprint and pulp. Indeed, it is significant that these two items accounted for nearly half New Zealand's exports to Australia, and forest products now rank high in a country which is traditionally

associated with agricultural produce only. There is plenty of land available for acquisition by the Forest Service, especially land that is marginal for agriculture. But some unsuitable land is being offered because would-be sellers too often look on forestry as a form of land use to take over where agriculture has failed. With the availability of land as it is, there is every likelihood that there will be an increased rate of planting by both State and companies, although private planting may have to be given incentives because during the past 30 years it has lost some of its public appeal as a commercial investment. The New Zealand Forest Service provides a vigilant timber inspection. Packing-cases are regarded as one of the most dangerous means of entry of forest insects into the country. There is the not unusual animal-control controversy, but the Forest Service insists that its policy is to enforce proper control and not to exterminate. Over-population of noxious wild animals on catchment areas can sometimes be associated with flooding. Forestry in New Zealand continues to prosper in a very convincing manner.

### Bibliography on British Soils

INFORMATION on soils is presented in such a large number of scientific journals and memoirs that it is often difficult for anyone not actually engaged in soil science investigations to decide where to look for appropriate reliable data. It was with this in view that *An Annotated Bibliography of Memoirs and Papers on the Soils of the British Isles*, by B. T. Bunting, was published in time for the twentieth International Geographical Congress (London) and the eighth International Congress of Soil Science (Bucharest) (Part 1: *The Classification, Morphology, Distribution and Reclamation of British Soils*. Pp. iv+100. London: Geomorphological Abstracts, Department of Geography, London School of Economics, 1964. 10s.). In the main, it includes papers dating from 1920. Authors and titles are given with occasional cross-references and, in many cases, an abstract is added. Part 1 of the *Bibliography* deals with classification, morphology, distribution and reclamation, and consists of two parts: the first is of 11 sections on systematic and general papers, the second is on regional and local papers for 16 regions. There is an author index. A second volume (Part 2) is planned to deal with the internal properties and external relationships of British soils.

### Odour in Theory and Practice

INCREASING specialization brings with it the advantage that every available scientific and technical approach can be brought to bear on a single problem. Odour is a case in point. Its practical importance is so great in so many ways that it is scarcely surprising to find numerous laboratories devoted solely or mainly to the investigation of various aspects of the chemistry, physiology, psychology, measurement, analysis, correlation, control and production of odours and fragrances. These topics are described in the report of a conference entitled *Recent Advances in Odor: Theory, Measurement, and Control* (*Annals of the New York Academy of Sciences*. Vol. 116, Article 2. By Richard L. Kuehner and 60 other authors. Pp. 357-746. New York: New York Academy of Sciences, 1964). What emerges from this comprehensive account is the complexity of the subject and the realization that perception of odour, particularly by a trained perfumer, often still exceeds the sensitivity of vapour phase chromatography. To someone who is not a specialist in the field of odour research, its ramifications come as a surprise. To begin with, atmospheric pollution, especially by particular industries, is a source of odour which was tolerated by local residents in the past but is now no longer acceptable. Odour control in the crowded urban environment, both outdoors and indoors, assumes equal importance with reduction of noise. Body odour and deodorants are a fertile field of research. The importance of odour or fragrance in market-

ing products (quite apart from cosmetics or toiletries) appears to extend to synthetic fertilizers and plastic records. Despite the wide scope of the conference, some important aspects of the subject, of particular relevance to food, were not touched on: for example, the relationship between odour and flavour or the nature, causes and prevention of taint. A method for characterizing odour qualities was developed in order to permit an accurate description of the odour of irradiated beef. Based on matching standard odours described as "oily, sweet, fragrant, spicy, burnt, rancid, metallic, etherish and sulphurous", it conjures up anything but an inviting gastronomic experience until one realizes that fresh meat, too, is accounted for in the same terms, but with rather less of the etherish and sulphurous elements.

### Low Temperature Biology Club

AN informal association of biologists, physicists, clinicians and surgeons interested in biological and medical effects of cold has recently been formed, to be known as the Low Temperature Biology Club. The next meeting will be held at Westminster Hospital, London, on March 22. Further information can be obtained from Dr. M. J. Ashwood-Smith, Medical Research Council Radiobiological Research Unit, Harwell, Didcot, Berkshire.

### Fulbright Travel Grants

UNDER the provisions of the Fulbright programme, travel grants are available to citizens of the United Kingdom and dependent territories to go to the United States of America for academic or educational purposes, provided that they have adequate financial support in dollars for the proposed period of the visit to the United States, and can show proof of admission to an institution of higher learning in the United States for a minimum period of nine months. All grants cover the cost of direct round-trip travel between a candidate's home and the American university or institution. They are available between June 1, 1965, and August 7, 1965, for which applications must be received before March 16, 1965, and between August 8, 1965, and April 1, 1966, for which applications must be received before June 1, 1965. Application forms and further information can be obtained from the United States Educational Commission in the United Kingdom, 71 South Audley Street, London, W.1.

### National Science Foundation Fellowships

NATIONAL Science Foundation fellowships, enabling the recipients to teach and conduct research at universities in the United States for 5-12 months, have been awarded to the following: Prof. F. A. Drahowzal, professor of chemistry at the Technical University of Vienna; Prof. F. R. Keogh, professor of mathematics at Royal Holloway College, University of London; Dr. T. Nakada, director of the Research Laboratory of Precision Machinery and Electronics at the Tokyo Institute of Technology; Prof. R. H. Stokes, professor of chemistry in the University of New England, New South Wales; Dr. P. J. Wheatley, senior research chemist with the Monsanto Research Laboratories, Switzerland.

### The Night Sky in February

NEW Moon occurs on Feb. 1d 17h U.T. and full Moon occurs on Feb. 16d 00h. The following conjunctions with the Moon occur: Feb. 3d 10h, Saturn  $4^{\circ}$  N; Feb. 9d 03h, Jupiter  $2^{\circ}$  N; Feb. 17d 22h, Mars  $1^{\circ}$  S. Mercury is in conjunction with the Sun on Feb. 24d 03h and Saturn on Feb. 26d 10h. Conditions continue to be unfavourable for observation of Mercury and Venus. Mars rises at 20h 50m, 19h 45m and 18h 15m on Feb. 1, 14, 28 respectively; its distance from the Earth on Feb. 14 is 69 million miles; its stellar magnitude is  $-0.7$  and it is in Virgo. Jupiter sets at 1h 50m, 00h 55m, 23h 55m at the beginning, middle and end of the month; its distance from the Earth on Feb. 14 is 469 million miles; its stellar magni-

tude is  $-1.9$  and it is in Aries. Saturn sets at 18h 50m, 18h 00m at the beginning and middle of the month, while towards the end of the month Saturn is too close to the Sun for observation, setting before sunset; its distance from the Earth on Feb. 14 is 995 million miles and it is in Aquarius. Occultation of the following star brighter than magnitude 6 occurs: Feb. 10d 19h 08.8 m, 129 H<sup>1</sup>. Tau (D). D refers to disappearance.

### Announcements

AIR COMMODORE SIR FRANK WHITTLE is to be the first recipient of the Goddard Award of the American Institute of Aeronautics and Astronautics, for his work in pioneering the gas turbine as a jet-propulsion aircraft engine.

THE American Physical Society Prize for 1964, sponsored by the Hughes Aircraft Company, has been awarded to Prof. Melvin Schwartz, professor of physics at Columbia University, in recognition of his experimental studies of weak interactions and in particular for the use of high-energy neutrinos.

THE first Edward B. Rosa Award of the National Bureau of Standards has been presented to Mr. I. H. Fullmer, in recognition of leadership in development and promulgation of screw-thread standards, and to Dr. A. T. McPherson, for significant educational and organizational achievements in standardization.

A SYMPOSIUM under the auspices of the Faraday Society on "Excess Functions in Molten Salts" will be held at the Imperial College of Science and Technology, London, on February 25. Further information can be obtained from Dr. E. Rhodes, Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S.W.7.

A SEMINAR for science writers on "Quasi-stellar Radio Sources", sponsored by the American Institute of Physics and the National Association of Science Writers, Inc., will be held in New York City on January 26. Further information can be obtained from Mr. E. H. Kone, American Institute of Physics, 335 East 45th Street, New York 10017.

A MEETING of the Midlands Section of the Society for Analytical Chemistry will be held in the University of Birmingham on February 2. A paper entitled "pH and its Significance" will be presented by Prof. W. F. K. Wynne-Jones. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

THE annual general meeting of the North of England Section of the Society for Analytical Chemistry will be held in Manchester on January 30. The retiring chairman's address entitled "Fat Extraction in the Analysis of Food-stuffs and Related Material" will be presented by Mr. C. J. House. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

THE following educational and training courses will be held at the Production Engineering Research Association of Great Britain during 1965: *five-day courses*, general appreciation; practical machining and metal cutting; modern machining techniques; pressworking techniques; toolroom techniques; senior apprentice, parts 1 and 2; *two-day courses*, tool grinding; machine installation and maintenance; machine alignment and performance tests; production inspection; quality control. Further information can be obtained from the Information Manager, Production Engineering Research Association of Great Britain, Melton Mowbray, Leicestershire.

CORRIGENDUM. It is regretted that the telephone number of Cahn Instrument Co., Ltd., 27 Essex Road, Dartford, Kent, appeared incorrectly in the January 9 issue of *Nature* (205, 1; 1965). It should read Dartford 21540.

## ADVISORY COUNCIL ON SCIENTIFIC POLICY

APPENDED to the seventeenth annual report of the Advisory Council on Scientific Policy\* are the usual tables to be found in recent reports giving details of support of research at universities and colleges of advanced technology by the Research Councils, Government expenditure on civil research, and an analysis of the expenditure and staff of the Research Councils. Figures are also appended for science and mathematics graduate teachers in grant-aided schools and establishments, for first degrees and diplomas awarded in science and technology in 1962-63 and new membership of scientific and technological professional institutions. In view of tendentious questions asked in Parliament, the Advisory Council has also done well to append the memorandum of advice regarding a World Health Research Centre sent to the Minister for Science in February 1964. In addition to topics discussed elsewhere (see p. 319 of this issue), the main report also refers briefly to the International Biological Programme proposed by the International Council of Scientific Unions, and to the position of research in oceanography and marine biology, particularly in the laboratories of the Fisheries Department.

Expenditure by the Research Councils in support of research at universities and colleges of advanced technology is estimated at £12.1 million in 1963-64 and £14.8 million in 1964-65, compared with expenditure from the Exchequer grant to universities through the University Grants Committee of £31 million and £37.1

million, respectively. Of the £12.1 million, £5.2 million was on research grants, £2.5 million on research units within universities and colleges of technology, and £1.6 million on research units associated with them; and £2.4 million on postgraduate studentships and fellowships: for 1964-65 the corresponding figures are estimated as £6.8 million, £2.7 million, £1.57 million, and £2.8 million, respectively. Government expenditure on civil research is estimated at £174 million in 1963-64 and £204.5 million in 1964-65; of these totals, £14.1 million and £15.8 million, respectively, are on agriculture, forestry, fisheries and food; £41.5 million and £49.7 million, respectively, on industry and communications; £7.7 million and £9.7 million on medicine and health; £2.3 million and £2.28 million on overseas research; £52.8 million and £56.9 million on nuclear science; £31.2 million and £37.6 million on universities and learned societies; and £24.6 million and £32.5 million were spent in other ways, mainly through the Ministry of Aviation. Science and mathematics graduate teachers in grant-aided schools and establishments in England and Wales increased from 20,870 in 1962 to 22,121 in 1963; corresponding figures for Scotland being 5,255 and 5,338. In 1962-63, 7,728 first degrees and diplomas were awarded in science faculties and 3,983 in technology faculties, compared with 7,080 and 4,188 in 1961-62; 1,073 diplomas in technology were awarded in 1963-64, compared with 927 in 1962-63 and 620 in 1961-62. New membership of scientific and technological professional institutions increased from 11,057 in 1962 to 12,135 in 1963.

\*Annual Report of the Advisory Council on Scientific Policy, 1963-1964. Pp. vi + 48. (Cmd. 2538.) (London: H.M.S.O., 1964). 4s. net.

## BRITISH AID TO THE WEST INDIES

IN *Aid to the West Indies*\*, Mr. D. J. Morgan surveys attitudes and needs for the Overseas Development Institute seeking to elucidate obstacles to the effective use of British aid. An introductory chapter is followed by successive surveys of Bermuda and the Bahamas; Jamaica, Trinidad and Tobago; British Guiana; and Barbados, the Leeward and Windward Islands. Finally, some proposals are advanced for changes to maintain and increase the effectiveness of Britain's aid, including an immediate conference on the subject. The survey was assisted by a six-week visit to the West Indies during February-March 1964, in which problems of obtaining and using British aid were discussed.

Mr. Morgan found a general feeling in the West Indies that over the past decade or so interest has passed from their problems to those of Africa, and although bilateral aid from the United Kingdom to the West Indies has increased from £4.38 million in 1945-46 to 1956-57 to £14.95 million in 1960-63, in the same period the proportion going to the West Indies has fallen from 10 per cent to 7.45 per cent. Nevertheless, although no single development plan in the West Indies was wholly financed from Colonial Development and Welfare Funds in the late 1950's, four of the plans for 1960-64 were. Research assistance provided through the Department of Technical Co-operation is still predominantly for Africa and the West Indies, with the main emphasis in the fields of natural resources and medicine. Apart from the effect of price changes and, to a limited extent, the collapse of the

Federation, there is little evidence of neglect. However, Mr. Morgan suggests that the relatively high *per capita* income found side by side with high unemployment calls for special assistance. The grant-in-aid system appears to sap local initiative, while there are serious delays in aid administered through London as compared with American and United Nations programmes.

Accordingly, a regional aid office is suggested to administer Britain's contributions and regional programmes in agriculture, medicine, education, technical training and rural credit, and this should be a major item on the agenda for the proposed conference.

The main emphasis of the survey is on such regional schemes and programmes for medicine, agriculture, education, technical training, development banking, etc. While the people of the West Indies receive more aid from Britain than almost any other part of the Commonwealth—£8 million in 1962-63, an annual average of more than £3 a head for the past three years, apart from a substantial subsidy through the Commonwealth Sugar Agreement—more information about the programme in the area would probably make it more effective. Technical assistance to the West Indies in 1961 was £461,400, rising to £713,000 in 1963, compared with bilateral totals for all countries of £15.4 million and £24.3 million, respectively. Mr. Morgan suggests that these figures indicate a relative neglect of the West Indies in technical assistance.

The survey is the first of those from the Overseas Development Institute concerned primarily with the recipient and not the donor aspect of British aid. It lacks something of the clarity which has characterized earlier reports from this Institute.

\*Overseas Development Institute, Ltd., *Aid to the West Indies: a Survey of Attitudes and Needs*. By D. J. Morgan. Pp. 56. (London: Overseas Development Institute, 1964.) 8s. 6d.



## ELECTRICAL DOUBLE LAYER IN SURFACE AND COLLOID CHEMISTRY

AN informal discussion of the Faraday Society on the electrical double layer, particularly in relation to colloid and surface chemistry, was held in the New School of Chemistry of the University of Bristol during September 21-22. The meeting was attended by 129 people, 57 of whom came from industrial organizations and 72 from academic institutions. There were 22 visitors from overseas.

The chair for the session on September 21 was taken by Prof. D. H. Everett (University of Bristol). After a brief welcome to the visitors by Prof. Everett, Dr. R. Parsons (University of Bristol) opened the scientific sessions with a lecture which reviewed the history, and attempted to assess the importance, of the influence of the discrete nature of the charges on the structure of the electrical double layer. He distinguished the effects on the diffuse layer, originally suggested by Frumkin<sup>1</sup> and later developed by Stillinger<sup>2</sup> and Levich and Krylov<sup>3</sup>, from the effects on the compact part of the double layer, which can be traced back to de Boer<sup>4</sup> and which have been worked out in more detail by Esin and Shikov<sup>5</sup>, Ershler<sup>6</sup>, Grahame<sup>7</sup>, Levich<sup>8</sup>, Levine<sup>9</sup>, and Macdonald and Barlow<sup>10</sup>. The uncertainties which exist in these models, that is, the role of the solvent, the effectiveness of the imaging by the outer Helmholtz plane, etc., were mentioned. The importance of devising critical experiments to test these models was emphasized.

Drs. S. Levine (University of Manchester), J. Mingins (Unilever, Port Sunlight) and G. M. Bell (Chelsea College of Science and Technology) discussed, in terms of the discrete ion effect, a number of phenomena at charged interfaces which at present appear inconsistent with the classical Gouy-Chapman-Stern double-layer theory. These authors found that application of the discrete ion theory gave approximately constant values of specific adsorption energy with variation of surface charge, predicted a maximum in the potential at the outer Helmholtz plane with increase in primary charge at fixed ionic strength in the electrolyte, and explained the decrease in concentration of electrolyte required for flocculation of hydrophobic sols with increasing surface charge density. A satisfactory explanation could also be obtained for the phenomenon of mutual antagonism encountered in the flocculation of sols with mixed electrolytes.

The results of potentiometric investigations on the electrical double layer at the silver iodide crystal-solution interface were described by Prof. J. Lyklema (University of Wageningen, Netherlands). The double-layer properties of the silver iodide interface were compared with those of the mercury interface, and the differences and similarities discussed. Adsorption of cations on a negatively charged silver iodide interface was found to be considerably larger than on mercury, probably owing to specific effects. Evaluation of the ionic components of charge at a given potential showed that the adsorption of cations increased in the lyotropic order; a sequence which was also reflected in the flocculation values and in ion exchange. An interesting feature was that the negative adsorption of co-ions did not level off to a given value, as it does on mercury, but passed through a maximum.

Drs. J. J. C. Oomen and Prof. J. Th. G. Overbeek (University of Utrecht, The Netherlands) described measurements of the impedance of silver iodide electrodes, in aqueous solution over a range of  $pI$  and electrolyte concentrations, at frequencies in the range 120 c/s-6,000 c/s. The double-layer capacities of the silver iodide-solution interface obtained using this technique turned out to be very large and dependent on frequency. An

explanation was advanced in terms of surface roughness, and it was shown that on this basis the impedance of the electrode at low frequencies would reduce to a frequency independent series combination of a resistance and a capacity. Experimentally a linear dependence of the square root of the frequency was observed and the differential capacities extrapolated to zero frequency paralleled those obtained with silver iodide suspensions using the potentiometric technique. The ratios of the micro-area to the macro-area were found to be in the range 4-5.

The chairman for the second session, held on September 22, was Prof. J. Th. G. Overbeek. The session commenced with a theoretical paper by Dr. P. H. Wiersema (University of Utrecht, The Netherlands) on the relationship between the electrophoretic mobility and the zeta-potential of a spherical colloid particle. Earlier work on this problem by Overbeek<sup>11</sup> and Booth<sup>12</sup>, which had taken into account both electrophoretic retardation and the relaxation effect, had given approximate analytical expressions for mobility as an incomplete power series in terms of zeta-potential, with coefficients which were functions of  $\kappa a$ ;  $1/\kappa$  is the Debye-Hückel double-layer thickness and  $a$  the radius of the particle. The present work used a numerical solution which corresponds to the complete power series. The differences between the approximations and the present results were appreciable in the region of  $\kappa a$  between 0.2 and 50, and it appeared that the earlier treatments had over-estimated the relaxation effect.

Dr. R. H. Ottewill and Mr. J. N. Shaw (University of Bristol) described an experimental investigation on the electrophoresis of spherical colloidal particles in media of different ionic strengths. Polystyrene latex particles which had been prepared by means of an emulsion polymerization method were used for the investigations; the charge of the particles originated from carboxyl groups on the surface. Five monodisperse preparations were examined with diameters ranging from 600 Å to 4230 Å. Electrophoretic measurements were made using both moving boundary and ultramicroscopic electrophoresis in a range of concentrations of 1:1, 1:2 and 1:3 electrolytes. When examined as a function of  $\kappa a$ , the experimental mobilities gave curves which were qualitatively of the form predicted theoretically by Overbeek<sup>11</sup>, Booth<sup>12</sup> and Wiersema<sup>13</sup>. However, even using the more complete treatment of Wiersema some difficulties were experienced in evaluating zeta-potentials for particles of high mobility with  $\kappa a$  values in the range of 1-10.

Simultaneous measurements of the thickness and conductivity of isolated black foam films were reported by Drs. J. S. Clunie, J. M. Corkill, J. F. Goodman and C. P. Ogden (Procter and Gamble, Ltd., Newcastle upon Tyne). Films having the shape of open rectangular prisms were drawn from solutions of decyltrimethylammonium decyl sulphate, containing sodium bromide (varied from 0.0005 to 0.5 M), and 3-(dimethyl hexadecylammonio)-propane-1-sulphonate, containing sodium bromide (varied from 0.0005 to 1.0 M). The conductance of each film was measured parallel to the surfaces using bright platinum rectangular electrodes and a screened a.c.-bridge. At the limiting film thickness the conductivity measurements indicated an apparent excess of electrolyte in the film core as compared with the bulk solution from which the films were drawn. An explanation of the experimental data was given in terms of Bikerman's theory of surface conductance<sup>14</sup>, and application of Bikerman's equations led to a zeta-potential of about 50 mV for the surface layer/film core interfaces in both systems.

Drs. B. A. Pethica, M. M. Standish, J. Mingins and D. H. Iles (Unilever, Port Sunlight) introduced prelimi-

nary results designed to test the various assumptions made in the thermodynamic theory of the Volta effect. The critical analysis of these assumptions given by Koenig<sup>15</sup> was closely followed. The results were obtained using a vibrating plate electrometer and depended on measuring the compensation potential at the air/electrolyte solution interface in the presence and absence of various spread monolayers. The variation with applied field of the true surface potential ( $\Delta\chi$ ) for some phospholipid monolayers in the zwitterionic form was shown to cast doubt on the general validity of the Bridgman assumption. Within experimental error, the compensation potential does not vary with the separation of the vibrating plate and the solution surface. The Lorentz-Kelvin and Kelvin assumptions though not directly disproved can only be fortuitously correct in particular cases. Tentative proposals for the orientation of water molecules at the solution surface were made.

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## NEW BRITISH ANTARCTIC BASE

A SMALL scientific exploration team from the British Antarctic Survey base at Halley Bay has established a research base in unexplored territory 170 miles south of Halley Bay and about 800 miles from the South Pole.

Sir Vivian Fuchs, director of the British Antarctic Survey, who led the Transantarctic Expedition in 1955, stated recently in London that: "This journey into entirely new territory has not only made possible a valuable contribution to ionospheric studies, but has paved the way for a future journey to link up our mapping programme with the work done further south by the Transantarctic Expedition".

The six-man team, led by Dr. G. Bowra, field leader, and Mr. W. Bellchambers, chief scientist, have established themselves at 77° 57' S., 24° 48' W., and have set up an ionosonde to measure the height and density of electrons in the ionosphere between 60 and 250 miles above the surface. Their main object will be to measure the drift of electron clouds over the Antarctic.

The new station, which will be a summer base, was reached after a difficult and hazardous 13-day journey over the ice field in two snow tractors, towing sledges. One of the sledges carried a 'caboose' housing scientific equipment. The equipment is powered by light-weight Villiers 'Mini-Gen' generators, giving about 1 kW of power.

Mr. Bellchambers heads a team of young scientists, mainly from the University College of Aberystwyth, who

are spending two years in Antarctica as part of the British contribution to international research during 1964-65 (designated International Years of the Quiet Sun).

The area in which the British team is working is of particular scientific interest because it is at a very high geographic latitude (so that the Sun does not set for several months in the Antarctic summer) but at a low magnetic latitude. This means that the relation between the ionosphere, the magnetic perturbations, the aurora and the meteorology of the high atmosphere are relatively simple. Such extreme relations between these factors are not found elsewhere in the world.

The Halley Bay equipment for studying the ionosphere includes ionospheric absorption measuring equipment belonging to the Department of Scientific and Industrial Research, an ionosonde and other equipment, some of which is used in co-operation with the United States base at the South Pole.

The team which established the new base consists of: Dr. G. Bowra, field leader and medical officer, Springfield, Ashted Park, Ashted, Surrey; Mr. W. Bellchambers, senior scientist, 40 Hilltop Road, Whyteleafe, Surrey; Mr. L. Dicken, physicist, Dunedin, 18 Risca Road, Rogerston, Newport, Mon.; Mr. P. C. Goodwin, meteorologist, Haughton House, Humshaugh, Hexham, Northumberland; Mr. G. A. Thompson, tractor driver-mechanic, Hope House Cottage, Ettrick, Selkirkshire, Scotland; Mr. B. Kraehenbuehl, Angaston, South Australia.

## GEOLOGICAL EXPEDITION TO CAPES DYER AND SEARLE, BAFFIN ISLAND, CANADA

By PROF. J. TUZO WILSON, O.B.E.\*, and D. B. CLARKE

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THE occurrence of Upper Cretaceous and early Tertiary lava flows and sedimentary beds on the Atlantic coasts of Scotland, East and West Greenland, and of similar, but younger, rocks on intervening islands led to the expectation that such rocks might be found in North America<sup>1</sup>. Small patches of Tertiary sedimentary rocks have long been known on the coasts of Ellesmere Island and Northern Baffin Island<sup>2</sup>. In 1952, flat-lying

lavas of undetermined age were photographed at Capo Searle at latitude 67° 12' north on the east coast of Baffin Island<sup>3</sup>. They were later briefly described<sup>4</sup>.

This discovery has special significance in view of A. Wegener's hypothesis<sup>5</sup> that Greenland and northern Canada were separated by the spread of Baffin Bay accompanied by transcurrent faulting along the straits between Ellesmere Island and northern Greenland, and J. T. Wilson's corollary suggestion<sup>6</sup> that a branch of the

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North Atlantic Ridge should extend up the centre of Baffin Bay. If this is true, then the volcanic rocks at Cape Searle should once have been in contact with the extensive areas of Upper Cretaceous and early Tertiary sedimentary and volcanic rocks which have been described by S. Munck and A. Noe-Nygaard<sup>7</sup> and others on the west coast of Greenland in the vicinity of Disco Island.

Examination of air photographs by D. B. Clarke, a graduate student, revealed about 20 separate patches of lava along the 55 miles of coast between Cape Searle and Cape Dyer. None appeared to be more than 1 square mile in area, and all were close to the sea.

On July 14 a field party, supported by a grant from the Department of Northern Affairs and National Resources, Canada, flew to Baffin Island with the object of searching for fossiliferous strata, mapping the stratigraphy and geological structures of the coastline, and collecting oriented samples of the lavas for palaeomagnetic examination and isotopic age determinations.

After spending a week examining on foot the lavas on top of cliffs at Cape Dyer, the party flew to Broughton Island, 30 miles north of Cape Searle, and travelled south along the coast in Eskimo whaleboats. One of us (J. T. W.) returned on August 1, but D. B. Clarke, assisted by J. Dodds, spent two months in the field.

The coastline consists of a series of high, exposed head-lands separated by deep fiords and valleys. The flat-lying lavas were poured out on an earlier, irregular surface. Their contact with underlying Precambrian gneisses ranges from below sea-level to 1,700 ft. above sea-level, the average elevation being 1,300 ft., but at Cape Searle and on Padloping Island, where the lavas reach sea-level, several large normal faults were observed to strike parallel to the coast. Along them, either the coastal margin has been downfaulted or the interior uplifted. The outcrops of lava are up to 1,400 ft. thick and characteristically form prominent, vertical, seaward-facing cliffs. Single flows are usually about 10 ft. thick, but a few are as much as 150 ft. Zeolites were observed in many of them. Some flows were seen to terminate

abruptly, and correlation of sections between different outcrops is not easy.

No dykes were seen cutting Precambrian rocks, and those few observed in the lavas were very fine-grained with strikes roughly parallel to the coastline and nearly vertical dips.

In several localities, the base of the lavas rests on or is interbedded with sedimentary rocks up to 500 ft. thick. In some places, these sandstones, shales and conglomerates contain many fossil plants.

Dr. W. A. Bell, formerly director, Geological Survey of Canada, has made a quick, preliminary examination of a small sample of the material collected. His tentative conclusion, which he has allowed us to quote, is as follows: "The assemblage is all too meagre for reliable conclusion as to age other than a horizon within confines of Upper Cretaceous-Eocene inclusive. I do favour somewhat greater probability of a Palaeocene than of a late Upper Cretaceous or other ages".

This winter it is hoped that the full collection of fossil material can be examined and described. Petrographic investigations, potassium-argon age determinations and a comparison of the geological succession with those in West Greenland will be undertaken. It is hoped to continue field work next summer.

Except for a short period in August, field work was made difficult by heavy snow on the upland and sea ice obstructing passage around the head-lands or across bays.

We thank those who supported and assisted the expedition, especially Mr. John Macdonald, Hudson's Bay Co., Broughton Island, and the Eskimo boat crews under Pauloosie.

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## POTASSIUM-ARGON AGE DETERMINATIONS FROM THE LEWISIAN OF GAIRLOCH, ROSS-SHIRE, SCOTLAND

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RECENT geochronological work on the Lewisian of the Scottish mainland from Assynt to Durness<sup>1-5</sup> has firmly established the ages of four major events—the Scourian metamorphism<sup>6</sup> (about 2,600+ m.y.), the Inverian metamorphism<sup>2,3</sup> (about 2,200 m.y.), the intrusion of a series of basic dykes (2,200 m.y.) and the Laxfordian metamorphism<sup>6</sup> (1,600–1,500 m.y.).

The later Lewisian ages obtained in this northern area show a marked spread. Giletti, Moorbath and Lambert<sup>1</sup> base their estimation of the date of the Laxfordian metamorphism mainly on two pegmatites, one in South Harris and the second, cutting a dyke in Scourian rocks, in Scourie, which give muscovite and feldspar ages in the range 1,650–1,550 m.y. In contrast, the gneisses and pegmatites of the 'type' Laxfordian area from Laxford to Durness give ages ranging from 1,530 to 1,280 m.y.

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with a mean of 1,430 m.y. These younger Lewisian ages were considered to be due to mild reconstitution of Laxfordian (1,600–1,500 m.y.) rocks occurring later than about 1,100 m.y. and possibly of Caledonian age. The possibility that the Laxfordian metamorphism continued throughout the period represented by the spread in determined 'Laxfordian' ages was rejected, but the alternative possibility, that two separate metamorphic events are represented, was not considered.

Four samples from the Gairloch area were dated in this preliminary attempt to correlate its structural history<sup>7</sup> with that of the Lewisian to the north.

The geochronology of the Lewisian south of Assynt is relatively poorly known. One date is available for gneiss at Gruinard Hill, north of Gairloch (1,510 m.y.), two for muscovite-schist at Letterewe on Loch Maree, just east of Gairloch (1,510 and 1,540 m.y.), and two for gneisses at Loch Torridon, south of Gairloch (1,300 and



1,160 m.y.). According to the scheme proposed by Sutton and Watson<sup>6</sup> the first of the Loch Torridon samples is Scourian (presumably modified in the Laxfordian) and the remaining samples are Laxfordian, modified in the case of the Loch Torridon samples by reheating after 1,100 m.y., probably during a period of crushing<sup>1</sup>. The identification by Sutton and Watson of Scourian gneisses at Loch Torridon, and the supposition that the remaining gneisses of the southern Lewisian were originally also Scourian but have been reconstituted during the Laxfordian, thus find neither confirmation nor contradiction in the age determinations as yet, and depend on the correlation of basic dykes and sheets in this area with the Scourie dyke swarm of the north.

A detailed sequence of events, based on structural evidence, recently proposed by Park<sup>7</sup> for the Gairloch area differs from the Sutton and Watson chronology in several respects. A fragment of a presumed basement complex (the Ialltaig gneisses) metamorphosed in granulite facies is recognized. The surrounding rocks, considered to be later sediments, together with the Ialltaig rocks, are affected by three later tectonic episodes (early, main and late phases). Certain dykes which Sutton and Watson correlate with the 2,200 m.y. Scourie dykes here post-date the foliation of the main phase and are affected by the late phase. Both phases were accompanied by metamorphism, but it is not yet known whether two quite separate episodes occurred, separated by dyke intrusion, or whether metamorphism continued during the period of dyke intrusion as has been suggested for dykes in Scourie<sup>8</sup> and Assynt<sup>9</sup>. The metasomatism shown by the gneisses also post-dates the main phase, but again it is not known whether or not the two episodes are part of a continuous process. It is probable that metamorphism also accompanied the early phase, but any mineralogical effects of this episode are likely to have been swamped by the pervasive main-phase metamorphism.

GA 150/6 from the Ialltaig gneisses is a garnet-clinopyroxene-hornblende-plagioclase rock, with granulitic texture. Hornblende, the only mineral bearing significant amounts of potassium, is present in two varieties. The dominant variety is strongly pleochroic with the scheme X-pale yellow, Y-grass-green, Z-brown. It is partly replaced by a less pleochroic yellow-green to bluish-green variety. Both kinds partially replace pyroxene. The retrogressive alteration from granulite facies to epidote-amphibolite-facies can be correlated on structural grounds with the main phase<sup>7</sup>. The blue-green hornblende appears to be the typical main-phase variety, but the date of origin of the older brown variety is uncertain.

GA 39/3 from a basic sheet in the schists is a hornblende-oligoclase-quartz-schist, very fine grained, with extremely well-developed foliation caused by planar alignment of hornblende and by feldspar-hornblende banding. Quartz is a minor constituent. The potassium is effectively concentrated in the hornblende which is an actinolitic variety, pleochroic according to the scheme X-very pale yellow, Y-yellow-green, Z-bluish green. The foliation and the hornblende can be correlated with the main phase.

GA 114/8 from a thin basic sheet in the gneisses is a hornblende-oligoclase-epidote-quartz-amphibolite, with poorly-developed foliation caused by planar alignment of hornblende. Quartz and epidote are present in relatively minor proportions. Again, the potassium can be assumed to be concentrated in the hornblende, which has strong pleochroism (X-pale yellow, Y-deep yellow-green, Z-blue-green). The foliation of the sample originated in the main phase, but the surrounding gneisses have been affected by later metasomatism which presumably also caused some modification in the amphibolite.

GA 153/7 from a metamorphosed post-main-phase basic dyke is a hornblende-plagioclase-epidote-quartz-amphibolite. The hornblende and the plagioclase-epidote occur in aggregates pseudomorphing the original igneous crystal pattern of rather coarse grain. A few

altered biotite laths occur streaked with opaque brown material (probably iron ore) and associated with magnetite and sphene. The hornblende is moderately pleochroic (X-pale greenish-yellow, Y-yellow-green, Z-blue-green) and forms complex intergrowth patterns. The epidote-amphibolite-facies recrystallization of the sample is assigned to the late phase. The hornblende and the alteration of the biotite thus date from this episode.

The determinations of potassium and argon were carried out on whole-rock samples (120 mesh). Potassium was determined with a flame photometer using an addition dilution method<sup>2</sup>. Argon was extracted by fusing a weighed sample under high vacuum in a Mo crucible with a radio frequency induction heater, and was measured by standard isotope dilution techniques on a Renolds-type mass spectrometer. The decay constants used were  $\lambda_e = 0.584 \times 10^{-10} \text{ yr}^{-1}$  and  $\lambda_\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$ .

The results of this preliminary investigation (Table 1) indicate: (1) that one phase of recrystallization of the Ialltaig granulites can be attributed to a metamorphic episode between 1,600 and 1,500 m.y. which agrees with the three previous Rb-Sr dates from north of Gairloch and with the date of the 'type' Laxfordian of Laxford; (2) that at least one post-1,600 m.y. thermal event affected the Gairloch area. The 1,400 m.y. age is in good agreement with rubidium-strontium determinations on Laxfordian gneiss from Laxford and with gneiss and pegmatites from the Hebrides, and adds to the growing body of evidence indicating a distinct and widespread thermal event younger than the Laxfordian *sensu stricto*, but distinct from, and much older than, the reheating episode of Giletti *et al.*<sup>1</sup>. This event may correspond to the late phase of metamorphism in Gairloch.

Table 1. WHOLE-ROCK POTASSIUM-ARGON AGES OF ROCKS FROM GAIRLOCH

Sample No.	Description	<sup>40</sup> Ar rad. s.o.c./g $\times 10^{-4}$	% <sup>40</sup> Ar rad.	K, 0%	Age m.y.
GA 150/6	Garnet-pyroxene-hornblende-granulite	0.2154	92.4	0.275	1,530 $\pm$ 60
GA 39/3	Hornblende-schist	0.1785	89.2	0.258	1,400 $\pm$ 60
GA 114/8	Amphibolite	0.8079	95.9	1.389	1,240 $\pm$ 50
GA 153/7	Metamorphosed dyke	0.2739	92.4	0.538	1,120 $\pm$ 45

Location of samples:

GA 150/6: From the Ialltaig granulitic gneisses<sup>1</sup> of Ard Ialltaig, Loch Gairloch, locality NG 805736.

GA 39/3: From the Aundray basite<sup>2</sup>, one of a group of basic sheets associated with the Gairloch metasedimentary schists, locality NG 850720.

GA 114/8: From one of a group of amphibolite sheets in the Buainchean gneisses<sup>3</sup> of Gairloch, locality NG 861730.

GA 153/7: From one of the metamorphosed basic dykes forming the South Sithean Mor dyke complex<sup>4</sup>, locality NG 813716.

See Park<sup>7</sup>: (1) 401 and Plate 34; (2) 404 and Fig. 2; (3) 399 and Fig. 2; (4) 405 and Plate 34.

The two younger ages, however, may represent argon loss during a mild reheating at or later than 1,120 m.y. It may be significant that this date is close to the minimum age for this part of the Lewisian by Rb-Sr (1,160 m.y.).

The tentative dates of the metamorphic episodes in Gairloch are summarized in Table 2. Until further age-determinations are available it is not possible to correlate definitely the events at Gairloch with those of the northern Lewisian.

Table 2. TENTATIVE CHRONOLOGY OF METAMORPHIC EPISODES AT GAIRLOCH

1. Ialltaig metamorphism:	Certainly older than 1,530 m.y. Possibly = Scourian (2,600+ m.y.).
2. Gairloch early-phase metamorphism:	Certainly older than 1,400 m.y. Possibly about 1,530 m.y. (also = muscovite dates from Loch Maree) Possibly 2,200 m.y. (Inverian)
3. Gairloch main-phase metamorphism:	Certainly at least 1,400 m.y. Possibly = 1,530 m.y. (but could be much older).
4. Intrusion of South Sithean Mor dykes:	Certainly older than 1,120 m.y. Possibly younger than 1,530 m.y. (but could be older if (3) is older).
5. Gairloch late-phase metamorphism:	Certainly at least 1,120 m.y. Possibly younger than 1,240 m.y. (but could be older if (6) occurred).
6. ? Reheating episode:	Younger than 1,120 m.y. (alternative explanation of younger ages postulated by Giletti <i>et al.</i> <sup>1</sup> ).

We thank Prof. L. R. Wager and members of the Geological Age and Isotope Research Group at Oxford for making equipment available for the age determinations. We also thank Mr. D. C. Rex for performing the analyses.

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## POTASSIUM-ARGON AGE MEASUREMENTS ON TWO IGNEOUS ROCKS FROM THE ORDOVICIAN SYSTEM OF SCOTLAND

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THE time of occurrence of the Ordovician system<sup>1-3</sup> is not precisely known, and it was the purpose of the investigation recorded here to provide additional reference points. Table 1 summarizes pertinent data obtained by other workers.

Table 1

Locality	Rock	Mineral	Method	Date (m.y.)	Ref.
Upper Caradocian Kinnekulle, Sweden	Bentonite	Sanidine	K—Ar	452	4
		Biotite	Rb—Sr	447	
Middle Ordovician, Tennessee	Bentonite	Zircon	<sup>238</sup> U	447 ± 10	5
Middle Ordovician, Alabama	Bentonite	Zircon	<sup>207</sup> Pb	455 420 447 477 481	3
		Biotite	K—Ar		
		Biotite	Rb—Sr		
Middle Ordovician, Quebec	Granite	Muscovite	K—Ar		
	Granite	Muscovite	K—Ar	481	6

When interpreting these results it should be noted that the zircons referred to gave discordant U—Pb ages and that the K—Ar age of biotite from the Alabama tuff is distinctly below the Rb—Sr age. The latter effect is probably the result of slight loss of argon from the biotite, possibly as the result of some later event.

In this work the ages of two igneous masses are reported. The Ballantrae complex in Ayrshire and the Bail Hill volcanics of Dumfriesshire have both been assigned to the Ordovician on geological grounds and both include suitable biotite-bearing formations.

**Ballantrae Igneous Complex.** The Ballantrae Igneous Complex is made up of spilitic lavas and agglomerates together with serpentines and other basic and acidic intrusives. It is unconformably overlain by a Lower Caradocian conglomerate containing pebbles derived from the igneous rocks. It has been described by Peach and Horne<sup>7</sup>, Balsillie<sup>8,9</sup>, Anderson<sup>10</sup>, Pringle<sup>11</sup> and others. A review of previous work is given by Bailey and McCallien<sup>12</sup>.

The serpentine occurs in association with spilitic lavas of Middle Arenig age, and a similar age has been proposed for the whole complex by Peach and Horne<sup>7</sup> and by Anderson<sup>10</sup>. Bailey and McCallien<sup>12</sup> suggest that the serpentine originated as a submarine lava which forms a single sheet interposed between a lower and upper lavas series of Arenig age. Although these interpretations have been questioned by Balsillie<sup>8,9</sup> and Pringle<sup>11</sup>, further evidence of the age is given by the fact that the whole complex, including the Middle Arenig sediments, is typical of the spilitic-radiolarite-serpentinite association ('Steinmann Trinity') occurring in geosyncline belts in many areas.

Within the serpentine outcrop are a series of small exposures of gabbro, some of which are deformed and others thermally metamorphosed but undeformed. These

are thought to be broadly contemporaneous with the serpentine and are most probably intrusives<sup>12</sup>.

Samples of the gabbro were collected from a small outcrop 1½ miles north of Colmonell (Ayrshire). The rock is fresh and undeformed, although a slight schlieren structure is present which probably indicates mild pressure after emplacement. Thin-section examination shows it to consist of plagioclase feldspar, hornblende, pyroxene and biotite.

In order to separate the biotite, approximately 1.0 kg of the gabbro was crushed to less than 72 mesh B.S. In view of the similar magnetic properties of the biotite and hornblende in the rock, it was not possible to use direct magnetic separation; instead, flotation was used. As a necessary preliminary to the flotation process, which is inhibited by very fine-grained material, the crushed product was scrubbed and the resulting slime removed by decantation. The clean sample was then conditioned for 0.5 min in a 1-kg flotation cell at pH 3.5, using an amine collector and an alcohol frother. Air was admitted to the cell for 2 min and a good concentrate of biotite was obtained from the froth. After washing and drying, the concentrate was finally cleaned on a Frantz magnetic separator. Microscopic examination of the concentrate confirmed that it consisted of biotite with impurity contamination of less than 1 per cent.

**Bail Hill Volcanics.** The Bail Hill lavas, agglomerates and minor intrusions represent the site of an Ordovician volcanic pile which has since been subjected to regional folding. They have been described in detail by Peach and Horne<sup>7</sup>, who concluded that the volcanics were erupted mainly during Arenig times, as Arenig cherts are found in contact with the lavas. Furthermore, they point out that some volcanics are interstratified with shales containing Glenkiln graptolites (L. Caradocian) and suggest the possibility of this age for some of the late outpourings. More recently, Mrs. V. A. Eyles<sup>11</sup> has shown them to be later than the associated Upper Glenkiln shales. However, Pringle<sup>11</sup> has assigned all the Bail Hill volcanics to a Glenkiln horizon.

Among the Bail Hill lavas lies a mica andesite, samples of which were collected from an exposure in Grain Burn on the western slopes of Bail Hill approximately half a mile north-east of Kirkconnel, Dumfriesshire. A description of the outcrop together with sections across it is given by Peach and Horne<sup>7</sup>.

In thin section the mica andesite, which is vesicular, is seen to consist essentially of large phenocrysts of biotite and feldspar approximately 0.5–1 mm in length set in a fine-grained ground-mass. The biotite is pleochroic dark green to light green and shows the usual birefringence and characteristically small 2V angle, while the ground-mass is made up of very fine biotite and feldspars, though the

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fineness of grain makes precise identification difficult. Opaque minerals, probably iron oxides, are present to the extent of 5–10 per cent.

Preparation of pure concentrates of biotite was carried out using a Frantz magnetic separator.

Potassium-argon age determinations made using the isotope dilution technique and flame photometry have been carried out on the separated mica fractions in Oxford, Cambridge and Toronto. Results are shown in Table 2. The close agreement among the results obtained by the different workers in different laboratories indicates that experimental errors in the ages found are definitely less than  $\pm 5$  per cent.

Table 2. RESULTS OF K/Ar DATING

Sample	Grain	Lab.	$\lambda_p = 4.72 \cdot 10^{-10} \text{ yr}^{-1}$		$\lambda_a = 0.584 \cdot 10^{-10} \text{ yr}^{-1}$		$^{40}\text{Ar}$ p.p.m.	Apparent age (m.y.)
			K%	Atmos. %				
Biotite from Bail Hill mica andesite	Coarse	T	3.44	17.8	0.126		455	
	Coarse	O	3.39	2.0	0.121		455	
	Coarse	C	3.31	10.7	0.116		437	
	Fine	C	3.55	6.5	0.125		440	
Mean age of Bail Hill andesite = 445 m.y.								
Biotite from Colmonell gabbro	Coarse	T	5.44	36.0	0.213		483	
		C	5.34	1.5	0.203		467	
	Coarse	T	4.98	10.3	0.192		476	
	Coarse	C	4.85	2.7	0.186		477	
	Fine	T	6.13	3.0	0.238		479	
Mean age of Colmonell gabbro = 475 m.y.								

T, Toronto; C, Cambridge; O, Oxford.

In assessing the reliability of the ages it is important to consider the possibility of argon loss from the micas due to their subsequent geological history. Thus, the Colmonell biotites may have been modified by a pre-Caradocian metamorphism, but as this probably occurred immediately after emplacement it is unlikely to have significantly affected the present argon content. The

widespread Caledonian earth movements which occurred approximately 415 million years ago<sup>12</sup> could conceivably have caused some argon loss from both the Colmonell and Bail Hill micas. Also the Bail Hill micas, which are green in colour and have a low potassium content, have been affected by hydrothermal activity or by weathering, which again could result in some argon loss.

Should these or other less-obvious events have affected the argon content of the micas, then our data would give minimum ages for these rocks.

We conclude that our results give reasonable grounds for assigning minimum ages of 445 million years to the Lower Caradocian and 475 million years to the Arenig. These new measurements and our interpretation are consistent with the data in Table 1.

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## LATIN SQUARE AS A NETWORK MODEL OF RANDOM PACKING

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THE analytical description of fluid flow and retention in porous media has been inadequate because of the complexity of pore geometry, while physical models, such as bundles of capillary tubes, have been oversimplified<sup>1</sup>. Fatt<sup>2</sup> recognized the stochastic nature of the replacement of one fluid in a porous mass by another, and proposed as a model a network of tubes arranged randomly in a matrix, with radii conforming to a predetermined frequency distribution. While the number of tubes was limited to 200–400, a test sample may contain 40,000 pores; nevertheless, the results with the 400 tube network were considered not seriously in error despite the 1 per cent sample size. Later authors report somewhat larger models<sup>3</sup> with computer analysis<sup>3,4</sup>. Monte Carlo methods were used to evaluate the emptying–filling sequence; the procedure will be given later.

Two of the variables examined by Fatt were tube radius distribution, and the number of tube interconnexions in the network. The conclusions were that the capillary pressure curves were more sensitive to changes in tube radius distribution than to changes in network. For low dispersion all networks have similar capillary pressure curves, while high dispersion leads to different curves for each network. Only one entry–exit configuration was used, the wetting phase being considered to escape from the network as a film on the pore walls. Later workers introduced entrapment where an encroaching fluid cannot displace an occupying fluid if no pore flow path is available for its escape.

The object of the present investigation was to study the effect of the entry–exit configuration on the capillary

potential curve, if possible by somewhat simpler matrix models and pencil and paper methods than the post-Fatt computer procedures.

### Network Model

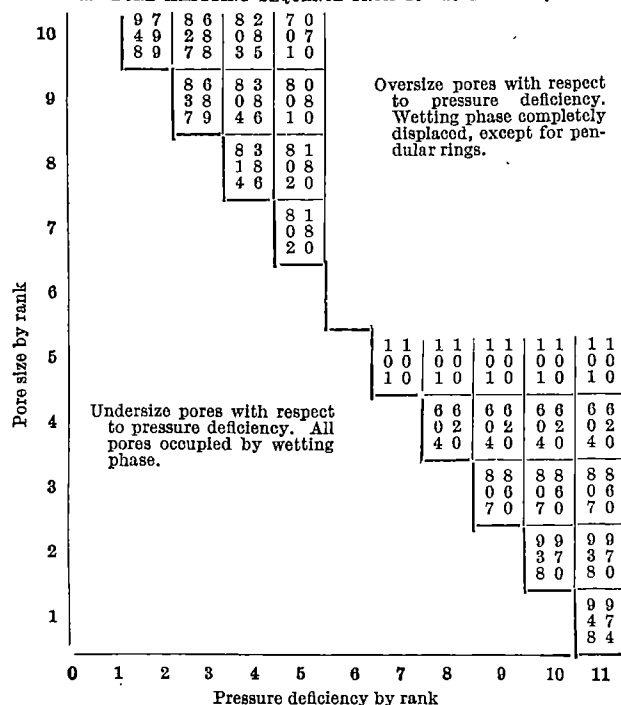
If any plane or any straight line is drawn through a random packing, the distribution of pore sizes so sectioned should be a representative sample of those in the packing. Consider the Latin square; the rows and columns (but not the diagonals) display these attributes in two dimensions accurately and with economy.

10 × 10 Latin squares were constructed by randomizing the columns and rows of a typical square<sup>5</sup>. Some selection was exercised, however, in rejecting squares having runs of successive integers, or of the same integer along diagonals.

*Procedure.* The imaginary apparatus and experimental procedure corresponded to the capillary potential experiment<sup>6</sup>. The square was completely occupied by the wetting phase (for example, water). A membrane which could conduct only the wetting phase (for example, a fine-pored sintered glass disk) was connected to certain edges, the remaining edges being connected to a reservoir containing the displacing phase (for example, air). Thus, as the wetting phase empties, the displacing phase takes its place. Increments of suction could be applied externally and conducted to the model through the membrane. The increments were graded by rank 1, 2, 3 . . . , so that pore size 10 could just empty by suction rank 2 (that is, pore 10 is oversize with respect to the applied suction rank 2), pore size 9 by suction rank 3, and so on. Each



Table 1. PORE EMPTYING SEQUENCE FROM 10×10 LATIN SQUARES



Average number of pores of stated rank still containing wetting phase after the application of stated pressure deficiency. The remaining pores contain pendular rings of wetting phase, and the displacing phase.

Key. Stepped line represents bundle-of-capillary-tubes model and divides the régime into oversize and undersize pores with respect to the applied pressure deficiency.

a	b
d	c
e	f

a, Displacing phase entering one edge. Wetting phase exiting from the opposite edge. b, Displacing phase entering three edges. Wetting phase exiting from one edge. c, Displacing phase entering one edge. Wetting phase exiting from three edges. d, Torus arrangement. Displacing phase entering about half the total number of pores. Wetting phase exiting from the remainder. e, Displacing phase entering two edges. Wetting phase exiting from two edges. f, Emptying sequence for fitting experimental data.

pore was connected to its eight neighbours, excepting the edge pores. Four connexions, along rows and columns, would appear more in keeping with the properties of the Latin square but not, however, with the complexity of a packing. An oversize pore will empty if both a complete wetting fluid path to the membrane and a complete displacing fluid path to the reservoir exist. Entrapment occurs when pores containing the occupying phase are surrounded by pores containing the displacing phase; edges and corners remote from the membrane are specially vulnerable. Other things being equal, with two pores of the same rank, the pore nearest the membrane was assumed to empty first, thus imposing a degree of selection on the model based on relative permeability concepts.

Having defined a set of rules, the ensuing desaturation sequences were obtained in the manner of a game (resembling, in some respects, the Japanese game 'Go'). The number of pores in the oversize ranks which still remained occupied after the effect from each increment of suction had proceeded to completion were counted; the results for various entry-exit arrangements are given in Table 1. The values shown are averages rounded to an integer, and here some judgment was exercised. Thus, considering oversize pores, it is possible that equal numbers of these will remain occupied (for example, pressure ranks 3 and 4, sequence (a) and (c)); however, on average it is not likely that more higher than lower rank pores will remain full. This criterion was used when rounding off.

The different arrangement (d) was designed to simulate a thin bed, the torus configuration being used to avoid edge effects. To deal with this apparently more difficult

case it is only necessary to repeat the left-hand column of the square on the right-hand side and the top row at the bottom, and vice versa to obtain cyclic continuity in both directions.

**Results.** Three conclusions may be drawn from Table 1. (1) At pressure deficiency rank 6 no oversize pores remain occupied by the wetting phase for all the different entry-exit arrangements investigated. All have at least this one point in common. (2) For pressure deficiencies below rank 6 the distribution of occupied oversize pores depends on the entry arrangement. The pattern is set at the 'entry pressure'; the curves merge towards the common point with no crossing over. (3) For pressure deficiencies higher than rank 6 entrapment occurs. The distribution of these occupied oversize pores depends on the exit arrangement; the curves diverge from the common point with no crossing over.

Thus, to single out just one factor for comment, the 'entry pressure' was dependent on the entry arrangement. Although interpretations of the present two-dimensional model in terms of a capillary potential experiment are uncertain, small thin samples would be expected to exhibit a somewhat lower entry pressure than thicker samples<sup>7</sup>.

The emptying sequence ceased when all the pores in contact with the membrane were empty. (In filtration the cake closest to the cloth is often the driest.) Had these remained full—that is, conducting—until otherwise unisolated pores elsewhere had emptied, a higher proportion of the wetting phase would have been withdrawn.

### Capillary Potential Curves

To display the emptying sequences of Table 1 as capillary potential curves, a pore-size frequency distribution will be considered and will be compared with the results of experiments on packings of equal spheres. This is strictly for purposes of illustration; it is not yet clear how to interpret such comparisons even if they are meaningful.

Fatt assumed cylindrical pores and reasoned that lengths were inversely proportional to radii; consequently

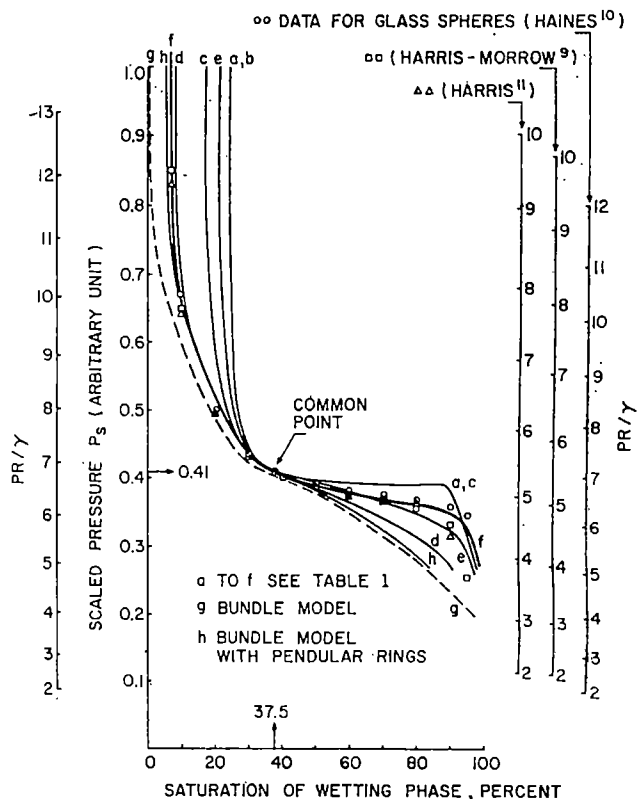


Fig. 1. Capillary potential curves derived from Latin square model

pore volume is proportional to radius. The pressure deficiency required to empty a pore was assumed inversely proportional to radius, in accordance with Laplace's equation (1) (Table 4). For the present purpose, Fatt's general relationships will be accepted, except that pore size (that is, radius) will here be interpreted as pore volume; there is no numerical difference on this account, only a change of scale because all Fatt's data were given in arbitrary units. A physical interpretation will be attempted by considering a packing of equal spheres (radius  $R$ ). Thus, scaled volume,  $\bar{V}_s = cV/R^3$  where  $V$  = pore volume and  $c$  is the scaling factor. Scaled pressure,  $\bar{P}_s$ , is a function of the dimensionless group  $PR/\gamma$ , where  $P$  is pressure deficiency, and  $\gamma$  is interfacial tension. Percentage frequency of pores of any specified size is  $f$ . Primary data similar to Fatt's first of three distributions (his Fig. 5) are given in Table 2; this is a moderately narrow distribution with slight positive skew. Considered as a bundle-of-capillary-tubes model, Table 2 leads to the capillary potential curve ( $g$ ) in Fig. 1.

To apply the desaturation sequences of Table 1, the primary data must be replaced with equivalent data expressed as a distribution of ten pore sizes of equal frequency. This is obtained by interpolation from a plot of  $\Sigma fV_s$  against  $\Sigma f$  at 10 per cent intervals giving equivalent scaled volumes,  $\bar{V}_s$ . The equivalent scaled pressure,  $\bar{P}_s$ , is best obtained from Fig. 1, curve ( $g$ ) interpolated from values of  $\bar{V}_s$  interpreted as a bundle-of-capillary-tubes model.  $\bar{V}_s$  and  $\bar{P}_s$  (Table 3) are consequently a set of data which can, when treated as a bundle-of-capillary-tubes model, generate substantially the same curve as the primary data.

**Pendular rings.** Fatt intentionally neglected all forms of residual wetting phase entrapment, while later authors<sup>3,4</sup> considered entrapment only in pores. The contribution of pendular rings to residual wetting phase saturation

has generally been neglected in network models. For equal spheres, the relationship between pendular ring volume,  $v$ , and the associated pressure deficiency has been tabulated<sup>5</sup>, while measurements indicate that the pressure deficiencies are substantially equal to the entry pressure for the pore<sup>6</sup>. Knowing the number of points of contact per sphere for the different regular packings, the saturation on account of pendular rings can be calculated (Table 4). (Theoretical pendular ring saturation cannot exceed 24.3 per cent for rhombohedral packing and 18.2 per cent for cubic packing<sup>8</sup>.)

With random packings of smooth equal hydrophilic spheres, the residual pendular ring saturation  $\Sigma v/\Sigma V \approx 4$  per cent. The remaining residual fluid  $\sim 3$  per cent is entrapped in pores, together with some possibly occurring as a non-conducting surface film.

The volume of pendular wetting phase associated with a pore depends on the throat radius, this in turn being related to the pore volume; the approximate relationships for the model assumed here are given by equations (2) and (3) and (6)–(8). However, because scaled values are being used, a linear relationship between pore and pendular ring volume will be assumed as a first approximation giving values intermediate between equations (6) and (7), and equation (8). Thus, the same scale ratio applies to both pendular rings and pore volume; that is:  $v_s/\bar{V}_s = v/V$ . A value of 4.2 per cent saturation for all pendular rings will be assumed, which is not unreasonable for packings of smooth rounded particles<sup>9</sup>.

**Theoretical curves.** The capillary potential curve derived from a bundle-of-capillary-tubes model with the addition of residual pendular rings is plotted in Fig. 1, curve ( $h$ ), and the sequences in Table 1 with pendular rings are shown as curves ( $a$ ) to ( $e$ ). Curve ( $b$ ) up to the common point is displaced about 2 per cent units to the left of curve ( $e$ ); it is omitted to avoid congestion. All the curves were

Table 2. PORE SIZE DISTRIBUTION  
Primary data. (Arbitrary units)

Scaled volume $\bar{V}_s$	0.5	1.0	1.4	1.8	2.4	2.6	2.9	3.2	3.5	3.9	4.5	4.9
Frequency % $f$	2.0	5.0	17.0	22.0	18.0	10.0	8.0	6.0	4.0	4.0	2.0	2.0
Scaled pressure $\bar{P}_s$	2.0	1.0	0.714	0.556	0.417	0.385	0.345	0.313	0.286	0.256	0.222	0.204

Table 3. PORE SIZE DISTRIBUTION  
Equivalent data. (Arbitrary units. Frequency, 10 per cent)

Scaled volume $\bar{V}_s$	1.0	1.4	1.6	1.8	2.2	2.3	2.6	2.8	3.2	4.1
Scaled pressure $\bar{P}_s$	2.0	0.69	0.59	0.51	0.45	0.41	0.39	0.36	0.32	0.27

Table 4. PARAMETERS OF RHOMBOHEDRAL AND CUBIC PACKINGS OF EQUAL SPHERES RADIUS  $R$

Packing	Porosity (%)	No. of points of contact ( $N$ )	Dimensionless throat radius ( $r_t/R$ )	Dimensionless pore-body radius ( $r_b/R$ )	Dimensionless pore volume ( $V/R^3$ )	Dimensionless entry pressure for desaturation ( $P_t R/\gamma$ )	Dimensionless entry pressure for imbibition ( $P_b R/\gamma$ )	Pendular ring saturation ( $v/V\%$ )
Rhombohedral	25.95	12	0.154	0.414*	1.052*	13.0	4.8	5.5; 0†
Cubic	47.04	6	0.414	0.732	3.81	4.8	2.7	4.2

\* Concave cubic part of pore. The two concave tetrahedral portions (vol. 0.209  $R^3$  each) can become isolated before they can empty. If this occurs there will be no pendular rings† contained in the rhombohedral pore; the range  $v/V = 5.5$ –0 per cent should cover all contingencies from random to regular packings: these values are assumed in equations (6) and (7), and equation (8) respectively.

Equations (2) to (7) are obtained from the above table assuming an equation of the form  $y = ax^n$ . All equations represent first approximations for random packings of equal spheres.

$$PR/\gamma = 2/\frac{r}{R} \quad (1)$$

$$V/R^3 = 12.0[r_b/R]^{1.25} \quad (2)$$

$$V/R^3 = 7.7[r_b/R]^{1.25} \quad (3)$$

$$P_t R/\gamma = 13.75[V/R^3]^{-0.775} \quad (4)$$

$$\frac{P_b R}{\gamma} = 1.11 \left[ \frac{P_t R}{\gamma} \right]^{0.67} \quad (5)$$

$$\frac{v}{V} \% = 5.6 \left[ \frac{V}{R^3} \right]^{-0.22} \quad (6)$$

$$\frac{v}{R^3} = 0.77 \left[ \frac{P_t R}{\gamma} \right]^{-1} \quad (7)$$

$$\frac{v}{V} \% = 1.52 \frac{V}{R^3} - 1.6 \quad (8)$$

} Based on cubic packing,  
and on cubic part of  
rhombohedral pore.

} Based on rhombohedral packing;  
pendular ring saturation = 5.5.

} Based on rhombohedral packing;  
pendular ring saturation zero†.

computed from the pore size distribution given in Table 3. The marked effect of the entry-exit arrangements is clearly displayed. A wide range of composite curves can be generated by selecting one sequence up to the common point, and another sequence for the remainder of the curve.

**Comparison with experiment.** All curves have a common point at pore rank 6 (saturation,  $S = 37.5$ ), force rank 6 (scaled pressure,  $P_s = 0.41$ ); this provides a point for purposes of comparison between calculated and experimental data. As a consequence of working with scaled values, the saturation axis remains unchanged. To express the scaled pressure in terms of the dimensionless group  $PR/\gamma$ , substitute  $V_s = cV/R^3$  into equation (4) whence:

$$\frac{P_t R}{\gamma} = 13.75 \left[ \frac{c}{V_s} \right]^{0.775}$$

but  $V_s \propto 1/P_s$ , hence:

$$\frac{P_t R}{\gamma} = 13.75 [c_m P_s]^{0.775}$$

where  $c_m$  is a modified scale factor.  $PR/\gamma$  for  $c_m = 1$  is plotted with  $P_s$  on the left-hand axis of Fig. 1 for comparison purposes. Three sets of data for glass spheres<sup>9-11</sup> are also plotted with their appropriate  $PR/\gamma$  scale;  $c_m$  was chosen for coincidence at the common point. The close correspondence between the experimental curves incidentally supports a correlation based on the ratio of pressure deficiency at any saturation, to the pressure deficiency at some fixed saturation<sup>11,12</sup>. It is seen that the experimental section below the common point crosses the theoretical curves\*, and entraps less fluid than the torus arrangement. By inspection, sequence (f) was inferred, which corresponds closely to the experimental points except near the entry pressure (where the experimental values are themselves different), and at the point  $P_s \sim 0.5$ . The details of possible interaction between pore size distribution and entry-exit configuration are clearly in need of elucidation.

**Imbibition.** On relaxing the suction the wetting phase imbibes in the reverse sequence to desaturation, though not at the same pressure deficiencies. Thus, no displacing phase entrapment occurs. For any two cells  $A$  and  $B$  ( $A > B$ ) the condition given by the inequality:

$$AP_b < BP_b < AP_t < BP_t$$

leads to a simple hysteresis loop; likewise the present model exhibits a desaturation-saturation cycle. Having traced a desaturation curve, the pressure deficiency axis for the imbibition curve may be calculated from equation (5). The ratios of pressure deficiency for imbibition to that for desaturation,  $P_b/P_t$ , associated with  $P_t R/\gamma$  values, are respectively: 0.61, 4; 0.55, 5; 0.51, 6; 0.48, 7; 0.45, 8; 0.43, 9. Experiment indicates this ratio to be roughly 0.5, so the theoretical values are of the correct order.

\* The situation may be better than indicated here. As will be seen next, with the present model no displacing phase entrapment occurs on imbibition. The curves are thus analogous to the secondary desaturation and the pendular imbibition curves<sup>12</sup>. The former are similar in appearance to the torus configuration curve (d) at pressure deficiencies below the common point.

The model requires refinement to represent scanning curves<sup>13,14</sup> (secondary desaturation and imbibition); a two-cell configuration (parallel arrangement) exhibiting an elementary scanning curve occurs when:

$$AP_b < BP_b < BP_t < AP_t$$

**Further investigations.** The square was used in a manner designed to simulate a drainage column. A suction gradient equivalent to a gravitational field increasing by one rank per row up the square was inherent in this model, and it was imagined that the wetting phase exited at the bottom edge of the square while the displacing phase entered at the top edge (one example of arrangement (a)). The gradient was balanced by a positive pressure which was relaxed one rank at a time allowing the column to drain. Eventually, a pressure deficiency was applied to complete the emptying sequence. Further work is required to elucidate the details; however, the results did not correspond to arrangement (a). Instead, they appeared to be more akin to arrangement (b) at the entry pressure, then somewhat between (d) and (f) up to pressure rank 6, and finally, at higher pressures were similar to arrangement (c).

Two refinements which could improve all network models concern the processes preceding the sudden emptying and filling of pores: (1) as the suction builds up to  $P_t$  the meniscus curvature increases and consequently a small quantity of liquid is withdrawn from the cell; when cumulated this leads to the steep slope extending from complete saturation to the entry suction, and would also help smooth the step nature inherent in the present models where relatively few pores are used; (2) as the suction is relaxed to  $P_s$  a quantity of liquid enters the cell; this could be as high as half the cell volume.

This investigation is being extended to a  $20 \times 20$  matrix; first results are generally similar to those from the  $10 \times 10$  matrix, with some local deviations of between 1/4 and 2 pores.

A three-dimensional model would provide a more appropriate representation of a packing. Arranging the rows or columns of a Latin square in cyclic order will lead to squares which can be assembled, layer by layer, into a simple model.

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<sup>11</sup> Harris, C. C., Ph.D. thesis (Leeds Univ., 1959).

<sup>12</sup> Harris, C. C., Jowett, A., and Morrow, N. R., *Trans. Inst. Min. Metall.*, **73**, 335, 520; **74**, 30 (1964).

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<sup>14</sup> Morrow, N. R., and Harris, C. C. (to be published).

## PACKING ARRANGEMENT OF TROPICOLLAGEN MOLECULES

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THE work of Highberger *et al.*<sup>1</sup>, Gross *et al.*<sup>2</sup>, Schmitt *et al.*<sup>3</sup> and Hodge and Schmitt<sup>4</sup> has led in the past decade to the theory that native collagen fibres are aggregates of tropocollagen molecules, each of which is about 2600 Å long and 15 Å in diameter. The molecules are similarly orientated and arranged so that each is staggered by a quarter of its length in relation to those molecules which abut immediately against it. This theory has been

supported by the demonstration of tropocollagen molecules of the forecast dimensions<sup>5,6</sup>, by the discovery that sonic irradiation disrupts such molecules into halves and quarters<sup>7</sup> and by the photographic synthesis of native collagen periodicity from the segment long spacing pattern<sup>8</sup>.

Recently, however, the complete validity of the theory has been questioned by some authors<sup>9,10</sup>. The theory is



usually illustrated in a two-dimensional sense by a diagram such as that in Fig. 1*a* showing all adjacent molecules quarter-overlapped in relation to immediately adjacent molecules. But consideration of the problem in three dimensions shows immediately that the simple arrangement visualized by the theory is a geometrical impossibility. Thus, if *A*, *B* and *C* in Fig. 1*b* represent three adjacent molecules in the same plane, *A* is in contact with *B* and *B* with *C*, but *C* is not in contact with *A*, and therefore *A* can be quarter-overlapped on *B* and *B* can be quarter-overlapped on *C*. But if the same molecules are packed as in Fig. 1*c*, as they would be in any actual fibre, each molecule is in contact with the other two. If molecule *A* is represented in Fig. 1*d* by *A*, then, allowing for a quarter-overlap, the position of molecule *B* must be that represented by either *B*<sup>1</sup> or *B*<sup>2</sup>. Similarly, if molecule *C* is quarter-overlapped on molecule *B*, its position in Fig. 1*d* must be represented either by *C*<sup>1</sup> or *C*<sup>2</sup> or *C*<sup>3</sup>. But *C*<sup>3</sup> is in register with *A* while *C*<sup>1</sup> and *C*<sup>2</sup> overlap *A* by half their length. It is therefore impossible for all molecular contacts to be quarter-overlap in nature in any three-dimensional packing arrangement.

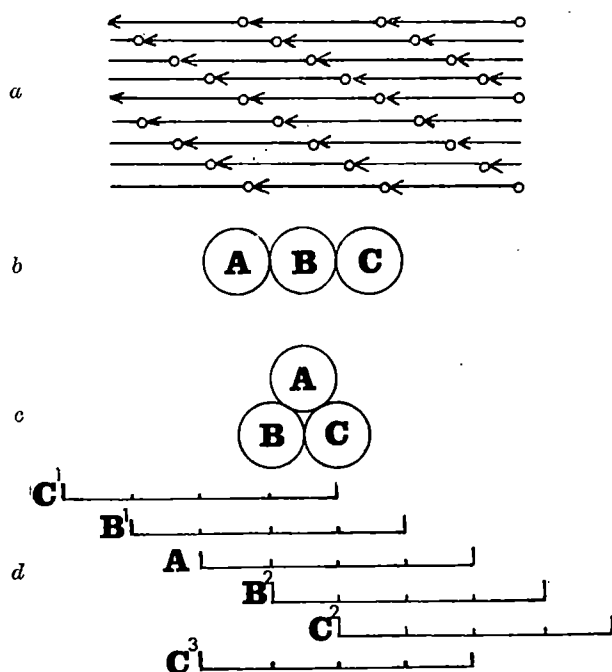


Fig. 1

For the purpose of estimating the number of quarter-overlaps possible in any particular collagen fibre, the hexagonal packing arrangement in Fig. 2, which accommodates the maximum number of molecules per cross-sectional area, has been used. Its use is, of course, purely a convenience, and does not infer that the collagen molecule is hexagonal in shape. The same packing arrangement could be formed with circles, each circle occupying the interior of a hexagon. In this packing, the molecules are arranged in successive rings around a central molecule and for convenience alternate rings have been stippled in Fig. 2. The first ring consists of six molecules and thereafter the number of molecules in each additional ring increases by 6 (Table 1). As each ring is added to the fibre its molecules can be regarded as forming two groups of contacts. The contacts between the members of the added ring are equal in number to the number of molecules within the ring, while the contacts with the molecules of the immediately deeper ring are equal to the number of all the molecules in the two rings concerned. Thus the third ring has 18 molecules, the second 12 molecules, and therefore the number of molecular

No. of molecular ring	No. of molecules in ring	No. of molecules in fibre	Diameter of fibre (Å)	No. of molecular contacts in fibre	No. of quarter-overlaps	% of quarter-overlaps
1	6	7	45	12	9	75.0
2	12	19	75	42	30	71.4
3	18	37	105	90	63	70.0
4	24	61	135	156	108	69.2
5	30	91	165	240	165	68.7
6	36	127	195	342	234	68.4
7	42	169	225	462	315	68.2
8	48	217	255	600	408	68.0
<i>X</i>	$6X$	$3X^2 + 3X + 1$	$30X + 15$	$9X^2 + 3X$	$6X^2 + 3X$	$\frac{100(2X+1)}{3X+1}$
13	78	547	405	1,560	1,053	67.5

contacts established, as the third ring is added, is  $18 + 18 + 12 = 48$ .

It has been found by trial and error that the arrangement of molecules in a longitudinal direction which gives the greatest number of quarter-overlapped contacts between adjacent molecules is that in which each molecule of a ring is quarter-overlapped on the adjacent molecules of the same ring. In these circumstances, of the contacts which the molecules of one ring form with those of the immediately deeper ring, half are quarter-overlapped while the other half are not so related. This arrangement is illustrated in Fig. 2 in which all the quarter-overlapped contacts are indicated by two short cross-lines.

Various features of fibres with this type of molecular packing are tabulated in Table 1. It will be noted that the figures in most of the columns form various types of arithmetical progressions, and as a result the features of a fibre with '*X*' molecular rings can be calculated: these are shown in the penultimate line. Finally, from these expressions, if the diameter of a collagen fibre is known, say, 405 Å, the other features of its molecular arrangement can be readily calculated as shown in the final line.

It is evident from the final column in Table 1 that in a fibre with only one ring of molecules and a diameter of

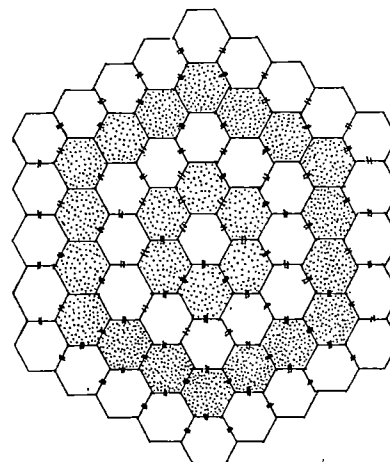


Fig. 2

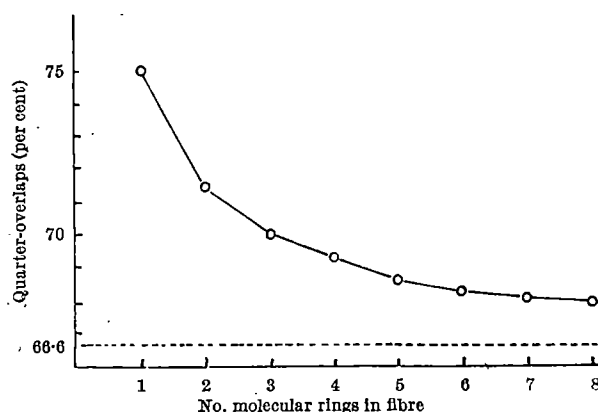


Fig. 3

45 Å, 75 per cent of the molecular contacts are quarter-overlapped. As more molecular rings are added this figure falls, making an asymptotic approach to a limiting value of 66.6 per cent (Fig. 3).

These observations do not affect the general concept that the 640 Å periodicity of native collagen is a result of quarter-overlapping among the constituent tropo-collagen molecules. But it does seem of some importance that it should be recognized that in a fibre of average size only 67–68 per cent of the molecular contacts can be of this nature. Moreover, it seems probable that the problem discussed here is closely associated with that of the minimum size of collagen fibre in which cross-striations are evident. Ross and Benditt<sup>8</sup> have calculated that with this type of molecular arrangement cross-striations

would be evident in fibres of 100 Å diameter, whereas if all molecular contacts were quarter-overlapped, striations would be evident in fibres of 70 Å diameter. In practice the minimum fibre diameter exhibiting cross-striations is about 100 Å.

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## NEW PHASES IN THE SYSTEM $\text{Al}_2\text{O}_3\text{--SO}_3\text{--H}_2\text{O}$ AT TEMPERATURES ABOVE 100° C

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**S**OLUTIONS of aluminium sulphate (especially 'basic' solutions, in which the molar ratio  $\text{SO}_3 : \text{Al}_2\text{O}_3$  is less than 3) hydrolyse readily at elevated temperatures<sup>1</sup> to produce in most instances the compound  $3\text{Al}_2\text{O}_3 \cdot 4\text{SO}_3 \cdot 9\text{H}_2\text{O}$  (phase *B*, Table 1). A further survey of the ternary system has now been made over the temperature-range 100°–290° C, at dilutions ranging from 0.1 to 1.0 molar (with respect to  $\text{Al}_2\text{O}_3$ ) and with compositions ranging from normal salt (molar ratio  $\text{SO}_3 : \text{Al}_2\text{O}_3 = 3$ ) to highly basic mixtures or solutions (ratios as low as 0.7). This work has demonstrated the existence above 100° C of at least five compounds, of which two are new (phases *A* and *D*) and another (phase *E*) has not previously been reported from sulphate solutions. The compounds are listed in Table 1 in order of increasing basicity.

Table 1. COMPOUNDS IN THE SYSTEM  $\text{Al}_2\text{O}_3\text{--SO}_3\text{--H}_2\text{O}$  AT ELEVATED TEMPERATURES

Phase	Oxide formula	Structural formula	Name
<i>A</i>	$\text{Al}_2\text{O}_3 \cdot 2\text{SO}_3 \cdot \text{H}_2\text{O}$	$\text{Al}(\text{SO}_4)(\text{OH})$	—
<i>B</i>	$3\text{Al}_2\text{O}_3 \cdot 4\text{SO}_3 \cdot 9\text{H}_2\text{O}$	$(\text{H}_2\text{O})_2\text{Al}_2(\text{SO}_4)_2(\text{OH})_{12}$	Hydronium alunite
<i>C</i>	$3\text{Al}_2\text{O}_3 \cdot 3\text{SO}_3 \cdot 10\text{H}_2\text{O}$	$(\text{H}_2\text{O})_2\text{Al}_2(\text{SO}_4)_2(\text{OH})_{12}$	—
<i>D</i>	$3\text{Al}_2\text{O}_3 \cdot 2\text{SO}_3 \cdot 11\text{H}_2\text{O}^*$	$(\text{H}_2\text{O})_2\text{Al}_2(\text{SO}_4)_2(\text{OH})_{12}$	Glassy phase
<i>E</i>	$\text{Al}_2\text{O}_3 \cdot \text{H}_2\text{O}$	$\text{Al}(\text{OH})_3$	Boehmite

\* As prepared over the temperature-range 130°–150° C.

It is interesting to note that a general formula,  $(\text{H}_2\text{O})_x \text{Al}_2(\text{SO}_4)_y(\text{OH})_{18+x-2y}$ , includes all phases (except *E*) in Table 1. As yet, however, we have no direct evidence of hydronium ions in phases *C* and *D* and we do not claim that the general formula necessarily indicates a series of closely related crystallographic structures.

A brief account of the conditions under which each compound forms is given here.

$\text{Al}_2\text{O}_3 \cdot 2\text{SO}_3 \cdot \text{H}_2\text{O}$  (*A*) is prepared by heating molar solutions of aluminium sulphate (ratio 2.9) at 290° C for 16 h. It does not appear at much lower temperatures (for example, 250° C) or from more dilute or highly basic solutions. It dissolves very slowly in boiling 5 N hydrochloric acid and loses 'hydroxyl' water only on heating above 550° C.

$3\text{Al}_2\text{O}_3 \cdot 4\text{SO}_3 \cdot 9\text{H}_2\text{O}$  (*B*) is the compound most commonly encountered over a very wide range of compositions and temperatures (170°–290° C). Although previously called 'hydrogen' alunite by Davey and Scott<sup>2</sup>, it is better described<sup>3</sup> as 'hydronium' alunite, with  $\text{H}_3\text{O}^+$  substituting for  $\text{K}^+$  in the alunite lattice. In the range 100°–170° C a very similar compound containing an extra 2–4 per cent water is encountered, which is probably  $3\text{Al}_2\text{O}_3 \cdot 4\text{SO}_3 \cdot 10\text{--}15\text{H}_2\text{O}$ , as described by Okabe<sup>4</sup>.

$3\text{Al}_2\text{O}_3 \cdot 3\text{SO}_3 \cdot 10\text{H}_2\text{O}$  (*C*) is formed in preference to phase *B* in more basic solutions and has been encountered at all temperatures in the range 100°–180° C. At the higher temperatures phase *C* is metastable with respect to *B* and is gradually converted to the latter over a period of several hours. Phase *C* gives a characteristic X-ray diffraction pattern which is related to that for the compound  $\text{Al}_2\text{O}_3 \cdot \text{SO}_3 \cdot 4\text{H}_2\text{O}$  described by Bassett and Goodwin<sup>5</sup>. The difference in water content may be ascribed to the fact that the latter compound was analysed in the air-dried state.

$3\text{Al}_2\text{O}_3 \cdot 2\text{SO}_3 \cdot 11\text{H}_2\text{O}$  (*D*) is an unusual material with a somewhat variable composition. The empirical formula given in Table 1 is averaged from preparations made by heating very basic solutions in the temperature range 130°–150° C. Below this temperature the water content shows a marked increase, but the  $\text{SO}_3 : \text{Al}_2\text{O}_3$  ratio does not change significantly. Phase *D* is usually found in preparations heated at temperature for a relatively short period, since it is nearly always metastable with respect either to phase *C* or to that form of phase *B* with the higher water content. It is optically isotropic and structurally amorphous in X-ray diffraction tests. We therefore describe it as 'the glassy phase' and it is undoubtedly related to a similar material obtained by Bassett and Goodwin<sup>5</sup> from their 'two-liquid system 1' at temperatures below 100° C.

$\text{Al}_2\text{O}_3 \cdot \text{H}_2\text{O}$  (*E*) has only been identified at temperatures above 190° C as a product of dilute and highly basic solutions. So far, it has always been obtained in admixture with phase *B*, from which it can largely be separated by selective solution of the latter with boiling 5 N hydrochloric acid. Phase *E*, though relatively resistant to solution in acids, is poorly crystallized but can be readily identified as boehmite by X-ray powder diffraction techniques. It is worthy of note in this connexion that well-crystallized boehmite with a very sharp XRD pattern has been prepared by us at temperatures below 190° C from basic solutions of either aluminium chloride or aluminium nitrate.

In the related system  $\text{Fe}_2\text{O}_3\text{--SO}_3\text{--H}_2\text{O}$ , phases analogous to our phases *A*, *B* and *E* have been described by Posjank and Merwin<sup>6</sup>. The respective iron compounds, however, start to form at much lower temperatures than their aluminium analogues. We have prepared the iron compounds  $\text{Fe}_2\text{O}_3 \cdot 2\text{SO}_3 \cdot \text{H}_2\text{O}$ ,  $3\text{Fe}_2\text{O}_3 \cdot 4\text{SO}_3 \cdot 9\text{H}_2\text{O}$ , and  $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$  by heating the appropriate ferric sulphate solutions and have shown them to be isomorphous with the corresponding aluminium compounds.

We thank Dr. W. G. Mumme for assistance with the X-ray diffraction work.

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## BLOOD : BONE EQUILIBRIUM IN CALCIUM HOMOEOSTASIS

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THE nature of the dynamic equilibrium between the mineral component of human bone and its ion in solution in tissue fluid is unknown. A few years ago the theory advanced by MacLean and Urist<sup>1</sup> was generally accepted, and experimental investigations of the mode of action of parathyroid hormone<sup>2,3</sup> were designed in terms of it. Their hypothesis held that normal tissue fluids were 'supersaturated' with respect to whole bone; that the bone mineral maintained only 'hypoparathyroid-levels' of calcium and phosphorus by physical processes and that a parathyroid-controlled 'vital activity' caused destruction of whole bone, release of mineral and increase in the concentration of calcium in the tissue fluids. Maintenance of a calcium concentration gradient therefore not only required continual secretion of parathormone but also continual osteoclastic resorption of whole bone.

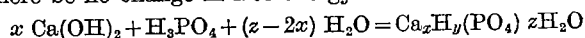
In 1957, Nordin<sup>4</sup> suggested that the situation be re-examined in terms of solubility theory and showed that calf bone powder would maintain reproducible levels of calcium and phosphorus *in vitro*. Later, MacGregor and Nordin extended the work and showed that, in both trihydroxymethylaminomethane : hydrochloric acid<sup>5</sup> and carbonic acid : potassium bicarbonate buffer systems<sup>6</sup> at ion strengths of 0.15 and at 20° C, human dead bone powder would maintain the levels of calcium and phosphorus found in normal tissue fluids if the pH at the solid/solution interface was about 7.0-7.1 (ref. 6). This pH is intermediate between the intracellular pH of 6.8 and the extracellular pH of 7.4, and the concept of a pH gradient across the blood : bone barrier is not unattractive.

The equilibration investigations showed that the ion-concentration product,  $[Ca^{++}]^3[PO_4^{--}]^2$ , expressed as a negative logarithm ( $pK$ ) was relatively constant over the pH range 6.4-7.6 and ranged from 26.0 to 26.8 and was not related to pH. On the other hand, the ion-concentration product,  $[Ca^{++}][HPO_4^-]$ , correlated highly with pH (ref. 5). On this evidence, MacGregor and Nordin postulated that, on purely empirical grounds, the cubed-squared product best represented the physico-chemical relation between normal human bone and its ions in 'equilibrium' with it. It was pointed out that no theoretical significance could be attached to the tricalcium phosphate product on this basis and that the authors did not necessarily consider that this salt was the relevant solid phase of 'the exchangeable calcium pool'.

The chemical nature of the micro-crystals in bone and the physical properties of its surfaces have been the subject of discussion for many years. The conventional view<sup>7</sup> is that hydroxyapatite (HA) is the relevant compound. The basic model is modified in detail by internal lattice faults with entrapped contaminants, and the free surfaces are believed to have suffered heteroionic exchange with ions such as  $Sr^{++}$ ,  $Mg^{++}$ ,  $Na^+$ ,  $P^-$ ,  $CO_3^{--}$ , and citrate. In consequence, quantitative analysis of whole bone has yielded no definitive data.

In view of the possibility that one or more sparingly soluble calcium phosphates  $\{Ca_5OH(PO_4)_3, Ca_8H_2(PO_4)_6-$

$5H_2O, \beta-Ca_3(PO_4)_2, CaHPO_4, CaHPO_4 \cdot 2H_2O\}$  could be present in bone, assumptions regarding the stoichiometry of the equilibrium between the solid phase and the aqueous solution are undesirable. It is easily shown<sup>8-10</sup> that solubility data can be treated in a way so that such assumptions become unnecessary. The formula for any calcium phosphate may be written in generalized empirical form as  $Ca_xH_y(PO_4)_zH_2O$ , where  $2x+y=3$  and  $y$  may have negative values (for example, for HA,  $x=5/3$ ,  $y=-1/3$  and  $z=1/3$ ). The condition for equilibrium between an aqueous solution and the generalized salt requires that there be no change in free energy for the reaction:



That is, the sum of the chemical potentials of the components given on the left side of the equation is equal to the standard free energy of formation of the salt on the right-hand side.

$$x \mu_{Ca(OH)_2} + \mu_{H_3PO_4} + (z-2x) \mu_{H_2O} = \Delta F_{xx}^0$$

If we then assume that the chemical potential of the water is essentially constant for dilute solutions and that the chemical potentials of  $Ca(OH)_2$  and  $H_3PO_4$  may be expressed as usual in terms of their ionic activities, then:

$$\mu_{Ca(OH)_2} = \mu_{Ca(OH)_2}^0 + RT \ln (Ca^{++})(OH^-)^2$$

$$\mu_{H_3PO_4} = \mu_{H_3PO_4}^0 + RT \ln (H^+)^3(PO_4^{--})$$

and we can derive the result:

$$\log (Ca^{++})(OH^-)^2 = -\frac{1}{x} \log (H^+)^3(PO_4^{--}) + K_{xx}$$

where the parentheses represent ionic activities and  $K_{xx}$  is a constant specific to the applicable salt.

Therefore if negative  $\log (Ca^{++})(OH^-)^2$  is plotted against negative  $\log (H^+)^3(PO_4^{--})$  for a series of solutions in equilibrium with the above salt, the points should fall on a straight

line with a slope of  $-\frac{1}{x}$ , where  $x$  is the Ca/P molar ratio in the salt. In other words, the slope is dictated by the stoichiometry of the reaction. The position of the line is determined by the value of  $K_{xx}$  which cannot be calculated without assumptions concerning the appropriate activity co-efficients. However, in a series of experiments at constant ionic strength it is usually assumed that the activity co-efficients are also constant, and therefore the slope of the line is valid even though its position is made somewhat obscure by the replacement of  $Ca^{++}$  and  $PO_4^{--}$  ion activities by their concentrations.

This method has been applied to the results of MacGregor and Nordin<sup>5</sup> and MacGregor<sup>11</sup>. In the first case negative  $\log (Ca^{++})(OH^-)^2$  was plotted against negative  $\log (H^+)^3(PO_4^{--})$  using the data from experiments in which adult dead bone powder was equilibrated with *tris* and cacodylate buffers. Unitary activity co-efficients were assumed for the calcium and phosphate ions. The graph is shown in Fig. 1. The slope of the line of best fit obtained by linear regression analysis was -0.616 which represents a Ca/P ratio for the solid phase of 9.72/6.

Secondly, the results of experiments with child dead bone powder were treated by this method and the results are shown in Fig. 2. In this case the slope was -0.743 representing a Ca/P ratio of 8.10/6.

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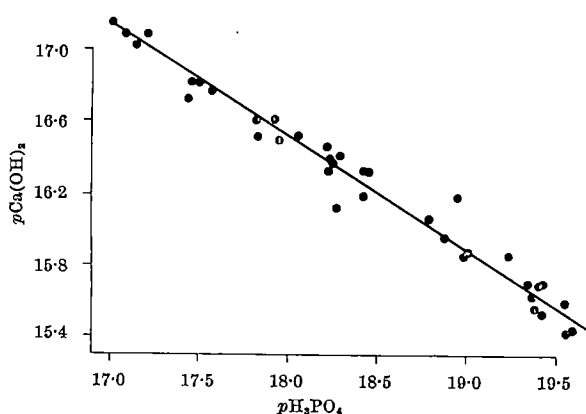


Fig. 1. Chemical potential relation in equilibration investigations with powdered adult bone

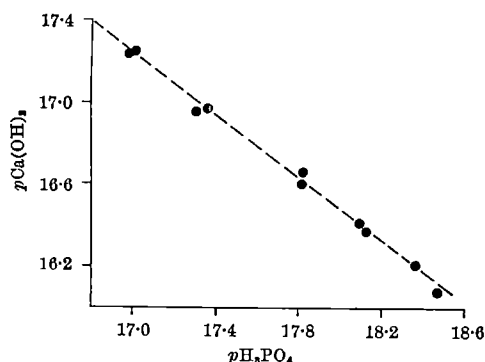


Fig. 2. Chemical potential relation in equilibration investigations with powdered child bone

It would appear then that in the child the solid phase in equilibrium with body fluids is octocalcium phosphate (OCP),  $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ , while in the adult the Ca/P ratio of the equilibrating solid phase is more nearly that of HA. This interpretation is dependent on the degree of confidence we can place in the slopes of the fitted lines.

A correlation analysis yielded regression co-efficients and standard errors for adult and child dead bone listed in Table 1. Given also is the Ca/P ratio (that is, the reciprocal of the regression coefficient) in each instance and the limits in the ratio Ca/P at the 95 per cent confidence-level.

Bone	Regression coefficient	S.E.	Equivalent Ca/P	95% confidence limits of Ca/P
Adult	-0.616	0.018	9.72/6	9.24/6, 10.32/6
Child	-0.743	0.018	8.10/6	7.62/6, 8.52/6

The Ca/P ratio derived from the adult-bone results corresponds quite closely to that of HA, 10/6. The ratio 9/6 is outside the 95 per cent confidence limits, and it is extremely unlikely that the true slope could correspond to 8/6, the ratio for OCP.

The results indicate that the solid phase saturating the solutions in the experiments with child bone is OCP, but they should not be construed to mean that bone salt in children is exclusively OCP. The behaviour is also consistent with the view that the major phase is unreactive or does not have a definite solubility, while OCP—even though a minor phase—governs the apparent solubility. It has been shown in fact that the rates of dissolution and recrystallization of OCP appear to be considerably greater than those of HA<sup>12</sup>.

Other indications support the idea that OCP may be present in bone. The 'platey' habit of crystallites in bone has been interpreted as indicating that OCP participated in their formation<sup>12</sup>. Evidence has been presented recently to support the view that growth of HA crystals depends on a mechanism in which OCP is

the initial precipitate<sup>13,14</sup>. The incorporation of phosphate into the apatite lattice has been shown by Dallemagne *et al.* to involve initially an acidic phosphate which we believe is OCP<sup>15</sup>. On the other hand, the adult bone results indicate that HA probably dominates the equilibration in aged formed bone.

Thus, the burden of evidence now available supports our considered view that bone mineral is first formed as OCP and that there are spontaneous ageing changes (hydrolysis) which convert OCP into HA. In the child, where bone growth is maximal, there tends to be more OCP in the microcrystals than in the older subject where the reorganization of the OCP to HA overtakes the precipitation process.

Since the new feature of our theory concerns the presence of OCP in 'young' bone, we have applied the calculation to the results of Nordin<sup>4</sup>, who examined the equilibration of powdered calf bone with 0.15 M *tris* and cacodylate buffers at 37° C (Fig. 3). His results yielded a regression coefficient of -0.738 with a standard error of 0.026 and 95 per cent confidence limits of 7.56/6 and 8.82/6. This regression coefficient represents a Ca/P ratio of 8.13 : 6. It is highly improbable that this observed ratio could have arisen by chance if the true Ca/P ratio was 7/6 or 9/6. This confirms the dominance of the OCP stoichiometry in equilibrations with 'young' bone.

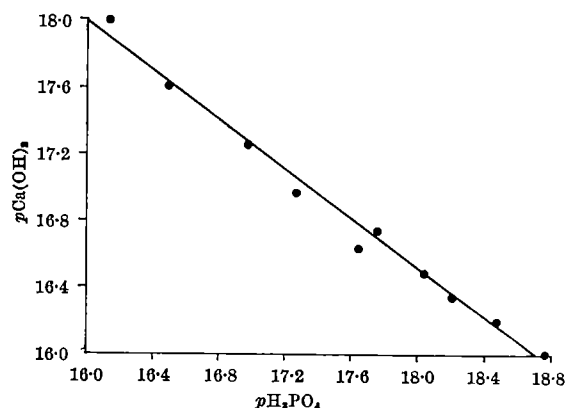


Fig. 3. Chemical potential relation in equilibration investigations with powdered calf bone

Since there appears to be a difference in the physico-chemical behaviour of young and adult bone, it may be possible to explain the raised serum inorganic phosphate concentrations in children by the greater solubility of OCP as compared to that of HA. MacGregor and Nordin were aware that their 'ion product' theory of blood : bone exchange and the role of the parathyroid glands in maintaining the level of the equilibrium<sup>16</sup> might not explain adequately the situation in child plasma. Possible explanations advanced by MacGregor<sup>17</sup> included the suggestions that child plasma might contain a larger proportion of calcium and phosphorus binding substances, that the metabolic pH gradient between the bone mineral tissue fluid interface and the circulating body fluids might be steeper due to increased metabolic activity in the young, or thirdly, that there might be a qualitative difference in child bone. The last-mentioned now appears more plausible, and explains the observed differences, at least in part.

Our present ability to re-interpret the results for child bone, which to some extent conflicted with the 'solubility' hypothesis of parathyroid function, now yields substantial support to that thesis, since it allows rational explanation of the fact that plasma inorganic phosphates in young mammals are higher than in adults, whereas the plasma calcium concentration is usually fairly constant at about 2.5 mM. In point of fact it is the calcium homeostasis which is the specific necessity for optimal cellular function.

The biological significance of these findings remains to be examined in detail. They suggest that OCP is present in greatest amounts in newly formed bone, whether child or adult, and that the chemical properties and interactions of two crystalline species must be taken into account for a full understanding of bone growth and remodelling, bone healing, and various pathological conditions.

In a sense this article can only be a preliminary communication on the basis of retrospective analysis of equilibration data. It is now necessary to re-examine this line of enquiry. In particular, the pure salts should be subjected to the same chemical treatment as the bone powder to verify the position of the fitted lines as well as their slopes. Regardless of the uncertainty suggested by this point, the present evidence for the presence of OCP *in vivo* is substantial and it introduces a new factor of considerable physiological potential. Far from detracting from the concept of a metabolic process modulating a physico-chemical exchange mechanism, these data may now help to explain many of the inconsistencies previously observed with the *in-vitro* models of skeletal behaviour.

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## RELATIVE BIOLOGICAL EFFECTIVENESS OF 592-MeV PROTONS ON MICE

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THE relative biological effectiveness (RBE) of high-energy protons has been investigated by exposing mice in the external proton beam of the CERN 600-MeV synchro-cyclotron. The proton beam followed the normal beam path in air, as is shown in the beam layout diagram (Fig. 1). The protons, after leaving the cyclotron, were focused by a pair of quadrupole lenses and deflected through 25° into the beam channel of the main machine shielding. Outside this shielding, the protons passed through another bending magnet which deflected the protons 27° in the opposite direction to remove unwanted radiation and to select the protons of a defined energy of 592 MeV. Another quadrupole lens was placed in front of this magnet to de-focus the beam in order to maximize the radiation field size at the place of irradiation. For the same reason, the irradiation site was chosen as far as possible away from the beam focus (18 m), immediately in front of a 10-m deep beam catcher.

The beam intensity was monitored with a secondary emission ionization chamber calibrated to measure total beam intensity<sup>2</sup>. The intensity distribution in the beam at the site of irradiation was determined using a 0.6-cm<sup>3</sup> air-filled ionization chamber (Ionex Baldwin Industrial Control, Dartford, Kent), mounted on a *x-y* scanning frame. This chamber had previously been calibrated with 600-MeV protons by comparison with a tissue-equivalent ionization chamber of the Rossi type<sup>3</sup>.

A rotatable disk 1 m in diameter, made of 0.3-cm 'Lucite', placed perpendicular to the beam direction was centred in the beam. Small perforated 'Lucite' mouse cages were attached to the disk with their axes parallel to the beam direction. Dose-rates were selected by fixing the mouse cages at various radii on the disk.

The dose-rate measurements were made by the ferrous-ferri dosimeter<sup>4</sup>. Small thin-walled sealed-off glass

tubes, 5 cm long and 1.2 cm in diameter, half-filled with the ferrous solution, were placed in the mouse cages. A 0.6-cm<sup>3</sup> ionization chamber was attached to the rotation disk at the same radius as the mouse cages. A second 0.6-cm<sup>3</sup> chamber was placed at the side of the beam to control any directional displacement of the beam during irradiation. The secondary emission chamber was used as the reference monitor.

Dose-rate measurements were first made at different radii with the disk rotating at a speed of 3 r.p.m. These measurements were used to centre the wheel in the beam. The relative dose-rate at various distances from the centre of the rotating wheel is shown in Fig. 2. By exposing the ferrous-ferri system and the Ionex ionization chamber simultaneously in the 600-MeV proton beam at the same radii, an agreement within 5 per cent of the measured dose was observed with these two instruments. A factor of 0.93 was used to convert the ferrous-ferri system readings to rad.

Male mice, 57 days old, of pure strain (C57B66), weighing on the average  $22.4 \pm 0.5$  g, were exposed. They were placed in the 'Lucite' tubes described here. The tubes were perforated to assure sufficient air circulation to the animals during exposure. The mouse cages were attached at 6 cm from the centre of the rotating disk and the animals irradiated along their antero-posterior axes with a dose-rate of 361 rads/min.

Six mice were exposed to each of the following doses: 600, 700, 800, 900 and 1,000 rads. For the 600-, 800- and 1,000-rad exposures, a ferrous sulphate dosimeter, used by the Institut du Radium and consisting of glass tubes of mouse-like dimensions filled with a solution of ferrous sulphate<sup>4</sup>, was irradiated simultaneously with the mice at the same radius on the disk. The difference between the chemical dosimetry made at CERN and the ferrous

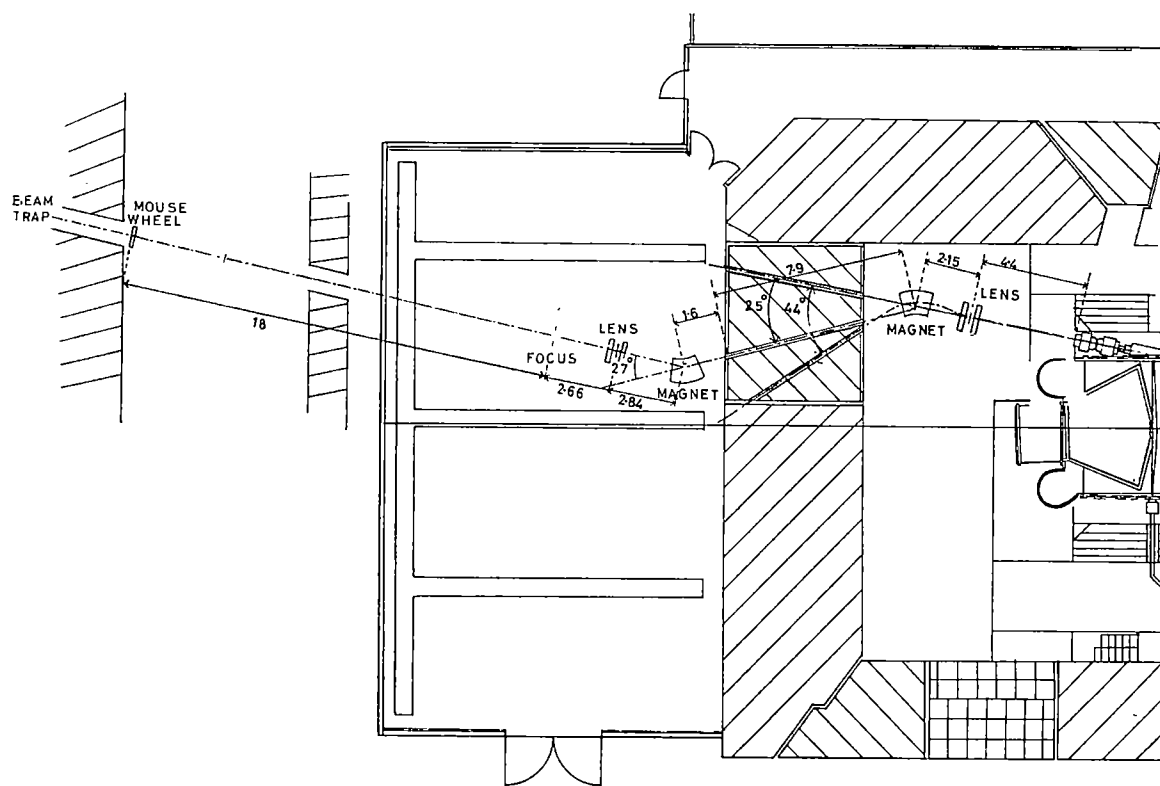


Fig. 1. Beam layout for the 600-MeV extracted proton beam of the CERN Synchro-cyclotron used for animal experiment

sulphate dosimetry of the Institut du Radium was less than 5 per cent ( $G = 15.6$ ).

After irradiation, the irradiated and control mice were kept under the same conditions and the following parameters recorded: (1) mortality statistics ( $LD_{50}$  and median survival time); (2) weight of the animals; (3) weight of organs sensitive to radiation: spleen, thymus, testicles; (4) haematological tests; white and red cell counts and differential white cell counts.

Mice of the same stock were also irradiated with X-rays, 250 keV (filtration: 2 mm aluminium + 0.3 mm copper), and at a dose-rate of 80 rads/min and tested in the same way for comparison.

The mice irradiated with 592-MeV protons showed the same symptoms as those irradiated with X-rays, that is, loss of weight, alteration of the fur, diarrhoea, blood in faeces, slowing-down of activity for the mice which eventually died, and an increase in weight and resumption of

activity for the survivors. The haematological changes (leucopenia, erythropenia, change of the differential white cell count) were found to be the same for both kinds of radiation, and no anomalous changes were observed in the data relating to 592-MeV protons.

In the dose-range of 600–1,000 rads, 5 of the 9 biological parameters investigated<sup>5</sup> did not show sufficient correlation with the dose to enable numerical values suitable for assessing the RBE to be determined. These were the loss of weight by the animals, the decrease in weight of the spleen on the day of death or on the 30th day, the cell counts and differential white cell counts. The biological variations in the control mice were, however, remarkably small, both with respect to the various weights and to the haematological data recorded.

Blood samples were taken by cutting the end of the tail, each sample being about 8 mg of blood. Such a sample is less than 1 per cent of the total blood volume of the animal but, if samples are taken too frequently (every day during the first 3 days of test, then every 8 days, that is 7 samples in 30 days), this leads to temporary anaemia which is noticeable in the blood counts of the control animals, both in the case of white cells and in the case of red cells (Figs. 3 and 4).

To eliminate as much as possible this additional cause of variation, all the counts made on irradiated animals were expressed as a percentage of those made on the control animals the same day.

For the reference X-rays, it was noted that the development of leucopenia remained approximately the same in the dose-range considered and did not show a significant correlation with the radiation dose. The same applied to erythropenia. Because of leucopenia, differential white cell counts are scarcely possible from the 3rd to the 20th day; inversion occurs on the first day, but the speed or magnitude of this inversion is not clearly correlated to the dose.

Cell counts made on mice irradiated with protons showed the same lack of correlation with dose, so none of the blood tests scheduled gave usable results under the conditions of our experiments; it is likely that this correlation

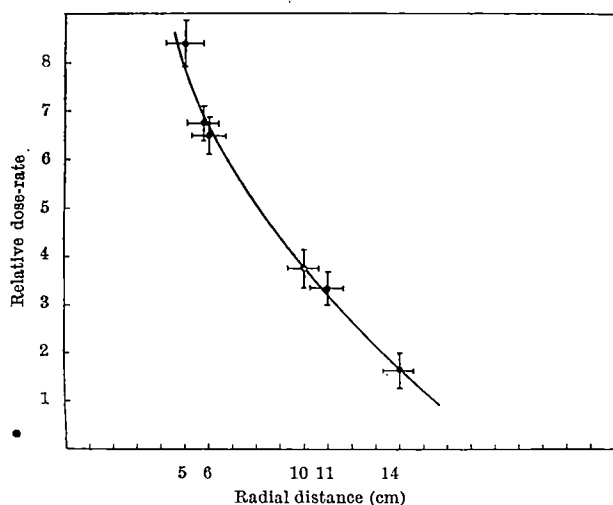


Fig. 2. Relative dose-rate variation with distance from centre of the rotating wheel exposed to the 600-MeV extracted proton beam



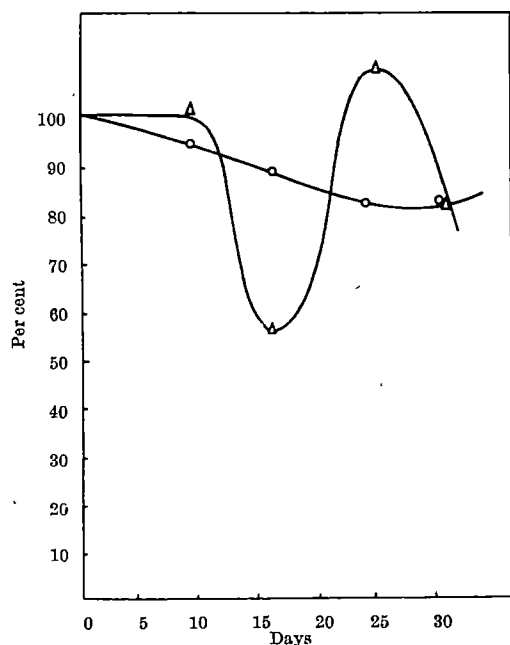


Fig. 3. Variation of erythropenia of mice irradiated with 600 rads, 600-MeV protons. O, Controls; Δ, 600 rads

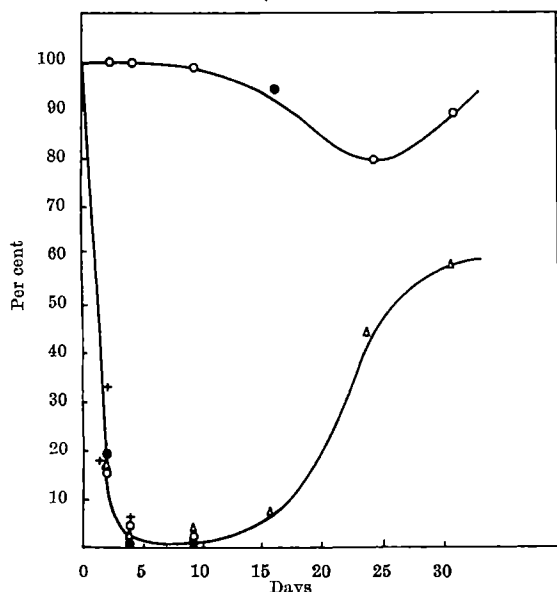


Fig. 4. Variation of leucopenia for mice irradiated with 600-MeV protons. Δ, 600 rads; ●, 700 rads; ○, 800 rads; +, 900 rads. Top curve controls

would be better for smaller radiation doses using a larger number of animals.

On the other hand, the correlation was satisfactory for the other 4 tests, that is,  $LD_{50}/30$  days, median survival time, testicular atrophy and thymus atrophy on the 30th day.

(1) The  $LD_{50}$  was determined from daily mortality statistics by the probit method<sup>6</sup> and for various periods of observation from the 3rd day, when death began to occur, to the 30th day. An  $LD_{50}$  versus period of observation curve was constructed from these data and the  $LD_{50}/30$  days was determined. With this method, better use can be made of the mortality statistics for high doses, which, in the first days of observation, give mortality rates below 100 per cent (Fig. 5).

For 592 MeV protons, a value of 580 rads for the  $LD_{50}/30$  days was found, whereas the value for the animals exposed to X-rays was found to be 570 rads. In other

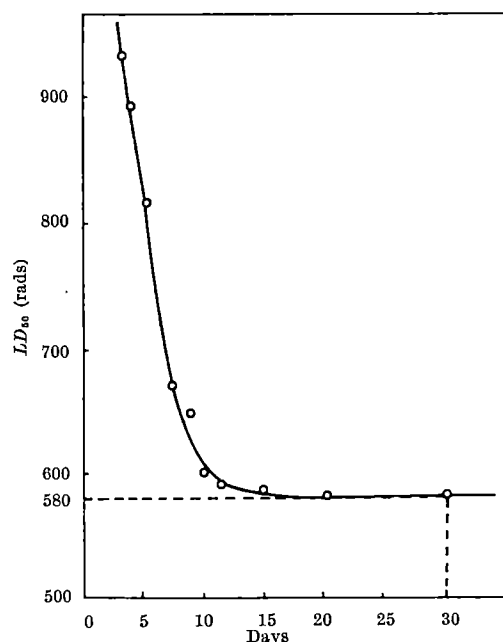


Fig. 5.  $LD_{50}$  for mice exposed to 600-MeV protons

words, the  $RBE\ X/P = 570/580 = 0.98$ . The errors of this measurement are estimated to be less than  $\pm 10$  per cent.

(2) The median survival time was calculated from individual survival times observed up to the 30th day, and the dose corresponding to a median survival time of 10 days was determined. This dose was 595 rads for X-rays and 560 rads for protons. The corresponding RBE from this test is  $X/P = 1.06 \pm 0.1$ .

(3) Testicular atrophy in the animals surviving on the 30th day was significant in the range investigated. For mice irradiated with a proton dose of 600 rads, the mean testicular weight was 60.6 mg, whereas it was 193 mg for the control animals; that is, there was a weight loss of nearly 70 per cent. For the X-ray irradiated animals, the corresponding dose was 620 rads. The RBE for these tests was then  $X/P = 620/600 = 1.03 \pm 0.1$ .

(4) For proton irradiated animals given 600 rads, the mean weight of the thymus on the 30th day was 24.4 mg against 53 mg for the control animals, that is, a loss in weight of approximately 52 per cent. The same loss in weight was observed for the X-irradiated mice for a dose of 622 rads. The RBE would therefore be  $X/P = 622/600 = 1.01 \pm 0.1$ .

The agreement between the RBE values from these four tests of different biological significance is rather remarkable, although these experiments are only preliminary and related to a small number of animals. The values of RBE found are a little greater than the value of 0.77 found for mice of different breeds irradiated with 152-MeV protons<sup>7</sup>. However, all the values are near to 1. It should be pointed out that the RBE value may vary with dose-rate<sup>8</sup>.

These experiments confirm that the RBE of near relativistic protons depends mainly on their linear energy transfer (LET) values. When 692-MeV protons enter tissue-like material, the LET is about 250 eV/ $\mu$ , whereas it is 550 eV/ $\mu$  for 152 MeV protons. These LET values are in the LET range of cobalt-60  $\gamma$ -rays and X-radiation<sup>7</sup>.

No peculiar biological effect which might be attributed to 'hot spots', corresponding to nuclear stars, could be detected in our observations. The possibility cannot be excluded, however, that less easily detectable phenomena (such as rare or late occurrence of cancer and genetic anomalies due to these 'hot spots') may later be discovered due to improved methods of investigation, particularly at cellular level.

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## REDUCTION OF RADIATION DAMAGE TO THE INTESTINAL MUCOUS MEMBRANE BY LOCAL HYPOXIA

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**T**O-DAY the most effective means known for reducing the sensitivity of living tissue to damage caused by ionizing radiation is reduction of the oxygen tension during irradiation. As early as 1909 Schwarz<sup>1</sup> found that ischaemia of the skin may, to some extent, prevent X-ray-induced erythema. Since then many investigators, notably Read and Thoday<sup>2</sup> and Gray *et al.*<sup>3</sup>, have given evidence that the dose-enhancing effect of oxygen, usually referred to as the 'oxygen effect', is an almost invariably present and most significant factor when living materials are being irradiated by ionizing radiation of low linear energy transfer (LET). So far there is no detailed explanation of this effect, but much attention is being paid to the hypothesis of Howard-Flanders<sup>4</sup> that the unpaired electrons of molecular oxygen might combine with the unpaired electrons appearing in the molecules split or ionized by radiation, so as to make radiolesions of the biochemical level irreversible. The possibility of increasing the lethal dose at whole-body irradiation by hypoxia has also been tested experimentally<sup>5,6</sup>. Thus, mice irradiated under nitrogen asphyxia availed themselves of a factor of 2.4 in effective dose reduction.

We have investigated the possibility of reducing acute radiation damage to the rectal mucous membrane by creating a state of local hypoxia in this region during irradiation.

Female albino rats of the Wistar strain weighing 200–250 g were used. Eleven animals were irradiated with a well-collimated 187-MeV proton beam<sup>7</sup> (LET at the rectal level = 0.5 keV/ $\mu$ ) and eight animals with  $\gamma$ -rays (mean LET = 0.2 keV/ $\mu$  from a 50-c. cobalt-60 source<sup>8</sup>). The whole abdominal cavity was irradiated uniformly at a dose rate of 200–300 rads/min. Before irradiation a thin rubber catheter, perforated 1 cm from its closed tip, was introduced about 5 cm into the rectum of the anaesthetized rat via the anus. The catheter was fixed about 0.5 cm from its end through a silk suture carefully applied around the rectum. Another small suture was used to mark the position of the temporary rectal stenosis thus provided. Treatment aiming at the removal of oxygen from the mucous membrane between the anus and the sutures was started 5 min before irradiation by injection of 1 ml. concentrated norepinephrine ('Norexadrin' 1 mg/ml., Astra, Södertälje, Sweden) through the catheter. 1 min later, continuous rectal injection of a reducing solution (0.1 M sodium sulphite in tap water) was started and continued until the end of irradiation. Finally, the stenosing suture and the catheter were removed and the abdominal incision closed.

Two  $\gamma$ -irradiated rats died four days after irradiation with signs of radiation sickness, and they were excluded from the investigation because of post-mortem changes. The other animals were killed 2–5 days after irradiation and specimens for histological examination were taken from the rectal wall in the areas situated 1 cm caudal (specimen *a*), and 1 cm cranial (specimen *b*) to the borderline between the 'deoxygenated' and the 'normally oxygenated' intestinal segments. The following grading

of the radiation-induced damage of the mucous membrane was attempted:

(a) Grade 0: Normal rectal wall (Fig. 1A) the long straight crypts of Lieberkühn of which show narrow lumina with high columnar goblet cells, apparently actively secreting.



Fig. 1. Photomicrographs showing the rectal mucous membrane of a rat, 71 h after irradiation with 2,000 rads of  $\gamma$ -rays (van Gieson stain  $\times 70$ ). A, Grade 0 damage of the 'deoxygenated' part of the rectum; B, grade ++ damage of the 'normally oxygenated' part of the rectum.

Table 1. SEMI-QUANTITATIVE COMPARISON OF CHANGES IN 'DEOXYGENATED' AND 'NORMALLY OXYGENATED' SEGMENTS OF THE RECTUM OF RATS IRRADIATED WITH PROTONS OR  $\gamma$ -RAYS

Experimental group	Dose in rads	Time (h) after irradiation					
		40-60		80-80		80-100	
		a	b	a	b	a	b
Rats irradiated with protons	1,400			+	++		
	2,000			+	++	+	+++
						+	+++
						+	+++
	2,400			+	++		
				+	++		
	2,800			+	++		
				+	++		
Rats irradiated with $\gamma$ -rays	2,000	0	0	0	++	0	+
		0	+	0	++	0	++

a, section from the 'deoxygenated' part of the rectum.

b, section from the 'normally oxygenated' part of the rectum.

0-5+, signify different grades of radiation-induced changes defined in the text.

(b) *Grade 1+*: The lumina of the bottoms of some of the crypts of Lieberkühn are slightly wide, apparently due to shrinkage of some of the epithelial cells.

(c) *Grade 2+*: The atrophy of the columnar goblet cell epithelium in the crypts of Lieberkühn is exaggerated with the formation of slightly cystic lumina (Fig. 1B).

(d) *Grade 3+*: Most of the epithelium in the mucosa is flattened or desquamated, leaving only small cystic remnants of the crypts surrounded by inflammatory cells. The surface epithelium is severely altered, showing polymorphism and hyperchromasia. A moderate oedema is present in the sub-mucous layer.

(e) *Grade 4+*: The mucosa is thin. The surface epithelium is absent or markedly atrophic and only single isolated epithelium-lined crypt-like structures occur. The sub-mucosa is markedly oedematous and invaded by inflammatory cells.

(f) *Grade 5+*: No epithelial structures occur in the atrophic mucosa which is covered by a fibrinous membrane containing granulocytes and cellular debris. Most of the mucosa is converted into a cellular granulation tissue containing small pools of mucin. The inflammatory cells of the markedly oedematous sub-mucous layer encroach on the muscularis propria, giving a phlegmonous inflammation of the rectal wall.

The sections were assessed blind and independently several times by two investigators.

The results are given in Table 1. In 82 per cent of the cases the changes in the 'deoxygenated' intestinal segments were one or two grades less than in those of the

'normally oxygenated' segments. In 18 per cent there was no clear difference between the two segments. In no case were the changes of the 'deoxygenated' segment more marked than in those of the 'normally oxygenated' one.

Fifteen rats were similarly treated with norepinephrine and sodium sulphite for 40 min without irradiation. Microscopic examination of the mucous membrane of those rats killed 3-38 days after treatment revealed no pathological changes.

The ability of norepinephrine and sodium sulphite to deoxygenate the rectal wall was estimated by electro-polarographic measurements<sup>9</sup> of the oxygen tension in the rectal lumen of normal anaesthetized rats. After introduction of the electrode and when constancy of the initial electrode potential, corresponding to about 20 mm mercury, had been established, cotton soaked in norepinephrine was carefully put around the rectum through an abdominal incision. 1 min later, 0.1 M sodium sulphite solution was dropped on the cotton for about 10 min. The oxygen tension was found to decrease during this treatment to less than 1 mm mercury within 7 min after application of the reducing solution to the exterior of the intestine. The experiment was repeated three times with essentially the same result.

From the results of these experiments we assume the protective effect of local application of norepinephrine and sodium sulphite demonstrated in the early interval after irradiation to be due to the hypoxic state of the tissue during irradiation. A practical consequence of this result might be that rectal damage can be reduced in patients irradiated with seemingly carcinocidal doses of high-energy protons<sup>10</sup> or other ionizing radiation of low linear transfer.

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## AN IMMEDIATE EFFECT OF X-RADIATION ON THYMUS CELLS

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THE lethal effects of X-irradiation on radiosensitive interphase cells have been investigated extensively with mammalian thymocytes, but the initial sequence of events leading to cell death is still uncertain. In 1959, Creasey and Stocken<sup>1</sup> reported that 'oxidative phosphorylation' in isolated thymus nuclei was rapidly abolished by low doses (25-100 rads) of X-irradiation administered either *in vitro* or *in vivo*. However, no evidence could be obtained for a similar effect of X-irradiation on the nuclei of intact thymus cells, and it was suggested, therefore, that the initial demonstration depended on loss of soluble materials from the isolated nuclei into the aqueous suspending medium following irradiation<sup>2</sup>. Further investigations have confirmed the absence of marked changes in intact thymus cells during the first hour after irradiation<sup>3-5</sup>, even though striking alterations are sometimes seen in the nuclei isolated from these cells<sup>7-12</sup>. Unfortunately the latter results are not always reproducible<sup>6</sup>.

In the investigation described here, an immediate effect of X-irradiation on one constituent of the cell nucleus could be demonstrated without any of the manipulations involved in isolation of the nuclei from irradiated cells.

It was noted previously that irradiation of rat thymocytes increased the speed with which the deoxyribonucleoprotein dissolved to form a gel in 2 M sodium chloride<sup>13</sup>. A similar effect can be demonstrated when using dilute (0.3 M) ammonium hydroxide<sup>14</sup> to dissolve the cells, but these gels have less reproducible results. This phenomenon has now been examined in more detail using 2 M sodium chloride at an alkaline pH and with a chelating agent present to increase the speed of gel formation. 2 ml. of cell suspension containing  $2 \times 10^7$ - $3 \times 10^7$  intact rat thymocytes in a standard Krebs-Ringer-phosphate solution<sup>4</sup> at 0° C were mixed rapidly with 3 ml. of a solution containing 3.33 M sodium chloride, 0.67 M *tris*(hydroxymethyl)aminomethane buffer and 0.084 M tetrasodium ethylenediamine tetraacetate (EDTA; versene) at pH 10.8 and room temperature. The mixture was allowed to stand at room temperature for a given period of time without further agitation and its apparent viscosity was then measured in duplicate by the method of Fisher *et al.*<sup>15,16</sup>. Microscopic examination of these mixtures showed that nearly all the cells disappeared within the first 5 min; since the viscosity continued to



increase markedly for many hours (Fig. 1), any differences in the rate of viscosity increase must be ascribed to differences in the rate at which the deoxyribonucleoprotein from the cell nucleus expanded to form a gel<sup>15,16</sup> in the alkaline sodium chloride solution. Cell suspensions were irradiated at 0° C with a 300-kV X-ray machine operated at 1.5 m.amp, the absorbed doses of X-radiation being measured with a ferrous sulphate dosimeter<sup>17</sup>. All doses, up to 1 krad were delivered in 1 min; higher doses were delivered at a rate of 1 krad/min.

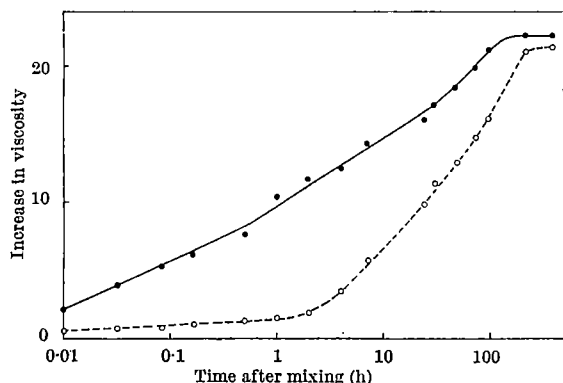


Fig. 1. Increase in apparent viscosity of gels formed from rat thymus cells in alkaline saline solution. The relative viscosities of the gels formed from control cell suspensions (○) and from the same cell suspensions after exposure to 1-krad X-radiation (●) were measured at room temperature at various times after mixing with alkaline saline as described in the text.

The relative viscosities of control and X-irradiated cell suspensions at various times after mixing with alkaline sodium chloride solution are shown in Fig. 1. In these experiments, the cell suspension and alkaline sodium chloride were mixed within 0.5 min after completion of a 1 krad X-radiation exposure. An appreciable alteration in viscosity due to irradiation of the intact cells was measured 0.5 min after mixing with alkaline sodium chloride, that is, within 1 min after irradiation. Since the results were essentially similar whether the cell suspensions were held at 0° C either for 0.5 min or for more extended periods of up to 2 h after irradiation before mixing with alkaline sodium chloride, it can be concluded that the viscosity increase represents an immediate effect of X-irradiation.

The rate of gel formation was appreciably affected by doses down to 35 rads of X-irradiation (Fig. 2). The radiation effect reached a maximum in the region of 1–2 krad; higher doses produced a marked decrease in gel viscosity (Fig. 2), presumably due to degradation of the long deoxyribonucleoprotein chains<sup>13,14</sup>.

This effect of X-radiation could also be demonstrated with cell suspensions prepared from the thymus gland of irradiated rats (Table 1). Cell suspensions prepared within 3 min after exposure of the whole animal to 1-krad X-irradiation exhibited the same increase in speed of gel formation as was observed after X-irradiation *in vitro*, again with little effect on the maximum viscosity of the gel after standing for longer periods in alkaline sodium chloride solution. When the irradiated rats were held for 1.5–7 h before preparation of the thymus cell suspensions, increasing degradation of the deoxyribonucleoprotein became evident (Table 1). A similar degeneration occurred when X-irradiated (1 krad) cell suspensions were incubated at 37° C *in vitro* for various periods of time before mixing with the alkaline sodium chloride solution. These *in vitro* experiments showed no measurable decrease in gel formation after 2, 5 or 10 min incubation of the cells at 37° C; the first small but consistent decrease was evident only after 20 min at 37° C. For the rest, the pattern of changes observed after *in-vitro* incubation of irradiated cells at 37° C paralleled closely that observed *in vivo* (Table 1).

The slow degradation of deoxyribonucleoprotein in intact irradiated thymocytes at 37° C is presumably due

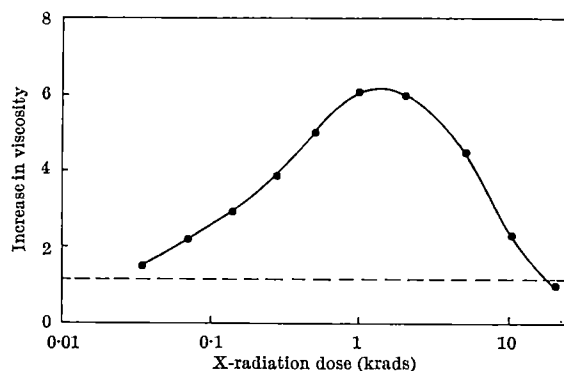


Fig. 2. Effect of X-irradiation dose on the speed of gel-formation from rat thymus cells in alkaline saline. Cell suspensions at 0° C were exposed to various doses of X-radiation and then mixed with alkaline saline solution at room temperature as described in the text. The relative viscosities of the deoxyribonucleoprotein gels were measured 5 min after mixing. The horizontal dotted line indicates the viscosity increase of the same cell suspensions before irradiation.

to enzymatic action. A similar degradation has also been demonstrated by other techniques<sup>5,17</sup> and is associated with gross changes in cell metabolism after 1 or more h at 37° C, either *in vitro* or *in vivo*<sup>4,12</sup>. However, an immediate effect of X-irradiation on these cells does not seem to have been demonstrated previously except in the more time-consuming and somewhat ambiguous experiments on isolated cell nuclei<sup>1,7,8,10</sup>. The work recorded here confirms the tentative conclusion that the nuclei of intact thymus cells are altered immediately after low doses of X-radiation.

Similar effects of X-irradiation can be demonstrated with freshly prepared mixtures of control thymus cells in alkaline saline solution. In this case, the *tris* buffer protects the cell residues against any indirect effects of the radiation<sup>16</sup>. When these preparations were exposed to 1-krad X-radiation 1 h after mixing, the apparent viscosity increased rapidly in much the same manner as it did (Fig. 1) after irradiation of intact cells. The dose-effect curve was also virtually identical with that shown in Fig. 2 for irradiation of intact cells, except that the apparent viscosity increased within 5 min to 3 instead of 6 times the corresponding value for non-irradiated preparations. In these experiments, the cells were no longer visible under the microscope, but presumably the islets of deoxyribonucleoprotein were still relatively intact at the time of irradiation.

If the control preparations in alkaline saline solution were allowed to stand for 10–14 days in order to reach maximum viscosities (Fig. 1) and were then irradiated, low doses of radiation had no appreciable effect on the apparent viscosity of the gel. Higher doses produced a decrease in viscosity which was independent of the time after X-irradiation. This decrease, which corresponds to the right-hand side of the dose-effect curve in Fig. 2, amounted to 50 per cent after exposure to 7 krad.

The increase in speed of gel formation after X-irradiation did not seem to be a general property of all cells. Homogenates of spleen, another radiosensitive organ, reacted in much the same manner as described here for thymus preparations. However, we have not as yet been able to demonstrate any striking effect of low radiation doses

Table 1. APPARENT VISCOSITY OF GELS FORMED FROM THYMUS CELLS AFTER X-IRRADIATION *in vivo*

Time after exposure to 1,000 r. (h)	No. of animals	Viscosity increase at various times after mixing with alkaline NaCl (h)		
		0.083	2	24
Control	14	0.9	1.6	8.3
0.05	9	3.9*	7.7*	12.3*
0.5	6	2.8*	5.4*	9.7
1.5	6	1.6*	3.8*	7.0
3.0	6	1.0	2.3	5.4*
5.0	4	0.4*	0.7*	1.6*
7.0	4	0.3*	—	0.8*

\* These values showed a statistically significant difference ( $P < 0.05$ ) from the corresponding control value.

All viscosities were measured with mixtures of 2-ml. cell suspension containing  $10^7$  cells/ml. plus 3 ml. of the NaCl-*tris*-EDTA solution described in the text.

on gel formation with homogenates of radio-resistant cells such as rat liver, rat kidney and chicken erythrocytes. In the case of the latter tissues, the only clear effect of X-radiation was the usual decrease in apparent viscosity after exposure to doses of 5–10 krad. The reason for this difference between the radiosensitive and radio-resistant tissues is still uncertain. Thymus nuclei do contain much more deoxyribonucleate per gram weight than do liver nuclei<sup>14</sup>, which suggests that the deoxyribonucleo-protein of control thymus cells must be more compactly coiled than that of control liver cells. The relative speed of gel formation in alkaline saline solution also tends to be slower with the thymus preparations. Possibly the molecular arrangement of the constituents of the irradiated thymus nuclei resembles more closely that of control liver nuclei. The data on metabolism of isolated nuclei<sup>1</sup> suggest a similar parallel between irradiated thymus and non-irradiated liver nuclei.

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## POLYMORPHISMS IN EGG ALBUMEN PROTEIN AND BEHAVIOUR IN THE EIDER DUCK

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**B**IOCHEMICAL polymorphism in animal populations presents several aspects. When the polymorphism is balanced we have the well-recognized problem of the role of natural selection, and this requires a search for correlations between gene frequency and differences in habitat, behaviour, susceptibility to disease and exposure, etc. On the other hand, heterogeneity in gene frequency between populations may often afford a useful indication of reproductive isolation, and the greater the number of loci at which such variation occurs the more precise are the tests for restriction of gene flow. This communication deals with a particularly well-marked example of the latter aspect; it is chiefly concerned with genetic polymorphism in egg-white proteins in the eider duck (*Somateria mollissima mollissima* L.).

Using starch-gel electrophoresis<sup>1</sup> and a two-phase buffer system<sup>2</sup>, and staining with amido-black, Robertson (unpublished) has examined several species of birds to see whether the kind of egg-white differences, reported by Lush<sup>3</sup> for the domestic fowl, commonly occur in wild species and, if so, whether this can be turned to advantage to shed light on the population structure of the species concerned. To ensure freshness, eggs were collected early in the laying season or before incubation had started. Generally one egg per clutch was examined after earlier work had shown that intra-clutch variation was unimportant; the phenotype of the albumen is determined by the genotype of the female parent. The species and the number of females scored in this way were as follows: herring gull (*Larus argentatus argentatus*), 196; lesser black-backed gull (*Larus fuscus graellsii*), 100; gannet (*Sula bassana*), 52; starling (*Sturnus vulgaris*), 30; great tit (*Parus major*), 19; and eider duck, 258. For the two gulls and the eider duck the eggs were collected from several widely separated localities. Only the eider showed evidence of egg-white variation. It is, of course, possible that minor differences were overlooked in the other species, since only one system of electrophoresis was used, but the heterogeneity in the eider duck was so well defined that it was the obvious candidate for further study.

This polymorphism presents an apparently simple Mendelian situation of one locus with two common

alleles which control differences in mobility of two protein types, as shown in Fig. 1. The alleles are referred to as *a* and *b* according to whether the protein they determine moves faster or slower. Equal mixture of albumen from the two alternative single-banded, presumably homozygous, types produces two less intensely stained bands which are indistinguishable from the naturally occurring double-banded, presumably heterozygous, types. In all but one of the samples from different localities, the frequencies of the alternative phenotypes conform to the Hardy-Weinberg distribution and this apparent exception is based on only a small sample (11 individuals). In addition to the three types just described, a fourth phenotype occurs very rarely (3 out of a total of 258 clutches scored) with two bands, one in the anterior position corresponding to the *a* band and the other behind the *b* position, as shown in Fig. 1. This slowest-moving band probably represents the effect of a third allele, *c*. So far no individuals have been found which would be identified as *cc* or *bc*.

Gene frequency has been estimated for five different breeding areas, namely Forvie (Aberdeenshire), Tentsmuir (Fife), and sites in Orkney, Iceland and Holland; the distribution of sites is shown in Fig. 2. The number of females scored and gene frequencies are set out in Table 1, which shows considerable variation between European populations. Excluding the Forvie samples, for the reasons described below,  $\chi^2$  for the differences between the other four localities is 20.9 for 3 degrees of freedom,  $P < 0.01$ . Types *ab* and *bb* were grouped in the analysis since the frequency of the *bb* type is very low. From these data, there is no evidence of a latitudinal cline since the gene frequency at the most southerly site in Fife ( $b = 0.09$ ) is very similar to that in the Iceland sample ( $b = 0.10$ ) and both populations conform to the Hardy-Weinberg distribution. We must now consider the situation in the Forvie colony, for which we have more detailed information.

Table 1. GENE FREQUENCY IN EIDER POPULATIONS

Site	N	Frequency of <i>b</i>
Tentsmuir	24	0.10
Orkney	36	0.35
Iceland	50	0.09
Holland	11	0.09*

\* Significant departure from Hardy-Weinberg distribution.

The breeding population of eiders on the Sands of Forvie is one of the largest, if not the largest, mainland colony in Britain. During summer the present population totals more than 3,000 birds, of which an estimated 1,000 pairs breed. About two-thirds of the population is migratory. The migrant birds leave the breeding grounds in July and August to overwinter apparently in the Firth of Tay. By marking individual birds, Milne (unpublished) has shown that the same individuals migrate south each autumn and return to breed the following spring, while the non-migrant birds remain at Forvie throughout the year. These two groups are referred to as 'migrant' and 'sedentary'. Field study of the sedentary flocks in the estuary of the River Ythan during winter showed that pair formation takes place during the period October–March inclusive, and that by April, when the migrants return, most of the sedentary group are already paired. The migrants pair during the period they are away from the breeding grounds. In three successive years they have been recorded arriving back at Forvie within a period of 2–3 days and 95 per cent were already paired. In view of this field evidence of reproductive isolation we decided to estimate the gene frequency in the two groups, and this posed the problem of distinguishing the nests of sedentary and migrant birds.

The Sands of Forvie National Nature Reserve, in which the eiders breed, is a wedge-shaped area of some 1,700 acres, bounded to the south and west by the estuary of the River Ythan and to the east by the North Sea. In winter most of the sedentary birds are to be found in the estuary with a few along the adjacent coast. Field observations suggested a strong tendency for the sedentary birds to nest on the west side of the moor near the estuary, while the migrants nest on the east side, nearer the sea; they overlap in the middle of the moor and records of marked individuals illustrate this difference (Table 2). In the seasons 1962 and 1963 eggs were collected separately from the estuary and the seaward area and the gene frequencies were compared. In 1962 an additional sample was taken from the middle region where overlapping was expected. Dealing first with the gene frequencies in the successive seasons on the west and east sides of the breeding area, we find no statistically significant difference in gene frequency between seasons within breeding areas (Table 3). The values of  $\chi^2$ , with  $ab$  and  $bb$  grouped, are 0.9 and 2.0 for a single degree of freedom in the estuary and seaward areas, respectively, and so the samples have been combined for the seasons to give a total of 51 and 67 clutches for the alternative groups which are believed to comprise almost entirely sedentary and migrant individuals. The gene frequencies are significantly different in the two groups, being respectively 0.14 and 0.27 for the less frequent  $b$  allele. Combining the  $ab$  and  $bb$  types as before,  $\chi^2$  for 1 degree of freedom = 5.7 ( $P < 0.5 > 0.02$ ). In addition, for season 1962, we have a sample of 19 from the middle region where overlap

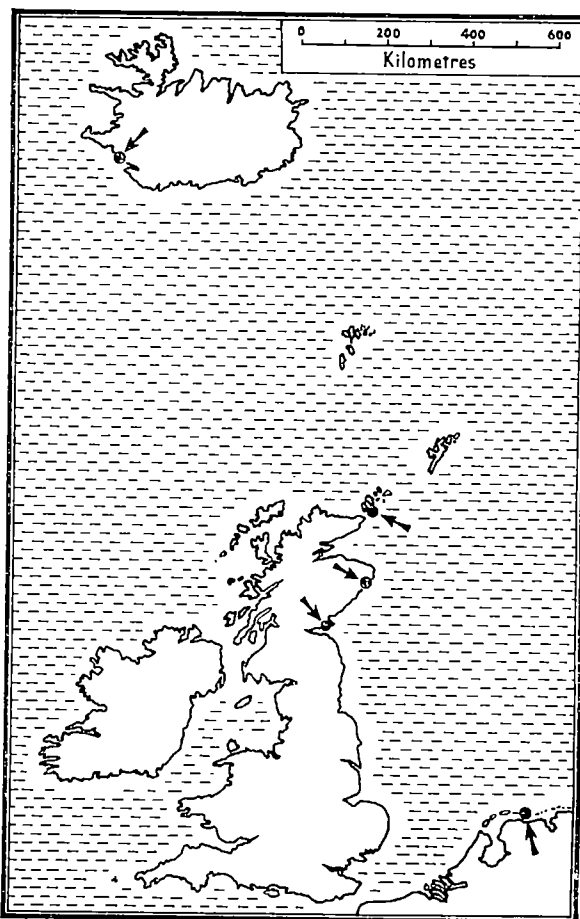


Fig. 2. The distribution of the sites from which eider eggs were collected. The Forvie colony is the more northerly of the two mainland Scottish sites.

occurs, and for this the frequency of  $b$  was estimated as 0.21, an intermediate value which is consistent with the general interpretation. Thus there is excellent evidence of effective reproductive isolation between the two groups of eiders which occupy the Forvie breeding area.

The nature and adaptive significance of this widespread protein polymorphism in the eider are at present unknown, but to clarify the situation the survey is being extended to other populations and geographical races. It would be particularly interesting to know whether there are other races with a high incidence of the  $c$  allele which is so rare in the European populations already sampled. In addition to the protein polymorphism we have also the behavioural polymorphism in the Forvie colony, and it remains to be seen whether this phenomenon also occurs in other colonies. The origin of this behavioural difference, and whether or not it has a genetic basis, is a matter for speculation.

Table 2. NEST SITES OF SEDENTARY AND MIGRANT EIDER DUCK AT FORVIE

	Nesting near estuary	Nesting near sea
Resident on estuary in winter	15	2
Not resident on estuary in winter*	1	14

\* This group refers to marked birds which were never recorded in the estuary during the period November to March inclusive.

Table 3. GENE FREQUENCY IN SEDENTARY AND MIGRANT EIDERS IN THE FORVIE COLONY

Year	Sedentary			N	Frequency of $b$
	$aa$	$ab$	$bb$		
1962	18	3	1	22	0.11
1963	21	7	1	29	0.16
Migrant					
1962	16	12	1	29	0.24
1963	21	12	5	38	0.29
Seasons combined					
Sedentary	39	10	2	51	0.14
Migrant	37	24	6	67	0.27

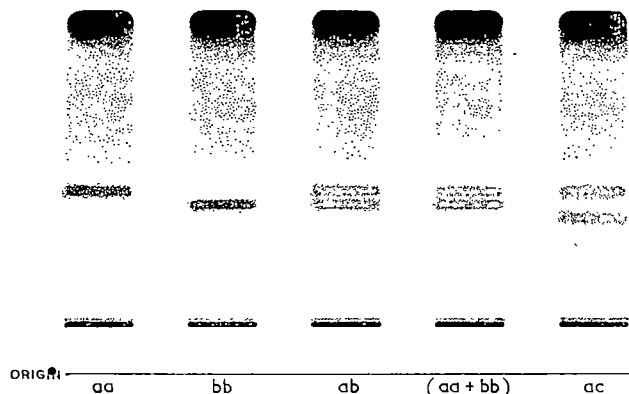


Fig. 1. The appearance presented by the different genotypes in starch-gel section, stained with amido-black. Equal mixture of albumen from the homozygotes is labelled as  $(aa + bb)$ .



Lack<sup>4</sup> has directed attention to the common occurrence of partial migration in various passerine and limicoline species. In the cases considered by Lack, the migrants travel much farther than the migrant eiders, generally to France, Spain and Portugal, while in some species there is a further group which migrates westward to Ireland, that is, the population may be di- or tri-morphic with respect to where the winter is spent. It is not yet known whether heterogeneity in behaviour is a regular feature of eider populations or peculiar to this local population due to particular historical or ecological factors. Conceivably migration might reduce food competition in the more sheltered estuary during winter, so that there could be some advantage to the population as a whole in limiting such (admittedly quite hypothetical) inter-individual competition. In view of their relevance to the stability of population size and density-determining factors, it would be valuable to check the proportions of sedentary and migrant birds in future seasons and record,

at the same time, the frequency of the egg-white alleles in the two groups.

These observations illustrate the advantages of combining ecological and genetic data in the study of animal populations. There is little doubt that the detection of biochemical variation of various kinds will be of great help in the analysis of population structure, not least by providing critical evidence for reproductive isolation in situations where this may be unsuspected or difficult to assess by more usual ecological methods.

We thank the various people who assisted in the collection of eider material or in other ways, especially Drs. H. N. Kluyver and J. Westhoff (Holland), Dr. F. Gudmundsson (Iceland), Mr. E. Balfour (Orkney) and members of the Scottish division of the Nature Conservancy.

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## SIMIAN-TYPE BLOOD GROUP FACTORS IN NON-HUMAN PRIMATES

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IN previous papers<sup>1-3</sup>, observations were described on human-type blood group factors in non-human primates, summarizing the results of two years of investigations of large series of apes and monkeys tested with suitably prepared reagents, originally produced for testing human blood. These tests demonstrated in the red cells and saliva of numerous species of non-human primates the presence of antigens serologically related to or indistinguishable from blood group antigens in man.

At the same time two other parallel investigations have been carried out.

(1) The foregoing procedure of testing of apes and monkeys with human blood grouping antisera has been reversed, that is, iso- and hetero-immune reagents were prepared against red cells of non-human primates<sup>3</sup>, with the view that some might cross-react with human red cells, and, eventually, disclose individual differences in human blood.

The blood factors determined by the reagents so obtained, type specific for the red cells of the homologous simian species, have been designated by us as "simian-type" to distinguish them from human-type blood factors such as A, B, M, N,\* etc., found also in non-human primates<sup>1-3</sup>.

(2) In another series of experiments, various species of primates have been cross-immunized with red cells of one another, in order to produce antisera capable of detecting individual differences in red cells of closely related species of primates including man. Such antisera were expected by us to be type-specific in tests on red cells from species closely related to the homologous red cell donor, while in tests on the homologous donor species, the selective type-specific reaction would tend to be obscured by the stronger, but not type-specific, anti-species reactions.

The type-specific antigen-antibody reactions of such antisera have been designated by us as 'cross-immune' to indicate the special immunological behaviour of reactions

between closely related species, which are different in important respects from other hetero-immune reactions, yet do not fall into the iso-immune category.

The original Rh-factor may be considered the first simian-type blood factor discovered<sup>10</sup>, because the original reagents were prepared by immunization of rabbits and guinea-pigs with rhesus monkey red cells.

More recently, an analogous cross-reacting situation was demonstrated by iso-precipitins produced in baboons, which detected individual differences in sera of man and other primates, while, conversely, human iso-precipitins resulting from blood transfusions detected individual differences in the sera of non-human primates. Moreover, baboons immunized with human sera have produced cross-immune precipitins type specific for sera of man and of other primates<sup>11-13</sup>.

So far observations have been made on simian-type and on cross-immune blood factors in chimpanzees, gibbons and Celebes black apes. The information gained from our previous work on human-type blood groups in these species makes possible a critical comparative evaluation of the newly discovered blood factors.

The purpose of this article is to present a preliminary survey of these most recent findings.

*Chimpanzees.* Individual differences in chimpanzee red cells have been demonstrated with reagents prepared by more than one method.

The first useful reagent proved to be anti-NV lectin from *Vicia graminea* seeds<sup>14</sup>, used in our previous investigations on human-type blood groups in chimpanzees<sup>15</sup>. The so determined human-type blood factor NV plays an important part also in a simian-type blood group system in chimpanzees, as will be shown later.

The second set of reagents was prepared by iso-immunization of chimpanzees. The antisera obtained detected simian-type blood factors designated A<sup>c</sup>, B<sup>c</sup>, C<sup>c</sup> (refs. 16 and 17) and, more recently, E<sup>c</sup> and two antisera temporarily designated as 'John' and 'Bill'. The superscript 'c' indicates the chimpanzee origin of these blood factors, and serves to distinguish them from blood factors of man as well as simian-type blood factors of other primates.

\* To avoid ambiguity, symbols for blood factors and their corresponding antibodies are printed in bold type, symbols for genes and genotypes are printed in italics, and symbols for agglutinogens, phenotypes and blood-group systems are printed in regular type.

Table 1. SIMIAN-TYPE AND CROSS-REACTING PRIMATE BLOOD FACTORS OF NON-HUMAN PRIMATE BLOOD

Species	Source or nature of the reagents	Blood factors found
Chimpanzees <i>Pan satyrus</i>	<i>Vicia graminea</i> seeds and some rabbit hetero-immune anti-human N	N <sup>v</sup> (V)
	Iso-immune chimpanzees	A <sup>c</sup> , B <sup>c</sup> , C <sup>c</sup> , E <sup>c</sup> , John, Bill, Chica
	Cross-immune chimpanzees (immunized with human red cells)	V <sub>1</sub> , Ginga, Mikel
Gibbons <i>Hylobates lar pileatus</i> <i>Hylobates lar lar</i> Celebes black apes <i>Cynopithecus niger</i>	Iso-immune	A <sup>c</sup> B <sup>c</sup>
	Rabbit hetero-immune	A <sup>ba</sup>

Additional type-specific blood factors of chimpanzee red cells have been detected with cross-immune sera from chimpanzees immunized with human red cells. One of these blood factors has proved to be related to the human-type blood factor NV like, in man, blood group A<sub>1</sub> is related to A. Therefore, the factor NV is now called by us v, and the newly discovered simian blood factor is designated as V<sub>1</sub>. The other cross-immune blood factors discovered more recently are temporarily identified by the name of the immune chimpanzees 'Chica', 'Ginga', and 'Mikel' (Table 1).

It is of interest that none of the cross-immune antisera is type-specific for man, but all agglutinate all human red cells tested so far. Perhaps, by selective absorption of the species heteroagglutinins, these antisera can be rendered type-specific for human red cells.

Of the 11 blood factors of chimpanzee red cells shown in Table 1, four are of special interest since they evidently belong to the same blood group system. The four blood factors are V, V<sub>1</sub>, A<sup>c</sup> and B<sup>c</sup>, which determine the system we have designated the V-A-B system of chimpanzees. That the factors V, V<sub>1</sub>, A<sup>c</sup> and B<sup>c</sup> belong to the same blood group system has been established by testing series of chimpanzees and submitting the data to gene frequency analysis<sup>18</sup>. Additional data on which this conclusion is based are shown in Table 2, where the results of tests for blood factor V<sub>1</sub>, A<sup>c</sup> and B<sup>c</sup> on series of 133 chimpanzees are presented. These three factors determine 8 blood types inherited by 6 allelic genes: V<sup>0</sup>, V<sup>A</sup>, V<sup>B</sup>, v<sup>0</sup>, v<sup>A</sup>, and v<sup>B</sup>. Gene frequency analysis shows that the theoretically possible additional allelic genes V<sup>AB</sup> and v<sup>AB</sup> are either non-existent or quite rare. When all four blood factors V, V<sub>1</sub>, A<sup>c</sup> and B<sup>c</sup> are taken into account, then 12 phenotypes can be distinguished, and at least 9 allelic genes v<sup>0</sup>, v<sup>A</sup>, v<sup>B</sup>, V<sup>10</sup>, V<sup>1A</sup>, V<sup>1B</sup>, V<sup>20</sup>, V<sup>2A</sup>, V<sup>2B</sup> may be postulated, determining 45 genotypes.

The serology and genetics of the V-A-B blood group system of chimpanzees are in many ways comparable to that of the Rh-Hr blood types of man. Nevertheless, there appears to be no relationship between the Rh-Hr system in man and the V-A-B system in chimpanzees. Instead, the V-A-B system seems to be the counterpart in chimpanzees of the human M-N-S blood group system. The blood factor V, which holds a key position in the V-A-B system, is detected by anti-NV lectin, which also detects the agglutinin N of the human M-N-S system. Like the tests for human M and N agglutinin, so also the test for V factor of chimpanzee is adversely affected when the red cells are treated with the proteolytic enzyme ficin. Moreover, the serological behaviour of anti-A<sup>c</sup> and anti-B<sup>c</sup> is similar to that of anti-S and anti-s of the human M-N-S system.

It is thus apparent that the V-A-B blood group system of chimpanzees comprises blood factors demonstrable by lectin, by iso-immune sera and by cross-immune sera. This is confirmatory of our hypothesis of the relationship among antigens in closely related species. The next logical step, namely, tests on human red cells with simian antisera, is at present under investigation.

Anti-C<sup>c</sup> serum, in contrast to the antisera of the V-A-B system, reacts well by the ficin technique, and the corresponding blood factor is distributed independently of the V-A-B blood group system. Approximately half the 133 chimpanzees tested so far have the C<sup>c</sup> factor.

Chimpanzees tested for the more recently discovered blood factors E<sup>c</sup>, 'John', 'Bill', 'Ginga', 'Chica' and 'Mikel' shown in Table 2, are still too few in number to allow any definite conclusions as to the relationship of these factors to the V-A-B system, to the C<sup>c</sup> blood factor and to one another. However, it is possible already with the antisera so far developed to distinguish more than 1,000 types of chimpanzee blood.

**Gibbons.** In gibbons (*Hylobates lar lar* and *Hylobates lar pileatus*), antisera of two distinct specificities have been obtained by iso-immunization. The antisera have been designated as anti-A<sup>g</sup> and anti-B<sup>g</sup>, the superscript 'g' representing the gibbon origin of the homologous antigen. So far, only eight gibbons have been available for testing with these reagents, so that no definite conclusions can be drawn as to their relationship.

Among the eight gibbons tested, the simian blood factor of gibbons A<sup>g</sup> is present in seven, and the factor B<sup>g</sup> in three animals.

Tests with anti-A<sup>g</sup> and anti-B<sup>g</sup> on red cells of other primates, including man, have been carried out only to a limited extent and do not permit any valid conclusions.

**Celebes black apes.** Rabbits have been immunized with red cells of Celebes black apes (*Cynopithecus niger*). From one of the rabbit antisera a type-specific reagent has been prepared by selective absorption with red cells of Celebes black apes. The reagent defined individual differences in the red cells of these monkeys and has been designated by us as anti-A<sup>ba</sup>, the superscript 'ba' representing the black ape origin of the homologous antigen. The simian-type blood factor A<sup>ba</sup> has been demonstrated on the red cells of four out of the ten animals tested. Further absorption experiments on other rabbit antisera are being carried out, as well as tests for cross-reacting activity with red cells of other primates.

The results obtained so far on chimpanzees, gibbons and Celebes black apes appear confirmatory of our initial hypothesis. The experiments are being continued.

We thank the following for samples of the animals tested: Dr. Arthur J. Riopelle, Dr. Charles W. Hill and Mr. Frank Shell, of the Delta Regional Primate Research Center of Tulane University, New Orleans, Louisiana; Dr. Clyde H. Kratochvil, Holloman Air Force Base, New Mexico; Dr. Charles W. DeWitt, Department of Surgery, Tulane University, New Orleans, Louisiana; Dr. Maitland Baldwin, National Institute of Neurological Diseases and Blindness, Bethesda, Maryland. Immunization of gibbons was carried out for us at the Delta Regional Primate Research Center.

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Table 2. THE V-A-B BLOOD GROUP SYSTEM OF CHIMPANZEES (WIENER THEORY)

Pheno- types*	No. of chim- panzees	Reactions with	Corresponding genotypes
		Anti-V <sub>1</sub> Anti-A <sup>c</sup> Anti-B <sup>c</sup>	
v.O	4	- - -	v <sup>0</sup> v <sup>0</sup>
v.A	21	- + -	v <sup>A</sup> v <sup>A</sup> and v <sup>A</sup> v <sup>0</sup>
v.B	36	- - +	v <sup>B</sup> v <sup>B</sup> and v <sup>B</sup> v <sup>0</sup>
v.AB	25	- + +	v <sup>A</sup> v <sup>B</sup>
V <sub>1</sub> .O	16	+ - -	V <sup>0</sup> V <sup>0</sup> and V <sup>0</sup> v <sup>0</sup>
V <sub>1</sub> .A	11	+ + -	V <sup>A</sup> V <sup>A</sup> , V <sup>A</sup> v <sup>A</sup> , V <sup>A</sup> V <sup>0</sup> , V <sup>A</sup> v <sup>0</sup> and V <sup>0</sup> v <sup>A</sup>
V <sub>1</sub> .B	19	+ - +	V <sup>B</sup> V <sup>B</sup> , V <sup>B</sup> v <sup>B</sup> , V <sup>B</sup> V <sup>0</sup> , V <sup>B</sup> v <sup>0</sup> and V <sup>0</sup> v <sup>B</sup>
V.AB	1	+ + +	V <sup>A</sup> V <sup>B</sup> , V <sup>A</sup> v <sup>B</sup> , V <sup>B</sup> v <sup>A</sup>

\* Since the reactions of anti-N<sup>v</sup> (V) have been omitted, type v actually includes types V<sub>1</sub> and v. Thus, when the reactions of anti-V are taken into account, there are 12 instead of 8 phenotypes, with 45 theoretically possible genotypes.

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## MYCOPLASMA (PLEUROPNEUMONIA-LIKE ORGANISMS) AND BLOOD GROUP I; ASSOCIATIONS WITH NEOPLASTIC DISEASE

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WE have reported that an association exists between the red blood cell group I and neoplastic disease, particularly leukaemia<sup>1</sup>. Whereas normal donors are rarely I negative (< 0.1 per cent), we found 38 of 124 patients with leukaemia to be I negative. Fluctuations in antigen I reactivity during exacerbation and remission in these patients suggested to us that the I antigen was blocked or destroyed during the course of the disease. There is one known relationship between an infectious disease and the I blood group, that is, the cold agglutinins of primary atypical pneumonia often have I specificity<sup>2</sup>. This prompted us to explore the disease relationship we observed in terms of possible association with micro-organisms.

In the work recorded here, 45 microbial agents including mycoplasma (PPLO or pleuropneumonia-like organisms), viruses and bacteria were added *in vitro* to I-positive red blood cells from normal donors to determine whether these agents could alter the I agglutinability of these cells. Eighteen of 25 mycoplasma tested, including three mycoplasma derived from human tumour tissue could block or destroy the I receptors of normal red cells *in vitro* and mimic our findings on patients with leukaemia.

The anti-I reagent was a  $\gamma$ -macroglobulin fraction of an isoimmune serum from an I-negative patient<sup>1</sup>. Parallel control tests were done with a human anti-A<sub>1</sub> (absorbed B) reagent.

The mycoplasma and bacteria were grown in broth medium containing a brain heart infusion base (Baltimore Biologics Laboratory), 15 per cent non-inactivated horse serum and 1 per cent yeast extract, incubated aerobically at 37° C for 3–5 days. The mycoplasma were sedimented by centrifugation at 27,000*g*, re-suspended in physiological saline at a 100-fold concentration and disrupted by freezing and thawing twice before use. The bacteria were sedimented at 8,000*g* and then treated in the same manner. The viral preparations were tissue culture suspensions except for the influenza viruses, which were chick embryo suspensions. They were also exposed to freezing and thawing. These microbial preparations had population titres ranging from 10<sup>7</sup> to 10<sup>9</sup> organisms per ml.

In the test system, one volume of the microbial material was added to an equal volume of a 2 per cent saline suspension of washed day-old I-positive human group A<sub>1</sub> red

cells. The mixture was incubated at 22° C for 30 min and then washed three times in a large quantity of saline and reconstituted to a 2 per cent suspension. One drop of treated cell suspension was mixed with one drop of agglutinating reagent. The tubes were incubated at 22° C for 30 min and centrifuged. Macroscopic agglutination (+++) occurred with saline controls and was used as evidence for no inhibition. Lesser agglutination requiring microscopic examination indicated inhibition and was scored as trace inhibition (++) agglutination) or strong inhibition (+ or negative agglutination). All tests were performed in parallel with cells and reagents from the same lots. Control tests were done using human group AB (non-immune) serum to detect any haemagglutination due to the microbial agents themselves. Broth medium and its yeast component were also tested for activity. After the preliminary experiments, the microbial materials and controls were number-coded before test and duplicate samples were intentionally included.

A total of 45 strains were tested as 58 unknowns for activity in both the I and A blood group systems. These included 25 mycoplasma strains (9 human, 5 murine, 2 avian and 1 each canine, calf and goat, 3 tissue culture and 3 saprophytes), 11 viral agents and 9 bacteria. Seven of the bacteria were non-specifically active, that is, caused pan-agglutination as in the Hubener-Thompson phenomenon<sup>3</sup> when the group AB serum was added. These latter are not listed therefore in Table 1, which shows results of specific activity in the I blood group system of tested material. None of the other agents caused spontaneous haemagglutination with the exception of one influenza strain.

Anti-I agglutination was inhibited by 18 of the 25 mycoplasma strains tested (72 per cent); 7 of the 9 human strains were active (Table 1). One of the 11 viral agents had trace activity. *Streptococcus MG*, an organism used in serological tests for primary atypical pneumonia, was inactive. No inhibition of anti-A<sub>1</sub> was seen. (In other investigations, agglutination by anti-Lewis sera, Le<sup>b</sup> and Le<sup>a</sup>, was not inhibited.)

The I agglutinability of red cells was altered by treatment with mycoplasma material for as little as 30 min. Stronger inhibition resulted when the treated washed cells were held overnight at 5° C. This may explain the vari-



ability of results seen with some of the mycoplasma, especially *M. hominis*, 2, and strain Rab-32.

It was not possible to demonstrate inhibition when mycoplasma were mixed with anti-I before addition of the indicator red cells. This indicates that the effect was not due to serologic cross-reactivity between PPLO and anti-I but was an alteration of the I receptor sites on the red cell.

Cold haemagglutinins are well known in patients with malignant disease of the lympho-reticular system and primary atypical pneumonia. These agglutinins often have anti-I specificity<sup>2</sup>. In primary atypical pneumonia it is presumed that *Mycoplasma pneumoniae*, the causative agent<sup>4</sup>, is responsible for the development of the cold agglutinins. *Mycoplasma pneumoniae* and other PPLO produce haemolysis in culture<sup>5</sup>; others produce haemadsorption<sup>6</sup>.

Mycoplasma have been isolated from human tumour tissue. Horoszewicz recovered mycoplasma strain 880 from the spleen of a patient with chronic lymphocytic leukaemia<sup>7</sup>, and one of us (M. F. B.) isolated mycoplasma strain A63-17 from lung tumour tissue of a patient with Hodgkin's disease and mycoplasma strain RI-12 from tissues of a patient with histiocytoma. These three strains of mycoplasma were among the agents showing the strongest inhibitory activity in the anti-I agglutination system.

Virus-like agents have been demonstrated in cell cultures and virus-like particles seen on electron microscopy of blood and bone marrow from patients with leukaemia<sup>8-12</sup>. Known mycoplasma under electron microscopy using the negative staining technique can appear quite similar to these particles<sup>13</sup>. Dmochowski reports that the blood of leukaemic patients which has virus-like particles also contains mycoplasma<sup>14</sup>. Murphy reported that his agent(s) which produces a fatal leukaemoid disease in mice is either a mycoplasma, an unidentified virus or both<sup>15</sup>.

Table 1. MICROBIAL INHIBITION OF THE AGGLUTINATION BY ANTI-I

Source	Identification	Inhibition of anti-I		
		None	Trace	Strong
<i>Mycoplasma</i>				
Human sarcoma	Strain A63-17			XXX
Human leukaemia	Strain 880			XX
Human histiocytoma	Strain RI-12		X	XX
Human pneumonia	<i>M. pneumoniae</i>			XX
Human synovitis	Strain 6R		X	X
Human penis	<i>M. fermentans</i>		X	X
Human mouth	<i>M. salivarium</i>	X		
Human urethra	<i>M. hominis</i> , 1	X		
Human urethra	<i>M. hominis</i> , 2	X		X
Murine arthritis	<i>M. arthritis</i>			
Murine arthritis	Strain JR-3		X	
Murine arthritis	Strain Preston	X		
Murine lung	<i>M. pulmonis</i>	X		
Murine brain	<i>M. neurolyticum</i>			
Murine brain	Strain PG-28	X		
Murine brain	Strain A		X	
Avian lung	<i>M. gallinarum</i>	X		
Avian sinusitis	Strain NTF		X	X
Canine	<i>M. spumans</i>		X	X
Bovine	Strain 'calf'		X	
Goat	Strain 'kid'			X
Tissue cell culture	Strain RK-13	X		
Tissue cell culture	Strain Hep-2			XX
Tissue cell culture	Rab-32	X		X
Saprophyte	<i>M. laidlawii</i> , A	X	X	
Saprophyte	<i>M. laidlawii</i> , B		XX	
Saprophyte	Strain Laidlaw			X
<i>Virus</i>				
Poliovirus	Type 1, TA 2	X		
Poliovirus	Type 2, TB 2		X	
Poliovirus	Type 3, TC 2	X		
Adenovirus	Type 3, GB	X		
Adenovirus	Type 4, MA	X		
Adenovirus	Type 7, LL	X		
Influenza	A/swine/1976/31	X		
Influenza	A2/Japan/305/57	X		
Influenza	A2/Japan/170/62	X		
Influenza	B/Maryland/1/59	X		
Smallpox	Reference Lot 2	X		
<i>Bacteria</i>				
	<i>A. aerogenes</i>	X		
	<i>Streptococcus MG</i>	X		
<i>Control</i>				
	Broth medium	X		
	Yeast extr. 25%	X		

X = individual test result on separate specimen.

Our findings suggest that an association may exist between the red cell antigen I, mycoplasma and leukaemia. Of course, it does not follow necessarily that mycoplasma are involved in the pathogenesis of this disease. Mycoplasma are important agents in veterinary medicine. They have been isolated from patients with non-gonococcal urethritis<sup>16</sup>, Reiter's syndrome, rheumatoid arthritis and lupus erythematosus<sup>17</sup>. However, primary atypical pneumonia remains the only disease of man known to be caused by mycoplasma. Thomas has suggested that since lung tissue antibody appears in patients with primary atypical pneumonia, the possibility that infection with mycoplasma is implicated in conditions characterized by auto-immune serologic reactions requires further investigation<sup>18</sup>. Our finding that red cell antigen I can be altered *in vitro* by mycoplasma when considered with the findings of both mycoplasma<sup>4</sup> and anti-I antibodies<sup>2</sup> in primary atypical pneumonia is an illustration of this concept.

The *in vitro* interference with anti-I agglutination by mycoplasma presents a working hypothesis to explain the inagglutinability by anti-I of red cells of patients with leukaemia. We suggest that although mycoplasma may not be primary agents in human neoplastic disease, they might be opportunist or secondary invaders. They could be involved in combination with another (viral?) agent, as in the mouse hepatitis system<sup>19</sup> or the Rous-helper system<sup>20</sup>.

One other microbial agent is known to alter the I red cell antigen. Marcus<sup>21</sup> found that filtrates of *Clostridium tertium* can destroy the I determinant. These filtrates were specific for I but were more active against the I factor of group A cells than of group O cells. The activity was probably enzymatic. We have found that mycoplasma can alter I antigen reactivity and that they are also more active against the I factor of group A cells than of group O cells. PPLO are enzymatically active, especially in the arginine dehydrolase system<sup>22</sup>, and the *in-vitro* effect we have observed may be due to an enzymatic degradation of the I antigen determinant on normal cells.

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## LETTERS TO THE EDITOR

## RADIO ASTRONOMY

## Observations of the Quasi-stellar and Other Radio Sources with an Interferometer of Resolving Power 0.4 sec of Arc

OBSERVATIONS made previously at Jodrell Bank with long base-line interferometers<sup>1</sup> have indicated that a small percentage of 384 sources examined with these interferometers have angular sizes of less than 1.0 sec of arc. Six were found to have fringe visibilities ( $\gamma$ ) > 0.8 for transit observations with an east-west base-line of 61,100 wave-lengths.

In order to examine such small sources with an instrument of still higher resolving power, several improvements have now been made to the basic interferometer<sup>2</sup>. The frequency of observation has been changed from 159

Mc/s to 408 Mc/s ( $\lambda = 0.73$  m) and this has enabled low-noise varactor diode parametric amplifiers to be used to greatest advantage. In addition, a transportable and fully steerable 25-ft. diameter radio telescope was constructed for the reception of the radio signals at the outstation, and a slightly more distant site was obtained near Pocklington, Yorkshire, 180,000 wave-lengths (132 km) to the north-east of the 250-ft. diameter *Mark I* radio telescope at Jodrell Bank which was used as the other element of the interferometer.

The transportable alt-azimuth telescope was remotely controlled by radio signals transmitted from Jodrell Bank, and, when it was so desired, an electro-mechanical analogue device at the outstation could steer that telescope to follow automatically any point in the sky, with an accuracy of  $\pm 2^\circ$ . A microwave link was installed to transmit to Jodrell Bank the radio signals received by the

Table 1. QUASI-STELLAR SOURCES

Source and $Z$	Summary of previous interferometric observations with $\lambda = 1.9$ m	Radio observation with tracking interferometer of aerial spacing 180,000 $\lambda$ ( $\lambda = 0.73$ m)	Provisional interpretation of radio measurements
3C 286 $Z = 0.86?$ (See note 4)	$\gamma \geq 0.8$ at all base-lines up to 61,100 $\lambda$	High $\gamma$ . No variation with hour angle (H.A.)	Not significantly resolved. Dimensions $\leq 0.4''$ . See note 1
3C 147 $Z = 0.545$	$\gamma$ fell to 0.5 at 61,100 $\lambda$	High $\gamma$ . Variation with H.A.	Dimensions $\sim 0.6''$
3C 47 $Z = 0.425$	$\gamma = 0.3$ at baselines of 2,200 and 9,700 $\lambda$	No fringes observed at this base-line	Halo radiating 70% of flux: dimensions $\geq 60''$ . Core radiating 30% of flux: dimensions $< 10''$ but $> 2''$
3C 48 $Z = 0.367$	$\gamma \geq 0.8$ at all base-lines up to 61,100 $\lambda$	High $\gamma$ . Small variation with H.A.	Source dimensions $\sim 0.5''$
3C 273 $Z = 0.158$	$\gamma = 0.5$ at 9,700 $\lambda$ $\gamma = 0.3$ at 32,000 $\lambda$ $\gamma < 0.1$ at 61,100 $\lambda$	$\gamma$ exhibited pronounced minima at some H.A.s	Double source of component separation $20''$ . Both components have appreciable structure $\leq 1''$ , and equal intensity when resolution 75,000 $\lambda$ N.-S. Good agreement with Hazard's occultation results
3C 9	$\gamma < 0.4$ at 9,700 $\lambda$	No fringes observed at this base-line. See note 2	Source dimensions $> 10''$
3C 93	Not previously observed	No fringes observed at this base-line. See note 2	Dimensions $> 1''$
3C 196	$\gamma$ fell to 0.2 at 32,000 $\lambda$ and 0.3 at 61,100 $\lambda$	Fringes of low $\gamma$ at some H.A.s	Possibly double source
3C 208	$\gamma = 0.2$ at 32,000 $\lambda$	No fringes observed at this base-line. See note 2	Dimensions $> 4''$
3C 216	$\gamma$ fell to 0.4 at 32,000 $\lambda$	No fringes observed at this base-line	Dimensions $> 3''$
3C 228	$\gamma = 0.3$ at 9,700 $\lambda$	No fringes observed at this base-line	Dimensions $> 12''$
3C 245	$\gamma$ fell to 0.3 at 9,700 $\lambda$	No fringes observed at this base-line	Dimensions $> 12''$
3C 287	$\gamma > 0.8$ at all base-lines except 32,000 $\lambda$ which is based on 1 measurement and may be in error	High $\gamma$ . No variation with H.A.	Not significantly resolved. Dimensions $\leq 0.4''$ . See note 1

See legend to Table 2.

Table 2. SOURCES NOT YET IDENTIFIED (see note 5)

Source	Summary of previous interferometric observations with $\lambda = 1.9$ m	Radio observation with tracking interferometer of aerial spacing 180,000 $\lambda$ ( $\lambda = 0.73$ m)	Provisional interpretation of radio measurements
CTA 21	Not previously studied with high-resolution interferometers	High $\gamma$ . No variation with H.A.	Not significantly resolved. $\leq 0.4''$ . See note 1
CTA 102	Not previously studied with high-resolution interferometers	High $\gamma$ . Not observed at all H.A.s because of poor signal-to-noise ratio	Possibly unresolved. $\leq 0.4''$ . See note 1
3C 119	$\gamma > 0.8$ at all base-lines up to 61,100 $\lambda$	High $\gamma$ . No variation with H.A.	Not significantly resolved. $\leq 0.4''$ . See note 1
3C 138	Not seen previously. See note 3	High $\gamma$ . Small variation with H.A.	Dimensions $\sim 0.5''$
3C 161	$\gamma = 0.1$ at 32,000 $\lambda$ $\gamma = 0.1$ at 61,100 $\lambda$	Small variation of $\gamma$ with H.A.	A component of dimensions $\sim 0.5''$ radiates $\sim 50\%$ of flux at 408 Mc/s. Marked change of intensity with frequency: at 160 Mc/s this component radiates only $\sim 10\%$ of flux
3C 225	$\gamma \sim 0.8$ at all base-lines 2,200 $\lambda$ to 61,100 $\lambda$	Fringes seen at some H.A.s	A component of dimensions $\sim 0.6''$
3C 237	High $\gamma$ out to 61,100 $\lambda$	High $\gamma$ . Variation with H.A.	Possibly double, or elongated
3C 283	$\gamma < 0.1$ at 32,000 $\lambda$ $\gamma = 0.2$ at 61,100 $\lambda$	High $\gamma$ over the restricted range of resolving powers at which it was observable	A component of dimensions $< 0.6''$
3C 298	$\gamma$ fell to 0.6 at 32,000 $\lambda$ 0.1 at 61,100 $\lambda$	Variation of $\gamma$ with H.A.	Possibly double

## Notes to Tables 1 and 2:

(1) The upper limit to the angular dimensions depends on the lower limit of  $\gamma$  and the source model assumed. The value quoted is the maximum diameter (to half-intensity points) that gives  $\gamma \geq 0.7$  for a source with Gaussian brightness distribution when observed with an interferometer of aerial spacing 180,000  $\lambda$ .  $\gamma = 0.7$  allows a generous margin for calibration and measurement errors. It may well be that  $\gamma$  is greater than 0.8 corresponding to dimensions of less than  $0.3''$ .

(2) A source of low flux density which would have given a poor signal-to-noise ratio even if unresolved.

(3) The position of this source as given in the original 3C catalogue<sup>10</sup> is in error by 1 lobe shift. The 408 Mc/s observations were made of the corrected position<sup>11</sup>.

(4) This value of the red-shift, suggested by I. Shklovsky, is not yet generally accepted.

(5) At the Second Texas Conference on Relativistic Astrophysics in December 1964, Dr. Alan Sandage announced that 22 more radio sources had been identified with quasi-stellar objects. Of these, 3 appear in Table 2 (CTA 102, 3C 237 and 3C 298).

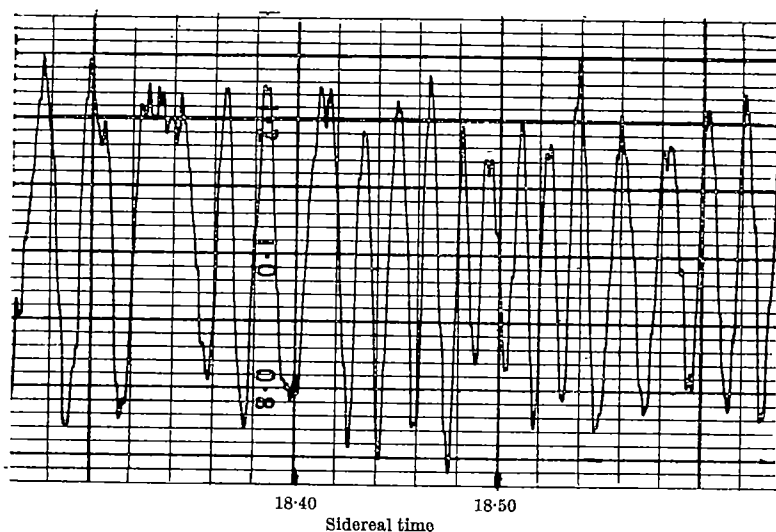


Fig. 1. An example of the fringe pattern formed by the source 3C 286 during interferometric observations with radio telescopes 180,000 wave-lengths apart

telescope, and this link was also used, on demand, to relay television pictures of the dials showing the pointing of the remote telescope.

The new instrument was used to examine all sources of adequate flux density at 408 Mc/s which had either given fringes at one of the two longest base-lines used previously or had been tentatively identified with optical objects of quasi-stellar appearance<sup>3,4</sup>. In addition, the sources CTA 21 and CTA 102 were observed, as theoretical estimates indicated that they might have extremely small angular diameters<sup>5,6</sup>. Measurable fringe patterns were observed for approximately half the sources investigated. An example of the fringe pattern formed by the source 3C 286 is shown in Fig. 1.

The results of these observations are summarized in Tables 1 and 2. Table 1 deals with the thirteen sources identified with quasi-stellar objects and Table 2 contains similar data on nine sources for which fringe patterns were observed, but which have not yet been associated with any optical object. The first column of these Tables gives the catalogue number of the source, and, where it is known, the red shift. Previous observations with interferometers of high resolving power are summarized in column 2. Column 3 contains the results of a preliminary analysis of the measurements made with the present base-line. The measurements have been calibrated by assuming that the sources 3C 119, 3C 286, 3C 287 and CTA 21 are unresolved ( $\gamma = 1.0$ ) and this can be justified by means similar to those in the paper of Allen *et al.*<sup>1</sup>. The data in columns 2 and 3 have been combined to give a preliminary interpretation of the angular structure of each source, as shown in the last column.

The only other source for which a measurable fringe pattern was observed at this base-line was 3C 295, which has been identified with the brightest member of a remote cluster of galaxies, with a red shift  $Z = 0.46$ . Fringes of low visibility were observed from this source at some hour angles, with several pronounced minima. These measurements confirm the double structure inferred from observations with a tracking interferometer at a base-line of 61,100  $\lambda$  (ref. 7).

Table 3

Source	Dimensions predicted	Dimensions observed
3C 48	0.4"	$\sim 0.5''$
3C 119	0.3"	$\leq 0.4''$
3C 147	0.2"	$\sim 0.6''$
3C 295	0.6" double	Components $1.7'' \times <1''$
3C 298	0.6"	Possibly double. Components $<1'' \times 1''$
3C 299	0.6"	No fringes observed: source of low flux density with poor S/N
CTA 21	0.01"	$\leq 0.4''$
CTA 102	0.01"	$\leq 0.4''$

Kellermann, Long, Allen and Moran<sup>8</sup> have discussed the correlation between radio sources of very high brightness temperatures and those which showed markedly curved radio spectra<sup>9</sup>. Sligh<sup>5</sup> and Williams<sup>6</sup> have suggested that this may arise because of the long wave-length absorption of radiation by relativistic electrons in the emitting region. They have used the frequency of maximum emission observed for some sources to predict the minimum angular dimensions of the emitting regions of six sources from the 3C catalogue<sup>10</sup>, and of two sources discovered at the California Institute of Technology. At that time the minimum angular dimensions which could be measured were 0.8 sec and the predicted values were all significantly smaller than that.

Table 3 is a revised edition of the table presented by Williams<sup>6</sup> showing in column 2 his predicted dimensions and in column 3 the dimensions deduced from the present observations. The dimensions quoted in column 3 do not necessarily refer to the sizes of the emitting regions of the sources. They may, in fact, refer to the separations of discrete regions of emission in sources of complex structure. Where such complexity is suspected, attempts have been made to estimate the sizes of the components. In all cases the values shown in column 3 may be taken as upper limits to the sizes of the emitting regions.

It will be seen that the measured sizes are now close enough to the dimensions at which synchrotron self-absorption is predicted to occur, to make it very likely that synchrotron self-absorption is in fact responsible for some at least of the observed decrease of flux density at long wave-lengths.

Finally, it may be noted that the unidentified sources in Table 2 and the six sources in Table 1 which gave measurable fringe patterns at this base-line have angular dimensions two orders of magnitude smaller than the values typical of known radio galaxies<sup>11</sup>. Furthermore, of the eleven sources the spectral curvature of which is well established<sup>9</sup> three occur in Table 1 and four occur in Table 2. Two of the remaining four (3C 295, 408) are identified with two of the most energetic radio galaxies known, and two are unidentified. It therefore seems likely that unidentified sources listed in Table 2 are more akin to the small diameter quasi-stellar sources in Table 1 than to less energetic radio galaxies.

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## PHYSICS

A Suggested Mechanism for the 337 $\mu$  CN Maser

STIMULATED emission at a wave-length of 337 $\mu$  has been observed<sup>1</sup> from pulsed discharges in volatile cyanides in the pressure range 0.1–1.0 mm. No other stimulated emission lines from such sources have been observed so far in our experiments. This is a surprising result when we consider how many transitions are possible in a molecular system. In view of the known high dissociation energy of CN it is plausible to assume that either CN or CN<sup>+</sup> is the emitter. We have now recorded the ultra-violet and visible spectra of the discharge using a Hilger large quartz spectrograph. No sign of the band at 3263 Å [ $f'\Sigma \rightarrow a'\Sigma$ ] characteristic of the CN<sup>+</sup> molecule was observed although the 'Melinex' windows of the maser are transparent at this wave-length. In fact all the observed bands belonged to the well-known violet and red systems of CN.

In searching for an assignment experiments were performed using nitrogen fifteen isotopically substituted methyl cyanide. The aim of these experiments was to determine, if possible, the effect of the small mass change on the emitted frequency and hence to deduce which transition was involved. Repeated experiments with the isotopically substituted material failed to give any stimulated emission in conditions where normal CH<sub>3</sub>C<sup>14</sup>N gave strong emission and this despite appropriate adjustments of cavity-length. Thus a small mass change has a drastic effect on the mechanism which leads to stimulated emission.

Since none of the known CN electronic levels is metastable it is most probable that stimulated emission arises from transitions within the ground electronic state. If this were not so, the far infra-red stimulated emission would have to compete with the rapid depopulation by spontaneous emission in the visible and ultra-violet regions. Examination of the known levels in the ground electronic state shows that a transition matching the observed wave-length is to be found between the levels  $K = 8$  and  $K = 7$  in the vibrational state  $v = 2$ . The fact that such specific levels were involved suggests that there is a peculiarity in the rotational energy level structure of CN. Such a phenomenon, namely, rotational perturbation, is very well known, having been first observed by Herzberg<sup>2</sup> in 1929 and further investigated and later explained by other workers<sup>3,4</sup>. Definitive experimental work has been carried out by Broida and Golden<sup>5</sup>, who recorded the emission spectrum of CN at pressures which are of the same order as those used in our experiments.

The electronic spectrum of CN contains two rotational perturbations, one connected with the level  $v = 11$  of the ground state and the other with  $v = 0$  of the second excited state  $B^2\Sigma$ . Both perturbations are due to the first excited state  $A^2\Pi$ , the vibrational levels involved being  $v = 7$  and  $v = 10$  respectively. For the  $v = 0$  level of  $B^2\Sigma$ , the rotational levels  $J = 3\frac{1}{2}(+)$ ,  $7\frac{1}{2}(-)$  and  $15\frac{1}{2}(-)$  are perturbed by the levels  $A^2\Pi_{3/2} J = 3\frac{1}{2}(+)$ ,  $7\frac{1}{2}(-)$  and  $A^2\Pi_{1/2} J = 15\frac{1}{2}(-)$  respectively. The perturbation leads to quantum mechanical mixing of wave functions and displacement of levels, so that transitions from the red system [ $A^2\Pi_{v=10} \rightarrow X^2\Sigma$ ] are allowed with matrix elements appropriate to the violet system [ $B^2\Sigma_{v=0} \rightarrow X^2\Sigma$ ]. Consequently extra lines appear in the violet band systems which involve the  $v = 0$  level of  $B^2\Sigma$ . These extra lines and their companion doublet components are slightly shifted from their expected frequencies and are, at low pressures, more intense than the unperturbed lines. This phenomenon first seen by Herzberg<sup>2</sup> is very clearly shown in the spectra of Broida and Golden<sup>5</sup>. If we now draw the conclusions from Broida and Golden's observations which are appropriate to the stimulated

emission results it will be seen that population of  $v = 2$  of  $X^2\Sigma$  from  $v = 0$  of  $B^2\Sigma$  leads to  $K'' = 8$  having the highest population and  $K'' = 7$  being scarcely populated. This is due, of course, to the usual selection rule  $\Delta K = \pm 1$  and leads to a population inversion.

The failure to observe maser action with C<sup>15</sup>N is now understandable. The conditions for rotational perturbation are stringent since the two levels involved must agree in energy very closely, must have the same angular momentum and the same parity. Thus for C<sup>14</sup>N the two levels at  $J = 7\frac{1}{2}$  of negative parity have energies  $B^2\Sigma = 26,938.8$  and  $A^2\Pi_{3/2} = 26,939.3$  cm<sup>-1</sup>. The energy of the  $B^2\Sigma$  level is mostly electronic (25,751.8 cm<sup>-1</sup>) with only a half quantum of vibrational energy; it is therefore not sensitive to isotopic substitution. However, the energy of the  $v = 10$  level of  $A^2\Pi$  is mostly vibrational (electronic = 9,241.6 cm<sup>-1</sup>, vibrational = 17,631.2 cm<sup>-1</sup>) and is therefore sensitive to isotopic substitution. Thus whereas in the C<sup>14</sup>N molecule we have two levels which agree in energy to within 0.5 cm<sup>-1</sup> the corresponding levels in the isotopically substituted C<sup>15</sup>N differ by 234 cm<sup>-1</sup>. Rotational perturbation is not therefore possible for any of the low vibrational levels of  $B^2\Sigma$  and the maser action is not possible.

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## Absorption and Refractive Index Measurements at a Wave-length of 0.34 mm

THE relatively intense, monochromatic source at a wave-length of 0.34 mm which has already been reported<sup>1</sup> has been used to make some measurements of refractive index and absorption coefficient. We are giving here a preliminary account of these measurements, and, at the same time, reporting some progress in our techniques for using the stimulated emission in CN discharges as a laboratory source.

There are three main items of progress. The first is that the length of the discharge tube, given as 9 m in our first publication, has been reduced to as little as 2 m. The second step forward is that we have found it possible to run the source for indefinitely long periods by flowing methyl cyanide vapour through the discharge tube. The rate of flow depends on the pulse repetition rate and is such that the equilibrium pressure is a few tenths of a millimetre. The pressure is fairly critical in a given arrangement and has to be adjusted carefully by varying the flow-rate to obtain maximum radiation intensity. The third result we wish to report in our attempts to make a simpler laboratory source is that we have observed stimulated emission when the breakdown of a condenser by an air spark gap is applied to the tube. Peak voltages as low as 6 kV have been effective, giving peak currents of about 300 amp and a pulse duration of about 1  $\mu$ sec. This condenser spark gap system represents a considerable simplification over the delay line type pulse modulator used in our first experiments.

The measurements we wish to report are of the complex dielectric constant of monohalogen substituted benzenes in the liquid phase. These polar substances were chosen because it had been suggested<sup>2</sup> that their dielectric behaviour could not be described in terms of a single Debye relaxation time, and measurements at sub-millimetre wave-lengths could give strong evidence for or

Table 1

	$n$	$\alpha$ nepers/cm	$\epsilon'$	$\epsilon''$
Fluorobenzene	1.46	21.1	2.11	0.165
Chlorobenzene	1.51	14.5	2.28	0.117
Bromobenzene	1.55	12.1	2.39	0.100
Iodobenzene	1.60	9.1	2.57	0.078

All measurements were made at temperatures between 20° C and 24° C.

against this. The quantities measured were refractive index  $n$  and absorption coefficient  $\alpha$  which are related to the complex dielectric constant  $\epsilon$  with real and imaginary parts  $\epsilon'$  and  $\epsilon''$  thus:

$$\epsilon = \epsilon' - i\epsilon'' = n^2(1 - ik)^2$$

hence:

$$\epsilon' = n^2(1 - k^2) \quad \epsilon'' = 2n^2k$$

where:  $k = \frac{\alpha\lambda_0}{4\pi n}$ ,  $\lambda_0$  measured in vacuum.

A Michelson interferometer was used to determine  $n$  by measuring the change in thickness of liquid path to give successive maxima in fringe intensity. Absorption coefficients were measured by a variable path liquid cell in front of a Golay detector. Results are given in Table 1. The estimated accuracy of these measurements is that values of  $n$  are within 1 per cent and values of  $\alpha$  are within 2 per cent.

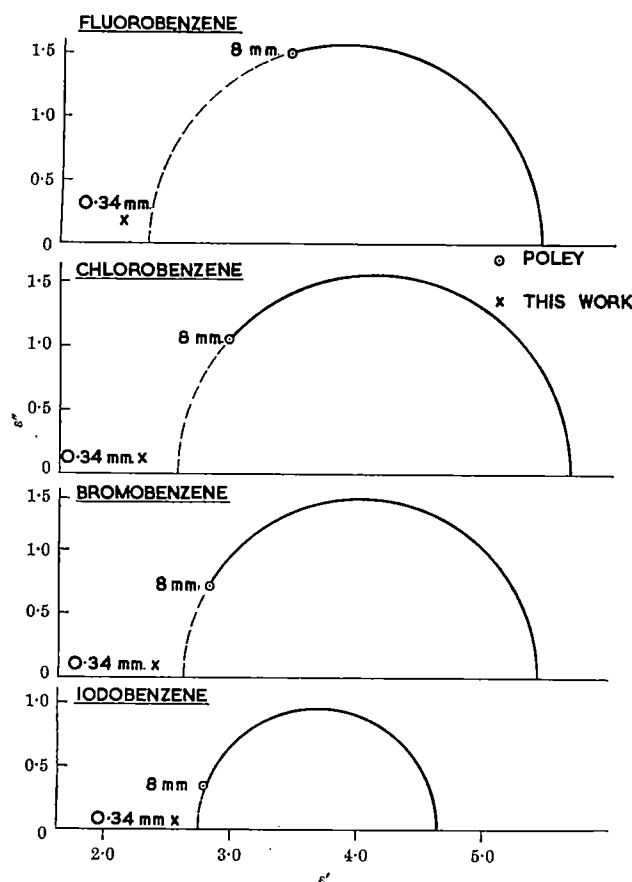


Fig. 1. Cole-Cole plots of the dielectric behaviour of the monohalogen substituted benzenes. They are constructed from the data of Poley and the new measurements at 0.34 mm wave-length show significant departures from the semicircular arcs

These results are most easily related to lower frequency observations by the Cole-Cole<sup>3</sup> plots of Fig. 1. The arcs are derived from the data of Poley with his shortest wave-length point ( $\lambda = 8$  mm) given explicitly. It will be seen that in all cases the new 0.34-mm values lie well away from the semi-circular arcs indicating the existence of an absorption process additional to that predicted by the

Debye expression. As Poley pointed out, there was already some evidence for this in the case of iodobenzene from his 8-mm wave-length point not lying on the arc, but the present results show much more clearly that such additional absorption is present in all the monohalogen substituted benzenes.

We thank the Central Electricity Research Laboratory, Leatherhead, for the loan of high-voltage equipment kindly arranged by Mr. J. Looms.

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### Stress Relaxation in Solids

THE purpose of this communication is to direct attention to a similarity in the shape of stress-log time graphs relating to static stress relaxation in solid materials. For a number of high polymers and metals the following relation between the inflexion slope  $F$  of the stress-log time curves and the total decrease in stress  $\Delta\sigma$  was found:

$$F \approx 0.1 \Delta\sigma \quad (1)$$

$F$  is defined as  $(d\sigma/d \ln t)_{\text{inflexion}}$ . A condition for the applicability of equation (1) seemed to be absence of internal stresses, that is, careful annealing. The substances investigated were rubber hydrochloride<sup>1</sup>, polyethylene<sup>2,3</sup>, cellulose and paper<sup>4</sup>, polycrystalline and single crystal cadmium, indium, lead and tin<sup>5</sup>, and polycrystalline molybdenum<sup>6</sup>. Experiments have also been carried out on a number of other substances including polyisobutylene, polyvinyl acetate, beryllium, aluminium, Lipowitz's alloy, cetyl alcohol, and single crystals of lithium fluoride<sup>7</sup>.

The conformity of the sigmoid stress-log time curves for solids of different types is illustrated by the examples given in Fig. 1. The approach to stress equilibrium varied appreciably from one solid to another. In cadmium, for example, stress equilibrium could be achieved in a few days, while times of the order of 10 days were needed for lead single crystals; even after this period no equilibrium was observed for polycrystalline samples. Initial transients, apparently not belonging to the main relaxation process represented by equation (1), were observed for tin and gallium at moderate strain rates of  $10^{-3}$ – $10^{-4}$  s<sup>-1</sup>. For other solids, such as cadmium or polyisobutylene, such transients appeared at strain rates of the order of 1 s<sup>-1</sup> corresponding to straining times of the order of 1–10 ms. On separating the transients from the main process agreement with equation (1) was achieved. Deviations from this relation were found for samples of polyethylene containing inhomogeneous internal stresses (insufficient annealing) and for polypropylene and beryllium, both of which were apparently difficult to free from internal stress.

Most experiments were performed at room temperature. Molybdenum was measured at 90° K. At this temperature, the constant of proportionality of equation (1) was only 7 per cent lower than at room temperature. Besides this independence of temperature, the constant of equation (1) was also little dependent on initial stress, the main effect of increasing this being to shift the curves toward shorter times<sup>8,9</sup>. Over the whole range of initial stresses and strain rates covered by the measurements, the coefficient of variation of the constant of proportionality of equation (1) was approximately  $\pm 10$  per cent.

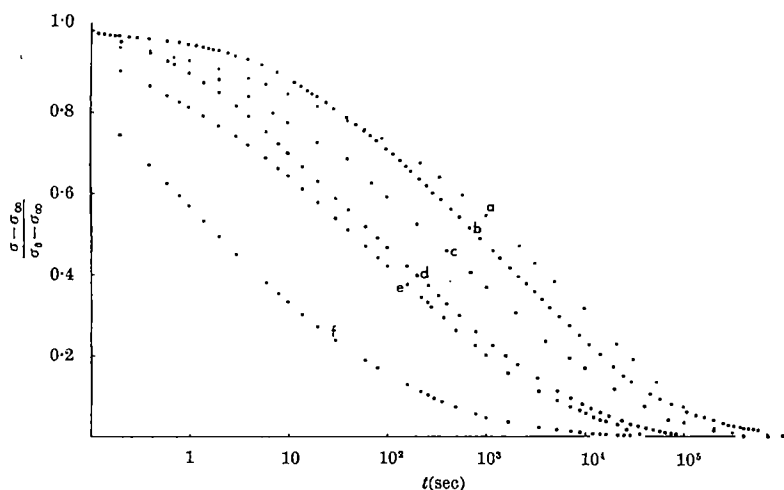


Fig. 1. Stress relaxation curves for some high polymers and metals in the  $(\sigma - \sigma_\infty)/(\sigma_0 - \sigma_\infty)$  (log  $t$ ) representation. Curves  $a, b, c, d, f$ —tension, curve  $e$ —compression.  $a$ , Lead, 99.999 per cent,  $\sigma_0 = 4.0 \times 10^7$  dyn  $\text{cm}^{-2}$ ,  $t_1 = 4.1$  s,  $\dot{\epsilon} = 4.2 \times 10^{-8} \text{ s}^{-1}$ , temperature  $40.7^\circ \text{C}$ ,  $\sigma_\infty/\sigma_0 = 0.15$ ;  $b$ , polyisobutylene, sp. gravity  $0.92 \text{ g cm}^{-3}$ ,  $M \approx 10^5$ ,  $\sigma_0 = 4.0 \times 10^4$  dyn  $\text{cm}^{-2}$ ,  $t_1 = 6.2$  s,  $\dot{\epsilon} = 5.0 \times 10^{-8} \text{ s}^{-1}$ ,  $\sigma_\infty/\sigma_0 \approx 0$ ;  $c$ , cadmium, 99.999 per cent,  $\sigma_0 = 1.3 \times 10^8$  dyn  $\text{cm}^{-2}$ ,  $t_1 = 1.7$  s,  $\dot{\epsilon} = 4.2 \times 10^{-8} \text{ s}^{-1}$ ,  $\sigma_\infty/\sigma_0 = 0.10$ ;  $d$ , polyethylene, sp. gravity  $0.920 \text{ g cm}^{-3}$ , crystallinity 42 per cent,  $\sigma_0 = 1.9 \times 10^8$  dyn  $\text{cm}^{-2}$ ,  $t_1 = 1.7$  s,  $\dot{\epsilon} = 4.2 \times 10^{-8} \text{ s}^{-1}$ ,  $\sigma_\infty/\sigma_0 = 0.39$ ;  $e$ , indium, 99.999 per cent,  $\sigma_0 = 2.0 \times 10^7$  dyn  $\text{cm}^{-2}$ ,  $t_1 = 6.2$  s,  $\dot{\epsilon} = 1.2 \times 10^{-8} \text{ s}^{-1}$ ,  $\sigma_\infty/\sigma_0 \approx 0$ ;  $f$ , rubber hydrochloride, sp. gravity  $1.12 \text{ g cm}^{-3}$ ,  $M = 2.4 \times 10^5$ ,  $\sigma_0 = 1.1 \times 10^7$  dyn  $\text{cm}^{-2}$ ,  $t_1 = 2$  ms,  $\dot{\epsilon} = 2.5 \text{ s}^{-1}$ ,  $\sigma_\infty/\sigma_0 = 0.32$ .  $\sigma_0$ , Initial (peak) stress;  $\sigma_\infty$ , equilibrium stress;  $t_1$ , straining time;  $\dot{\epsilon}$ , strain rate

The curve shape exemplified in Fig. 1 is usually explained either by assuming a spectrum of relaxation times or an activation energy that decreases (linearly) with stress. The activation concept based on a single Boltzmann term with a stress-dependent energy of activation gives, formally, a close agreement with the observed curve shape. The basic relation between the rate of stress decrease with time and stress is:

$$d\sigma/dt \sim \exp(\sigma/F) \quad (2)$$

with:

$$F = kT/v \quad (3)$$

$v$  is volume of activation. Here  $F$  is nearly identical with the inflexion slope of  $\sigma$  (ln  $t$ ). Considering the simple case of complete stress dissipation, as in cadmium or polyisobutylene, where  $\Delta\sigma$  is equal to the initial (maximum) stress,  $\sigma_0$ , the relation  $v\sigma_0 \approx 10 kT$  is obtained from equations (1) and (3). Relations between  $d\sigma/dt$  and  $\sigma$  of the type  $d\sigma/dt \sim \sigma \exp(\sigma/F)$ , which also closely represent the observed curve shape, give  $v\sigma_0 \approx 8 kT$ . The difficulty in finding a reasonable explanation of this in terms of equation (2) is underlined by the independence of  $F/\sigma_0$  (or  $F/\Delta\sigma$ ) of temperature<sup>10,11</sup>. Despite modifications of the basic theory, difficulties of this kind seem to persist.

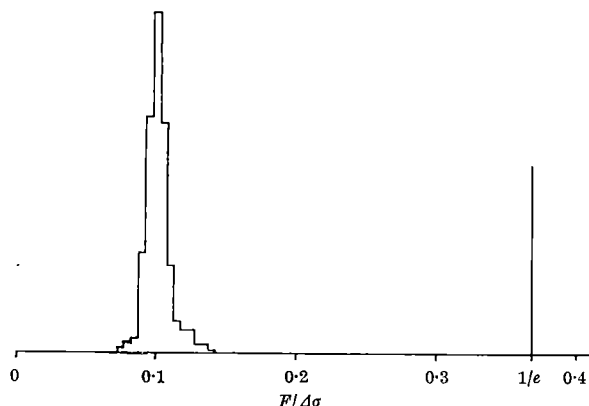


Fig. 2. Illustration of the stability of the ratio  $F/\Delta\sigma$ . The line at  $F/\Delta\sigma = 0.37$ , that is,  $1/e$  corresponds to Maxwellian relaxation. The histogram is based on 320 measurements tabulated in the previous papers (refs. 1-6) together with 75 unpublished  $F/\Delta\sigma$  values

Besides stress activation, pronounced spectral effects were observed in the present investigation—for example, in experiments in which the straining time was varied. Formally, the results could be interpreted in terms of a spectrum of relaxation times, which shifts along the log time axis when temperature or initial stress is changed. The extent of this shift and the original position of the spectrum are dependent on the characteristics of the material. The influence of stress is most pronounced in metals. It is less distinct in semicrystalline polymers and, at low deformations, apparently absent in polymers of the amorphous type. The shape of the spectrum is, however, largely independent of structure and composition. When approximated by a box distribution it extends over 4.3 decades of time.

Another illustration of the validity of equation (1) is the frequency distribution, shown in Fig. 2, and relating to relaxation results obtained with both polymers and metals. The mean of  $F/\Delta\sigma$  is  $0.10 \pm 0.01$ . Support for the validity of equation (1) is found in a number of reports (see refs. 1-6). Most have been concerned, however, with limited time intervals and with sub-

stances for which the main inflexion region of the  $\sigma$ (log  $t$ ) curves is not covered by the period of measurement, as is the case with steel.

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## GEOPHYSICS

### Determination of the Density of Air at an Altitude of 3,500 km

In 1963 an investigation was begun of densities of air at heights above 3,000 km. Observations were started in January 1964 on the high *Midas* satellites (*Agena* satellites in near-polar orbits at heights mainly between 3,000 and 4,000 km) with a special camera at the prime focus of the 24-in.,  $f/5$  reflector of the University of London Observatory. A tilting mirror gives timed breaks in both satellite and star trails and the precision has been found to be about 1" in position and 5 msec in time. The *Midas* objects are well recorded to magnitude 9, at angular velocities at 300"/sec, using special film (SO 118) obtained from Eastman Kodak and processed to give a speed of 2,000 A.S.A. A typical frame is shown in Fig. 1. In work recorded here observations were made at selected positions on high passes to maximize the accuracy in determining the satellite latitude, and hence mean anomaly. To derive the mean anomaly from each observation approximate orbit elements were obtained from various sources, including *Norad* (via the Goddard Space Flight Center) and the Smithsonian Astrophysical



Observatory. Near-zenith observations were also used to derive accurate positions for the ascending node and its motion.

In early 1964 it was learned that the object 1963 30D (magnitude 8) that originated from the 1963 30A launch (*Agna D*) was an inflated balloon satellite—an experiment by the Lincoln Laboratories, of the Massachusetts Institute of Technology. Correspondence with the Smithsonian Astrophysical Observatory and with the Lincoln Laboratories produced information on the size of the balloon (2.4 m diam. nominal) and its mass (1.25 kg). Consequently its area/mass ratio is some 1,000 times greater than the ratio for the parent *Agna* vehicle and observations were therefore concentrated on 1963 30D. It has been possible to determine the change in orbital period due to air drag much earlier than was expected from the original programme and a determination of the air density at 3,500 km has been obtained. This altitude is greatly in excess of those so far surveyed, the greatest previously being about 1,500 km<sup>1</sup>.

One obstacle in extending measures of orbital period changes to great altitudes is the large competing perturbation due to solar radiation pressure. Many investigations have been made of these perturbations, for example by Musen<sup>2</sup>, and a point of importance is the absence of any perturbation in the anomalistic period when the whole orbit is in sunlight<sup>3</sup>. For the *Midas* objects the sunlit periods may exceed ten weeks.

Observations on 1963 30D were made in two series. Series I covered the period April 26–July 2, with the orbit precessing into full sunlight on about June 11. Series II covered the morning passes first accessible on August 26 and continued until October 8, with the orbit again including shadow on about September 11.

To derive an orbital period which represents the orbital energy it is necessary to remove the motion of perigee, which is very non-uniform even during the sunlit periods, due to solar radiation pressure. Therefore the derived

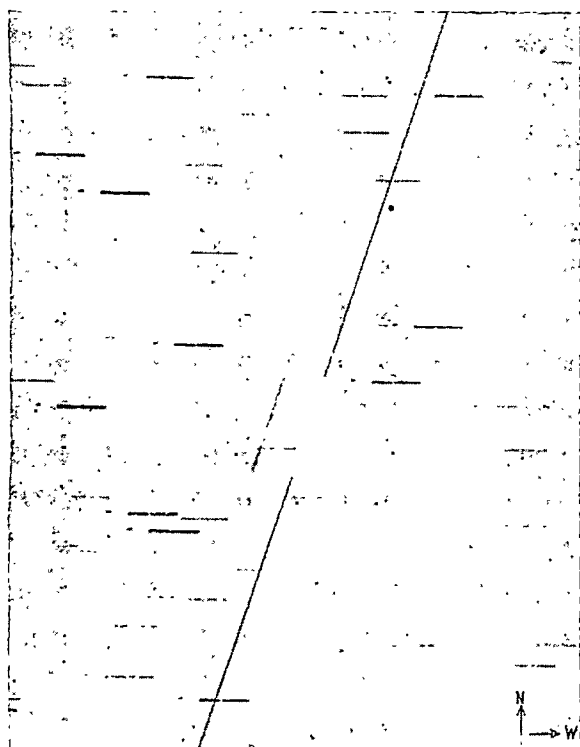


Fig. 1. Part of a frame obtained on September 23, 1964, showing the trail of satellite 1963 30D with a timing break lasting about 2 sec, and the star trails with the corresponding breaks. The reproduced part of the satellite trails is about 0.5° in length

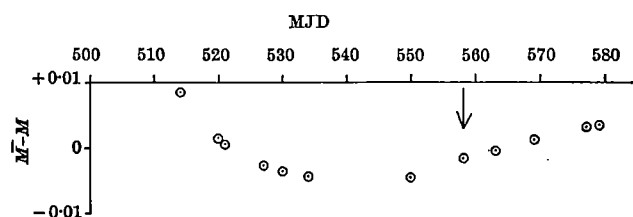


Fig. 2. The residuals in mean anomaly in units of revolutions, from a least-squares fit of mean anomaly to a linear function of time with the data in Series I. The date is the Modified Julian Date (MJD) and the arrow indicates June 11 when the orbit moved into fullsunlight. The points at MJD 534 and 550 indicate a nearly constant orbital period followed by a sharp change before the solar radiation pressure perturbation is removed on June 11. Results received from the Smithsonian Astrophysical Observatory confirm this curious behaviour, at present unexplained

anomalistic mean motions were modified by subtracting the mean motion of perigee used in each analysis, a procedure also followed by Zadunaisky *et al.* in their investigations of *Echo I*<sup>4</sup>.

The orbit elements used limited the accuracy to which the anomalistic period could be obtained from the observations; in particular, uncertainty in the argument of perigee introduced the largest errors into the anomalistic period—about one part in 10<sup>3</sup>, for the orbital eccentricity of 0.04.

Fig. 2 shows a plot of the residuals in mean anomaly from a least squares fit to a linear function of time to the data in Series I. The strong curvature in the left-hand part reflects the acceleration due to solar radiation pressure, about  $-1 \times 10^{-8}$  period/period. After June 11 the orbit is entirely in sunlight and the orbital period is essentially constant.

Series I gave a modified anomalistic period of 0.116 622 35 days for the date June 21.442; Series II 0.116 620 34 days for August 31.674. The rate of change of period was therefore  $-2.82 \times 10^{-8}$  period/period, and is estimated to be accurate to 10 per cent. There is no contribution by solar radiation pressure to this value, so it represents the effect of air drag only.

Using the air density relation given by King-Hele and Walker<sup>5</sup>, the following expression is obtained:

$$A/m \cdot C_D \cdot \rho^* = 3.02 \times 10^{-18} \text{ gm/cm}^3$$

$\rho^*$  is the air density at a height above perigee of one-half the scale height (assumed here 500 km);

$A/m$  is the area/mass ratio in cm<sup>2</sup>/g;

$C_D$  is the drag coefficient.

Recent brightness fluctuations suggest that 1963 30D is no longer fully inflated. Discussion with D. E. Smith (Radio Research Station, Slough) suggests that  $A/m$  may be only about 30, from investigations of the effect of solar radiation pressure on the orbital eccentricity. Furthermore, correspondence with Dr. I. Shapiro at the Lincoln Laboratories also indicates that the balloon is no longer spherical. A value of 30, which seems unlikely to be in error by more than 20 per cent, was adopted.

The value of  $C_D$  is debatable but seems likely to lie between 3 and 5 (ref. 6); the uncertainty in  $C_D$  at present limits the air density accuracy, but we may take 4.0 as a nominal value, representing the situation where the velocity of the satellite is of the same order of magnitude as the most probable molecular velocity and where the reflexion of the molecules from the satellite surface is primarily diffuse and not specular. Then the density is  $2.5 \times 10^{-20}$  g/cm<sup>3</sup> at 3,500 km. This result does not disagree with figures extrapolated over about three scale heights from models by Nicolet<sup>6</sup>. It should be stressed that the density is an average value over two months and refers approximately to the sunrise and sunset regions, excluding the diurnal bulge.

I thank Prof. C. W. Allen of the University of London Observatory for permission to use the 24-in. reflector during this work.

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## GEOCHEMISTRY

### Increasing the Settling Rate of Flocculated Suspensions

THERE is a wide range of fields in which sonic, ultra-sonic or pulsatory techniques have been found to improve the performance of a transfer process. In liquid-liquid extraction processes, for example, the effect of pulsation has been shown to increase the capacity of a unit by as much as 200 per cent<sup>1-3</sup>. Mass transfer rates and the coefficient of heat transfer are also claimed to be improved by sonic vibrations<sup>4-6</sup>.

These results suggest that vibrations might improve the performance of other processes. It is well known, for example, that the sedimentation rate of fine particles suspended in liquids can often be increased by the addition of a flocculating agent but, so far as we are aware, the influence of vibration on the settling rate of such suspensions has not been explored.

As part of a general programme dealing with the rheology of suspensions the effects of mechanical vibration at 50 c.p.s. on the settling rate of aqueous suspensions of polyvinyl chloride spheres (possessing a maximum diameter of 1 micron) and a kaolin china clay are being examined. When these are flocculated with potassium alum solution they develop definite yield stresses. The sedimentation

tests are carried out in cylindrical tubes of 3 cm internal diameter and 60 cm in height, under static and vibrated conditions. Typical results for polyvinyl chloride spheres at a volume concentration of 15.8 per cent and for kaolin at a volume concentration of 3.5 per cent are shown in Fig. 1. The differences in the sedimentation curves are quite pronounced, showing increases in the settling rate, with the vibration, of at least 200 per cent.

Although longitudinal and transverse vibrations both give improved settling rates compared with static conditions, it appears that the greatest improvement is obtained with transverse vibrations. Amplitude variations have very significant effects, particularly in the initial stages of settling, and the tube diameter may in some cases be important. The mechanism involved is at present under investigation and a more detailed report of the work will be published in the near future.

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## MINERALOGY

### Interlamellar Sorption in a 'Methylated' Montmorillonite

As a sequel to the work already carried out on interlamellar sorption of organic molecules on methylated graphitic acid<sup>1</sup> and on the structure of methylated graphitic acid by electron diffraction<sup>2</sup>, I decided to try to methylate montmorillonite, and to examine its capacity for interlamellar sorption. The problem of methylating montmorillonite has been discussed a good deal, and many arguments for and against have been put forward, and have been linked with the two structural models for montmorillonite.

A Wyoming montmorillonite was used in this work. It was acidified by the method described by Girod and Lacroix<sup>3</sup>.

The H<sup>+</sup> montmorillonite, once prepared, was dried at 110° C for 12 h and methylated, following Vogel's method<sup>4</sup>. The methylated product was washed several times with ether, then dried *in vacuo* in a desiccator with P<sub>2</sub>O<sub>5</sub>.

The basal spacing obtained was 12.35 Å, and remained constant after treatment with numerous organic substances, both polar (amines, alcohols, water) and non-polar (paraffins, toluene).

However, on heating the methylated sample in a capillary to 110° C and 130° C for 48 h, the layers become disordered, and the basal spacing diminishes by 1 Å (the disorder is indicated by a spreading of the basal spacing reflexion). These heated samples recover the basal spacing if treated with octylamine, but no higher spacing than 12.35 Å appears.

It is to be noted that original montmorillonite gives high spacings with aliphatic amines, in accordance with the observations of Aragón, Cano-Ruiz and MacEwan<sup>5</sup>, and Armin Weiss<sup>6</sup>. After 'methylation', the montmorillonite fails to react. This result does not agree with those of Brown, Greene-Kelly and Norrish<sup>7</sup>, or that of Greenland and Russell<sup>8</sup>. Methylation was carried out until no reaction of the diazomethane with the clay was observed<sup>9</sup>, only a slight bubbling corresponding to the diazomethane itself—in contrast to the intense bubbling of the first days.

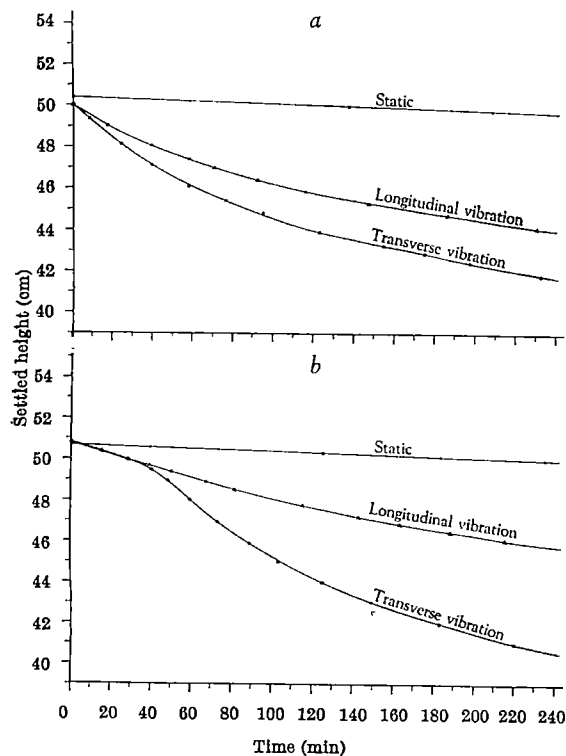


Fig. 1. Sedimentation under static and vibrated conditions. *a*, Polyvinyl chloride spheres; *b*, kaolin.

If the water contained between the sheets of the montmorillonite reacts with the diazomethane, or with the methyl group, to hydrolyse them, giving methanol as a final product, the addition of an excess of diazomethane would result in eliminating the interlamellar water, and finally the diazomethane would react with the clay.

If any methanol remained between the layers it would be displaced by the long-chain aliphatic amines with which the clay was treated, giving large spacings, especially after heating to 110° C.

While in G. F. Walker's laboratory in Melbourne, I investigated the sorption of aliphatic amines by a montmorillonite containing amorphous carbon between the sheets, resulting from the decomposition of sorbed glycerol, and the basal spacings obtained were similar to those given by normal montmorillonite. The polymerization of the diazomethane, as suggested by Vivaldi and Hendricks<sup>9</sup> and by Greenland and Russell<sup>8</sup>, is more acceptable as an explanation, but might not be adequate to explain the diminution of the spacing after treatment at 110° C, and the increase to the original value on sorbing an amine. I suspect methylation of the clay. This conclusion is supported by the experiments of Barrer and Realy<sup>10</sup> with a phenylmontmorillonite prepared by Deuel.

I thank Dr. D. M. C. MacEwan for his advice.

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## CHEMISTRY

### Homogeneous Electron Exchange Reactions of Aromatic Molecules

RECENT reviews summarize the large body of information available on the rates and mechanisms of inorganic electron exchange processes<sup>1-3</sup>. In contrast, purely organic systems have received minor attention. The homogeneous electron exchange rates ( $k_{\text{exc}}$ ) for a few aromatic hydrocarbons and other individual systems have been measured<sup>4,5</sup>. We have been interested in the values of  $k_{\text{exc}}$  for aromatic molecules containing polar functional groups and particularly the effect of solvents, substituents, and molecular geometry on these rates. We wish to report some preliminary results which show that we are able to measure  $k_{\text{exc}}$  with a precision which is capable of detecting subtle differences due to molecular structure and environment.

The measurement of  $k_{\text{exc}}$  for the electron transfer process  $X^- + X \rightleftharpoons X + X^-$  can be measured via electron paramagnetic resonance if the species  $X^-$  is the paramagnetic radical ion  $X^{\cdot-}$ . The theory of the electron paramagnetic resonance exchange phenomenon is not particularly simple but the bimolecular rate constant  $k_{\text{exc}}$  is obtained fairly easily by measuring the broadening of hyperfine lines in the electron paramagnetic resonance spectrum of  $X^{\cdot-}$  as a function of concentration of the parent compound,  $X$  (refs. 4-6). All the radical ions involved in the work recorded here were generated via controlled

Table 1. VALUES OF  $k_{\text{exc}}$  FOR SELECTED AROMATIC ELECTRON EXCHANGE SYSTEMS

Exchange system*	Solvent †	$k_{\text{exc}}$ ‡
A/A <sup>-</sup>	DMF§	$4.8 \pm 1 \times 10^8$
Q/Q <sup>-</sup>	DMF	$3.8 \pm 0.2 \times 10^8$
Q/Q <sup>-</sup>	DMF—10% H <sub>2</sub> O	$5.6 \pm 0.8 \times 10^7$
DQ/DQ <sup>-</sup>	DMF	$6.2 \pm 0.3 \times 10^7$
NB/NB <sup>-</sup>	DMF	$3.0 \pm 0.2 \times 10^7$
NB/NB <sup>-</sup>	DMF—10% H <sub>2</sub> O	$3.2 \pm 0.6 \times 10^5$
<i>p</i> -DNB/ <i>p</i> -DNB <sup>-</sup>	DMF	$6.0 \pm 1 \times 10^8$
<i>m</i> -DNB/ <i>m</i> -DNB <sup>-</sup>	DMF	$5.2 \pm 0.3 \times 10^8$
<i>p</i> -CNB/ <i>p</i> -CNB <sup>-</sup>	DMF	$7.9 \pm 0.2 \times 10^7$
<i>m</i> -CNB/ <i>m</i> -CNB <sup>-</sup>	DMF	$8.8 \pm 0.4 \times 10^7$
3,5-DCNB/3,5-DCNB <sup>-</sup>	DMF	$1.6 \pm 0.1 \times 10^8$

\* Symbols have the following meaning: A/A<sup>-</sup>, anthracene/anthracene anion; Q/Q<sup>-</sup>, *p*-benzoquinone/*p*-benzoquinone anion; DQ/DQ<sup>-</sup>, duroquinone/duroquinone anion; NB/NB<sup>-</sup>, nitrobenzene/nitrobenzene anion; *p*-DNB/*p*-DNB<sup>-</sup>, *p*-dinitrobenzene/*p*-dinitrobenzene anion; *m*-DNB/*m*-DNB<sup>-</sup>, *m*-dinitrobenzene/*m*-dinitrobenzene anion; *p*-CNB/*p*-CNB<sup>-</sup>, *p*-chloronitrobenzene/*p*-chloronitrobenzene anion; *m*-CNB/*m*-CNB<sup>-</sup>, *m*-chloronitrobenzene/*m*-chloronitrobenzene anion; 3,5-DCNB/3,5-DCNB<sup>-</sup>, 3,5-dichloronitrobenzene/3,5-dichloronitrobenzene anion.

† 0.01 M tetraethylammonium perchlorate (TEAP) supporting electrolyte except for A/A<sup>-</sup> where TEAP = 0.1 M.

‡ Bimolecular rate constant in l. mole<sup>-1</sup> sec<sup>-1</sup>.

§ *N,N*-dimethylformamide.

potential electrolysis. Details of the experimental techniques will be published soon.

Table 1 summarizes some of the systems examined. The unsubstituted aromatic hydrocarbons like A/A<sup>-</sup> (see legend to Table 1 for explanation of abbreviations) show very rapid exchange rates. (The naphthalene/naphthalide ion exchange measured by Ward and Weissman has  $k_{\text{exc}} = 10^8$ – $10^9$  l. mole<sup>-1</sup> sec<sup>-1</sup>) (ref. 6). Some substituted aromatics like Q/Q<sup>-</sup> also are quite rapid. However, when a strong electron acceptor function like the nitro group is present, the value of  $k_{\text{exc}}$  decreases by about a factor of 10. It is known from the electron paramagnetic resonance hyperfine coupling constants that the unpaired electron density in NB<sup>-</sup> is strongly localized in the nitro group ( $a_N$  = about 10 g) (ref. 7). In *p*- and *m*-dinitrobenzene anions, the <sup>14</sup>N coupling constants are considerably smaller than in NB<sup>-</sup> (the two <sup>14</sup>N coupling constants are equivalent in each anion radical and for *p*-DNB<sup>-</sup>  $a_N$  = 1.7 g; for *m*-DNB<sup>-</sup>  $a_N$  = 4.7 g) (ref. 8). Hence it was reasoned that  $k_{\text{exc}}$  for these systems should be faster than for NB/NB<sup>-</sup>. This prediction is well illustrated by the results in Table 1. Furthermore, it appears that other substituent groups which decrease  $a_N$  in a mono-nitro compound tend to increase the value of  $k_{\text{exc}}$  above that of NB/NB<sup>-</sup>. This is illustrated by the value for the system 3,5-dichloronitrobenzene and its anion radical, where  $a_N$  = 7.6 g. We are investigating the realities of these structural effects in far more detail. The importance of the results presented here is that it establishes clearly that such investigations can be made reliably.

The most striking effect in Table 1 is the dependence of  $k_{\text{exc}}$  on the solvent system. Addition of 10 per cent water to the DMF decreases the exchange rate for Q/Q<sup>-</sup> slightly. This same alteration causes  $k_{\text{exc}}$  to decrease by a factor of 100 in the case of NB/NB<sup>-</sup>. Electron paramagnetic resonance investigations have established strong solvation of NB<sup>-</sup> (refs. 9–12), and this preferential hydrogen-bonding or solvation of the anion radical represents an energy barrier to the exchange process and an expected decrease in  $k_{\text{exc}}$ . Strong solvent effects on the exchange rate of fluoranil and its semiquinone have been observed by Calvin *et al.*<sup>13</sup>.

The reliability of the present results is demonstrated by measurements of  $k_{\text{exc}}$  for Q/Q<sup>-</sup>. This rate was measured at the University of Kansas and at the Varian Research Laboratories in Zürich using entirely different solvents, compounds and experimental methods (the latter investigation used a flow system). Although the precision of the flow measurements was poorer,  $k_{\text{exc}}$  was found to be  $3.7 \pm 1 \times 10^8$  to be compared to  $3.8 \pm 0.2 \times 10^8$  given in Table 1.

We are now attempting to determine if the variation in rates is due primarily to differences of anion solvation



or unpaired electron distribution in these systems. The investigation is being extended to molecules of interest in biological electron transport processes.

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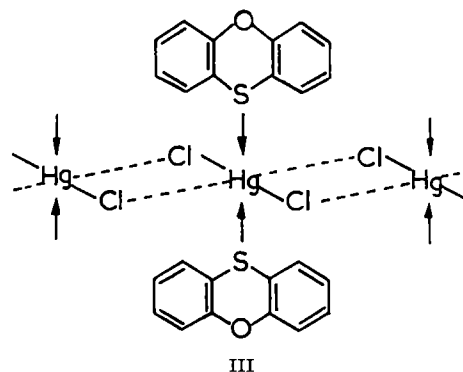
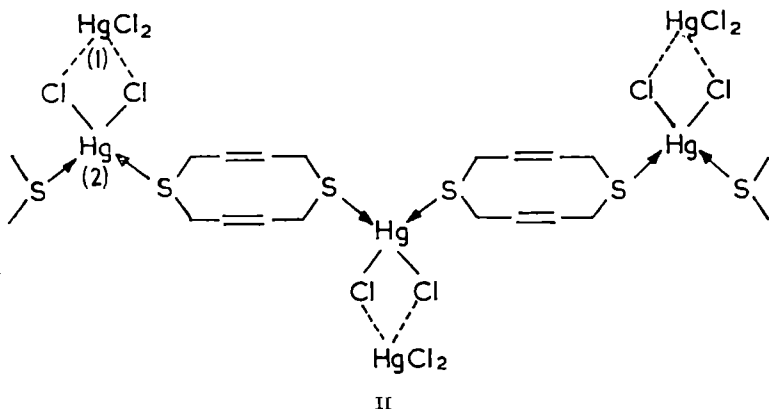
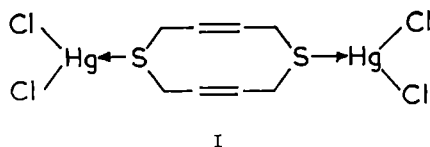
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### Structures of some Mercury(II) Complexes

1,6-DITHIACYCLODECA-*cis*-3, *cis*-8-diene<sup>1</sup> forms a *bis*-(mercuric chloride) adduct. The obvious postulate for the structure of this complex is shown in diagram I, with each mercury atom in three-fold co-ordination. The actual crystal structure determined by X-ray analysis, however, is of the polymeric type (diagram II), with one mercury atom, Hg(2), in tetrahedral co-ordination and the other, Hg(1), in an only slightly distorted mercuric chloride molecule. The Hg(2)-S and Hg(2)-Cl distances are 2.53 and 2.51 Å, respectively. The complex crystallizes in the monoclinic system, space group  $P2_1/m$ , with two molecules



of  $C_8H_{12}S_2Hg_2Cl_4$  in a cell of dimensions  $a = 7.29$ ,  $b = 17.01$ ,  $c = 6.20$  Å,  $\beta = 92^\circ 43'$ . The structure was solved by Patterson and Fourier methods and refined by least-squares calculations; the final value of the discrepancy  $R$  over 1,158 independent reflexions is 13.6 per cent.

The zinc atom in dichlorobis(thiourea)zinc is tetrahedrally co-ordinated<sup>2</sup>. The analogous mercury compound does not have the same type of structure but contains planar  $(N_2H_4CS)_2ClHg^+$  cations ( $C_{2v}$  symmetry) and  $Cl^-$  anions. The unit cell is orthorhombic, space group  $Pmnm$ , contains two 'molecules' of  $(N_2H_4CS)_2HgCl_2$ , and has dimensions  $a = 12.79$ ,  $b = 5.89$ ,  $c = 6.44$  Å. The structure was elucidated by Patterson and Fourier methods and refined by least-squares calculations. The value of  $R$  over 444 independent reflexions is 18.3 per cent. The dimensions of the  $(N_2H_4CS)_2ClHg^+$  cation are Hg-S 2.37 Å, Hg-Cl 2.56 Å,  $\angle SHgS$   $128^\circ 45'$ ,  $\angle SHgCl$   $110^\circ 37'$ .

In so far as the hybridization concept is a valid description of the covalent contribution to the bonding in metal complexes (compare with Jørgensen<sup>3</sup>) the valency angles in the thiourea complex can be interpreted to imply that the mercury ( $s, p$ )-hybrid orbitals directed towards the sulphur atoms have 38 per cent  $s$ -character and the orbital directed towards the chlorine atom 23 per cent  $s$ -character; these estimates are in good accord with the bond lengths, the Hg-Cl distance being slightly longer than that appropriate to  $sp^3$ -bonding (2.51 Å). In dichlorobis(thiourea)zinc the valency angles  $SZnS$  and  $ClZnCl$  are  $111.5^\circ$  and  $107.3^\circ$ , respectively, implying that here also the orbitals directed towards the sulphur atoms have more  $s$ -character (27 per cent) than the orbitals directed towards the chlorine atoms (23 per cent), though the difference is not so great as in the mercury compound. These results can be ascribed to a relationship between hybridization and electron-donor capacities of ligands<sup>4</sup>; orbitals directed towards the least electronegative ligands have more  $s$ -character than those directed towards the more electronegative ligands. That the variation of  $s$ -character with ligand electron-donor ability is much more pronounced for mercury orbitals than for zinc orbitals is a clear reflexion of the greater  $s-p$  energy separation in mercury.

The co-ordination number of mercury in these complexes increases from three to four with concomitant lengthening of the Hg-S bond when thiourea is substituted by 1,6-dithiacyclodeca-*cis*-3, *cis*-8-diene, a sulphur ligand of poorer electron-donor ability. The co-ordination number increases and the Hg-S bond lengthens (and weakens) further when a sulphur-containing ligand of still poorer electron-donor ability is used. X-ray analysis of the coloured complex dichlorobis(phenoxathiin)mercury reveals a crystal structure not unlike that of dichlorobis(pyridine)mercury<sup>5</sup>, and containing infinite chains (diagram III). The complex crystallizes in the monoclinic system, space group

B2/b, with four molecules of  $C_{24}H_{16}O_2S_2HgCl_2$  in a cell of dimensions  $a = 31.00$ ,  $b = 19.46$ ,  $c = 3.95$  Å,  $\gamma = 111^\circ 18'$ . The mercury atom has two short collinear bonds to chlorine atoms (that is, a discrete mercuric chloride molecule can be recognized) while two further chlorine atoms and two sulphur atoms at greater distances complete an octahedral co-ordination. The Hg-S bond is very long ( $\sim 3.0$  Å) and undoubtedly very weak and thus differs qualitatively from the more conventional 'covalent/ionic' Hg-S bonds in the complexes described above. The complex readily dissociates and the crystals at room temperature and exposed to the atmosphere lose their phenoxathiin content in a few days. A plausible description of the Hg-S bonding is that it is of the relatively weak charge-transfer type<sup>6</sup>, for the lower ionization potential of sulphur relative to oxygen then accounts for the sulphur being the atom involved in the mercury-ligand bond; a description of the complex based on purely electrostatic attraction, on the other hand, would naturally favour the oxygen atom for bonding.

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## Catalytic Activity of Haemin on the Decomposition of Hydrogen Peroxide

It is known that the velocity of decomposition of  $H_2O_2$  increases in the ratio  $1 : 10^3 : 10^{10}$  if one uses  $Fe^{3+}$  ion, haemin or catalase as a catalyst<sup>1</sup>. There have been several attempts to construct a general mechanism for these reactions<sup>2-4</sup>. The theory of Jones and Wynne-Jones seems to offer satisfactory explanation for many experimental facts<sup>4</sup>. However, there is still need for more experimental evidence to test this theory.

Some light might be shed on this problem by an examination of the haemin-catalysed reaction, since haemin stands, structurally, between a simple  $Fe^{3+}$  ion and a catalase molecule.

The investigation of the haemin-catalysed decomposition of  $H_2O_2$  is complicated by the fact that the catalyst is destroyed by the substrate  $H_2O_2$ . Thus true catalysis by haemin is observed only in the initial part of the reaction. Euler and Josephson found that the  $-(d[H_2O_2]/dt)_{\text{initial}}$  versus  $[H_2O_2]_{\text{initial}}$  graphs had the form of a saturation curve<sup>5</sup>. This fact indicates the formation and subsequent decomposition of a catalyst-substrate complex between haemin and  $H_2O_2$ , although no concurrent change in the visible spectrum of haemin has been observed in an aqueous solution.

From Euler and Josephson's results the dissociation constant of the complex ( $K_M$ ) and the rate constant of its irreversible decomposition ( $k_3$ ) can be obtained. Data taken from their Tables 8, 9 and 10 are plotted in Fig. 1 according to the method of Lineweaver and Burk<sup>6</sup>. For the calculation of  $-(d[H_2O_2]/dt)_{\text{initial}}$  only the first two points of each experiment were used.

From the results shown in Fig. 1,  $k_3 = 2.1 \pm 0.1 \text{ min}^{-1}$  and  $K_M = (8.3 \pm 1.2) \times 10^{-3} \text{ M}$  at  $0^\circ \text{C}$  and  $pH=7$  were

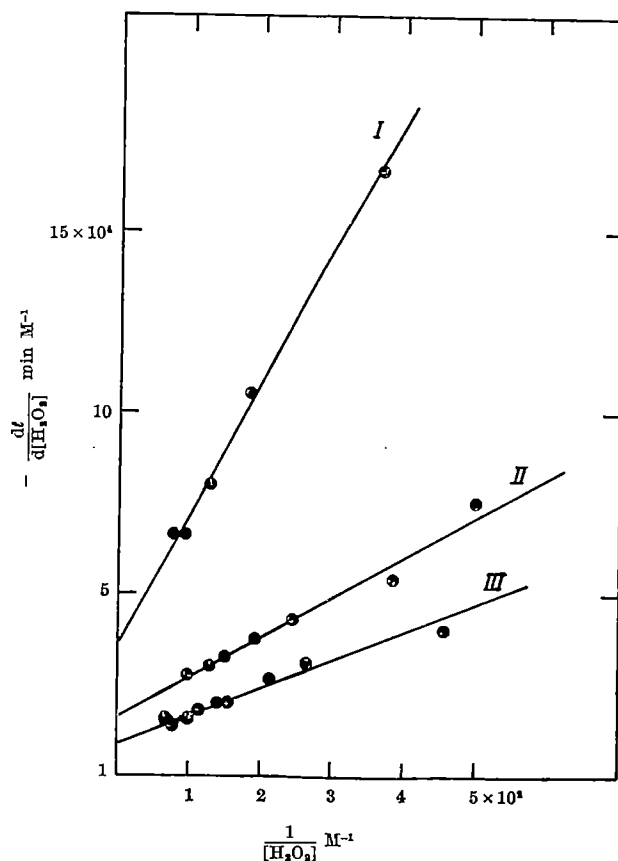


Fig. 1. Lineweaver-Burk plots for the haemin-catalysed decomposition of  $H_2O_2$ .

I,  $[E]_0 = 0.7 \times 10^{-3} \text{ M}$   $pH=7.0$

II,  $[E]_0 = 1.3 \times 10^{-3} \text{ M}$   $t=0^\circ \text{C}$

III,  $[E]_0 = 2.6 \times 10^{-3} \text{ M}$

Data of Euler and Josephson (ref. 5)

obtained. In calculating  $k_3$  the intercept was made equal to  $1/(2k_3[E]_0)$ , on the assumption that two molecules of  $H_2O_2$  are decomposed on the decomposition of each molecule of the complex<sup>7</sup>.  $[E]_0$  is the total concentration of the catalyst. Euler and Josephson's own estimate for  $K_M$  was  $(7.8 \pm 0.4) \times 10^{-3} \text{ M}$ . They used the direct plot of  $-(d[H_2O_2]/dt)_{\text{initial}}$  versus  $[H_2O_2]_{\text{initial}}$ , but this method, owing to the difficulty of estimating  $-(d[H_2O_2]/dt)_{\text{initial}}$  at  $[H_2O_2]_{\text{initial}} = \infty$ , is unreliable.

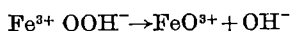
We can compare the value of  $k_3$  to that of an analogous rate constant in the  $Fe^{3+}$  ion catalysed decomposition. This latter reaction has been shown to proceed also by the formation and subsequent decomposition of a catalyst-substrate complex ( $Fe^{3+}HO_2^-$ )<sup>7-9</sup>. The rate constant for the irreversible decomposition of the complex (also denoted as  $k_3$ ) could not be obtained directly, but a related quantity  $k_3/(\epsilon_I - \epsilon_{Fe}) = 8.1 \times 10^{-2} \text{ M cm min}^{-1}$  at  $27^\circ \text{C}$  was calculated.  $\epsilon_I$  and  $\epsilon_{Fe}$  are the molar extinction coefficients of  $Fe^{3+}HO_2^-$  and  $Fe^{3+}$  at  $440 \text{ m}\mu$ . This value, thus  $k_3$ , was independent of  $pH$  (ref. 8).

In order to calculate  $k_3$ , an independent knowledge of  $\epsilon_I - \epsilon_{Fe}$  is needed. Using the value  $450 \text{ M}^{-1} \text{ cm}^{-1}$  of Evans *et al.* at  $440 \text{ m}\mu$ ,  $k_3 = 36.4 \text{ min}^{-1}$  is obtained at  $27^\circ \text{C}$  (ref. 10). The energy of activation of step 3 is  $16.8 \text{ kcal}$ . It was calculated from the temperature dependence of  $k_3/(\epsilon_I - \epsilon_{Fe})$ , assuming that  $\epsilon_I - \epsilon_{Fe}$  is independent of temperature. Thus  $k_3$  at  $0^\circ \text{C}$  becomes  $2.2 \text{ min}^{-1}$ , independent of  $pH$ .

Using an alternative estimate of Jones *et al.* of  $k_3 = 10 \text{ min}^{-1}$  at  $25^\circ \text{C}$ , we obtain  $k_3 = 0.8 \text{ min}^{-1}$  at  $0^\circ \text{C}$  (ref. 11). The close agreement of  $k_3$  for haemin and  $Fe^{3+}$  ion is

striking. The overall rate constants differ by a factor of several hundred.

Although a detailed comparison of the ferric ion and haemin-catalysed reaction is not possible on the basis of experimental data at present available, nevertheless it seems probable that in both cases in step 3 a breaking of the O—O bond in a co-ordinated  $H_2O_2$  molecule occurs. This process is assisted by the formation of new bonds between the catalyst and one of the oxygen atoms of peroxide, after the O—O bond is broken<sup>9</sup>.



$\pi$  molecular orbitals between  $Fe^{3+}$  and O are of particular interest since they can be formed only after the peroxide bond is broken. (A strong metal-oxygen bond involving one  $\sigma$  and two  $\pi$  molecular orbitals has been shown to occur in  $VO^{2+}$  (ref. 12).)

$H_2O$  ligands in aqueous  $Fe^{3+}$  are not involved in  $\pi$  bonding to the central atom. Similarly, there is negligible  $\pi$  bonding between iron and the nitrogen atoms of porphyrin in haemin<sup>13</sup>. Therefore, the  $Fe^{3+}$ —O  $\pi$  m.o.s in both cases are, in a good approximation, independent of other existing bonds in the molecule. As a consequence the energetic requirement for breaking the O—O bond both in the haemin and  $Fe^{3+}$  ion complexes may be very similar.

The incorporation of haemin in a catalase molecule increases  $k_3$  drastically. According to Jones and Wynne-Jones,  $k_3$  for catalase is  $5.5 \times 10^6 \text{ min}^{-1}$  at  $20^\circ \text{C}$  (ref. 4). This phenomenon seems to present one of the most interesting problems of catalase kinetics.

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### Free Radical Sources based on Zero-valent Metal Derivatives

We have previously shown that systems containing metal carbonyls<sup>1</sup>, particularly those in which the metal is in the zero oxidation state, and suitable organic halides, are sources of free radicals, as evidenced by their ability to initiate vinyl polymerization in the homogeneous liquid phase. It seemed of interest to extend these observations to other derivatives of zero-valent metals, especially those having more easily removable ligands than carbon monoxide, since under these conditions free radical formation would be expected to occur more readily. We report here investigations with two such derivatives—the hexaphenylisocyno derivatives of molybdenum (0) and tungsten (0).

As a ligand, phenyl isocyanide resembles carbon monoxide, except that the extent of back-donation from the  $d$  orbitals of the metal to the ligand is less important; thus the double-bond character of the metal-carbon bond is reduced, and the ligand is less firmly held.

The hexaphenylisocyno derivatives were prepared by reduction of the appropriate metal compounds in anhydrous ethanol in the presence of phenyl isocyanide, according to the methods of Malatesta and Sacco<sup>2</sup>, and afterwards purified as described by these authors. Molybdenum trioxide was reduced by hydrazine hydrate and tungsten hexachloride by magnesium powder and acetic acid.

Neither derivative alone is an initiator of the polymerization of methyl methacrylate, but both initiate it readily in the presence of a low concentration of a suitable organic halide such as carbon tetrachloride. The variation of the rate of polymerization with increasing  $[CCl_4]$  is remarkably similar to that observed with the metal carbonyls<sup>1</sup>, increasing rapidly at first and finally becoming almost independent of  $[CCl_4]$  for values of the latter exceeding about  $0.1 \text{ mole l}^{-1}$ .

The kinetics of these reactions (in inactive light) are somewhat more complicated than those generally found with metal carbonyls, especially with the tungsten derivative, since the rate of initiation decreases with time from its initial value to a greater extent than corresponds to any simple order of reaction. The mean rates of polymerization of methyl methacrylate  $\bar{w}$  (effectively in bulk) over 30 min at  $80^\circ \text{C}$  are given by:

$$\bar{w} = 1.9 \times 10^{-2} [Mo(CNPh)_6]^{1/2} \text{ mole l}^{-1}\text{s}^{-1} \quad (a)$$

$$\bar{w} = 1.5 \times 10^{-2} [W(CNPh)_6]^{1/2} \text{ mole l}^{-1}\text{s}^{-1} \quad (b)$$

for 'high'  $[CCl_4]$ . The overall activation energies are comparatively low—in the range 11–15 kcal mole<sup>-1</sup>—and polymerization may be readily initiated at room temperature. The free radical nature of the reaction is indicated by the half-order in the initiator concentration, and the 'normal' value for the ratio  $k_p k_t^{-1/2}$  calculated from mean rates and degrees of polymerization ( $k_p$ ,  $k_t$  are the velocity coefficients of propagation and termination, respectively).

Hexaphenylisocyno tungsten (0) does not show the 'inhibitory' features which are so marked with tungsten carbonyl<sup>3</sup> so that the relation (1b) is obeyed over the whole range of concentrations investigated (up to  $1.2 \times 10^{-3} \text{ mole l}^{-1}$ ). The molybdenum derivative inhibits the reaction at higher concentrations similarly to  $Mo(CO)_6$  so that (1a) holds only for  $[Mo(CNPh)_6] < 5 \times 10^{-4} \text{ mole l}^{-1}$ , approximately.

These systems are considerably more active initiators than those incorporating the corresponding metal carbonyls. Thus the mean rates given by reaction (1) are greater than the initial rates for the carbonyls; this is very marked with the tungsten compounds, the difference amounting to a factor of nearly 3, or about 8 in the rate of initiation. These results, therefore, are completely consistent with the view that phenyl isocyanide is a more easily displaceable ligand. At concentrations of metal derivative exceeding about  $10^{-3} \text{ mole l}^{-1}$  the differences in rate with the two types of ligand are even greater on account of inhibition in the carbonyl systems.

Addition of phenyl isocyanide ( $9 \times 10^{-4} \text{ mole l}^{-1}$ ) to reactions initiated by the tungsten derivative reduces the rate of polymerization at  $80^\circ \text{C}$ , but to an extent not much in excess of that observed in free-radical polymerization initiated with azo *bis* isobutyronitrile. With the molybdenum derivative the effect is somewhat greater, but it is still much less than the marked inhibition produced by carbon monoxide in the case of the corresponding metal carbonyl systems<sup>3</sup>.

Use of polyvinyl trichloroacetate as the halide in place of  $CCl_4$  leads to systems showing rapid gelation. As already shown<sup>4</sup> this implies that radical formation takes place by abstraction of a halogen atom from the halide by the metal derivative, leaving an organic radical (for example,  $\dot{C}Cl_3$  from  $CCl_4$ ) capable of initiating polymerization.

A change in the valency state of the metal atom naturally accompanies radical formation. Paramagnetic



species are produced from hexaphenylisocyno molybdenum (0) and molybdenum hexacarbonyl which may be shown from the hyperfine structure of their electron spin resonance spectra to be molybdenum compounds. No electron spin resonance signals are obtainable from the corresponding tungsten derivatives. Work is in progress to elucidate the nature of the valency change in the metals.

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## BIOPHYSICS

### Intrinsic Viscosity Measurements of Bovine Serum Albumin at Different Temperatures

INTRINSIC viscosity of bovine serum albumin has been measured by a number of workers<sup>1-6</sup> and values ranging from 3.7 to 4.5 were obtained. All the measurements were made within certain ranges of pH (5-7), ionic strength (0.1-0.2) and temperature (20°-28° C). It has been reported that the intrinsic viscosity of bovine serum albumin does not change within the aforesaid ranges of pH and ionic strength<sup>3,4</sup>. Naturally the question arises whether the difference in the values of intrinsic viscosity obtained by different authors can be attributed to: (1) the difference in the temperature of the solutions; (2) the difference in the samples, that is, the difference in the heterogeneity factors of the samples (albumin is always found associated with a small fraction of material of higher molecular weight). No experiment has yet been reported which can help in the solution of this problem. The heterogeneity factor of the different samples used by different authors is also unknown.

In the experiment recorded here the intrinsic viscosity of a particular sample of bovine serum albumin has been measured under the same condition of pH and ionic strength at different temperatures.

The sample was crystalline bovine plasma albumin, fraction V, obtained from L. Light and Co., Colnbrook, England. The diffusion coefficient of the present sample of serum albumin was obtained<sup>7</sup> as  $D_{25}^{0,w} = 6.73 \times 10^{-7}$  cm<sup>2</sup>/sec. The ultracentrifugal pattern of the present sample revealed the presence of about 6 per cent material of higher molecular weight.

The viscosity was measured with the help of an Ostwald type of capillary viscometer in a constant-temperature water bath ( $\pm 0.01^\circ$  C). The upper bulb of the viscometer had a volume of 20 c.c. The flow time of 0.1 M sodium chloride at 25° C was of the order of 16 min. The protein solution was prepared by measuring the dry weight with the help of a semi-micro balance and dissolving in 0.1 M sodium chloride. The solution was then filtered through a sintered glass filter. The protein concentration in the filtrate was measured with the help of a Zeiss PMQ 11 spectrophotometer ( $E_{1\text{cm}}^{278} = 6.67$  at 278 mμ). The flow times of the filtered protein solution and the solvent were measured. The ratio of the averages of five such readings in each case was taken as the relative viscosity ( $\eta/\eta_0$ ) of the protein at that particular concentration. The reduced viscosity of

the protein solution<sup>1</sup> was obtained by subtracting 1.00 from  $\eta/\eta_0$  and dividing the result by the corresponding protein concentration,  $c$  (g/ml.). Freshly prepared protein solution was used for each experiment.

Fig. 1 shows the plot of  $(\eta/\eta_0 - 1)/c$  against  $c$  for five different temperatures (14°-28° C). Experimental points for all the temperatures were found to fit well in a single straight line (drawn by the least square method) passing through them. The intrinsic viscosity  $[\eta]$  of bovine serum albumin was obtained equal to  $4.18 \pm 0.05$  (average deviation of the points from the straight line).

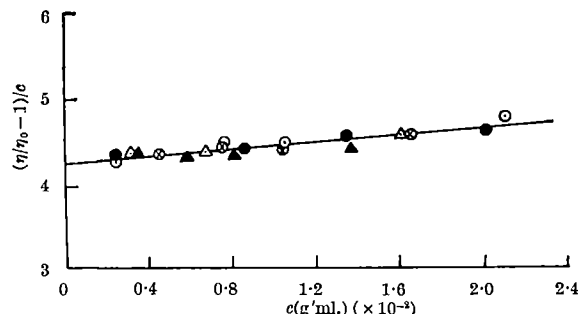


Fig. 1. Plot of reduced viscosity,  $(\eta/\eta_0 - 1)/c$ , against concentration,  $c$ , of bovine serum albumin in 0.1 M sodium chloride at different temperatures. The extrapolated value and the slope of the least-squares line passing through all the points are  $4.18 \pm 0.05$  and 19 respectively.  $\Delta$ ,  $14^\circ \pm 0.01^\circ$  C;  $\circ$ ,  $16.50^\circ \pm 0.01^\circ$  C;  $\bullet$ ,  $20^\circ \pm 0.01^\circ$  C;  $\blacktriangle$ ,  $25^\circ \pm 0.01^\circ$  C;  $\oplus$ ,  $28^\circ \pm 0.01^\circ$  C

The experimental value of  $\eta$  thus lies within the range of values reported by others. The present set of data further indicates that the temperature (within the range examined) affects significantly neither the slope nor the extrapolated value,  $\eta$ , of the plot of  $(\eta/\eta_0 - 1)/c$  against  $c$ . Indirectly this work indicates that the varied results obtained by different authors are due to the differences in the heterogeneity factor of the samples used and hence indicates the necessity of mentioning the heterogeneity factor of the sample while reporting the value of  $\eta$ .

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## BIOCHEMISTRY

### Detection and Documentation of Lipids after Thin-layer Chromatography

A DETAILED description of methods for the detection of lipids after thin-layer chromatography was recently given by Mangold<sup>1</sup>. Many workers prefer spraying with 2',7'-dichlorofluoresceine or rhodamine G solutions and viewing under ultra-violet light, while others expose the chromatoplate to iodine vapours. Subsequent elution of the lipids and methylation of fatty acids for gas-liquid chromatography is possible because the phosphors do not disturb these procedures. In semi-quantitative and preparative scale thin-layer chromatography we prefer localizing by means of fluorescence which many of the separated lipid spots exhibit under ultra-violet light<sup>2</sup>. In

Table 1. PERCENTAGE COMPOSITION OF A MIXTURE OF FATTY ACID METHYL ESTERS BEFORE AND AFTER ULTRA-VIOLET EXPOSURE

Fatty acid	Before ultra-violet exposure	After ultra-violet exposure
Palmitic acid	19.6	16.6
Stearic acid	3.7	3.9
Oleic acid	12.0	12.4
Linoleic acid	61.3	63.2
Linolenic acid	3.4	3.9

this way there is no contamination by phosphors and no danger of structural alterations. This method, too, is superior to 'leading chromatograms' which do not reveal irregularities of separation. In order to have permanent records the fluorescent lipid spots may be photo-copied in a manner similar to that of Milton<sup>3</sup>.

Samples of chloroform-methanol extracts (2:1, v/v) of tissue or plasma were spotted in 2 cm bands 1.5 cm above the lower edge of thin-layer plates coated with 0.25–0.5 mm silica gel G. The lipids were separated by ascend-

ing elution with the solvent mixture petroleum ether (b.p. 30°–60° C), diethyl ether and acetic acid (85:15:1, by volume) according to Mangold and Tuna<sup>4</sup>. After drying at room temperature a sheet of Agfa 'Copyrapid' negative *CpM* paper was placed on the silica-gel layer. In order to keep chromatoplate and paper in contact the paper was covered with a second glass plate. Next, this device was turned and the thin-layer was exposed through the glass to ultra-violet light for about 10 sec at a distance of 20 cm from the lamp. A quartz-lamp, model 'Heraeus', a big laboratory model with a predominating wave-length of 366 mμ, was used as the ultra-violet source. The negative paper was then drawn through the Agfa 'Copyrapid' developer and dried. The lipid spots for subsequent elution, etc., were localized by a second exposure to ultra-violet light and traced with a needle.

Fig. 1 shows the absorbing spots of four total lipid extracts from plasma and the solvent front as white areas against a dark background. There is no advantage in preparing positive copies because minor components are often lost. This is exemplified in Fig. 2 which represents the positive copy of Fig. 1; the weak spot representing free fatty acids of the first plasma sample is absent.

The short exposure of the thin-layer plates does not lead to alterations in fatty acid structure. Table 1 gives the composition of a mixture of fatty acid methyl esters before and after thin-layer chromatography and a 5-min exposure to ultra-violet light. There is no significant difference between fatty acid methyl ester composition with and without ultra-violet exposure.

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### Nitrate Reduction with Molecular Hydrogen in a Reconstituted Enzymatic System

In recent work from this laboratory<sup>1</sup> it was found that flavin nucleotides, the well-known co-factors of cyclic photophosphorylation, could mediate the transfer of electrons from illuminated spinach grana to nitrate in the presence of spinach nitrate reductase. Spinach ferredoxin was not the effective electron carrier in the process. The electrons were supplied by either water or reduced indophenol dyes, and neither pyridine nucleotides nor adenosinetriphosphate were required for the reduction of nitrate. Since the dark reduction of nitrate by molecular hydrogen has been reported to occur at the cellular level in photosynthetic purple bacteria<sup>2,3</sup> and in the alga *Ankistrodesmus braunii*<sup>4</sup>, we investigated the mechanism of nitrate assimilation in a reconstituted enzyme system using hydrogen gas-hydrogenase as the electron donor instead of illuminated grana. In similar experiments carried out previously<sup>5</sup> on the mechanism of the dark and light reduction of nitrite, it was found that the co-factor required in both cases was ferredoxin.

Table 1 shows that the dark reduction of nitrate to nitrite by electrons supplied by hydrogen gas (in the presence of added *Clostridium pasteurianum* hydrogenase) was mediated by flavin mononucleotide and required the presence of spinach nitrate reductase. The participation of flavin nucleotides in another metabolic process, the reduction of diphosphopyridine nucleotide by hydrogen, has been found in several microbial preparations<sup>6-8</sup>. Under our conditions, *Clostridium* ferredoxin was not active as electron carrier in the transfer of electrons from molecular hydrogen to nitrate.

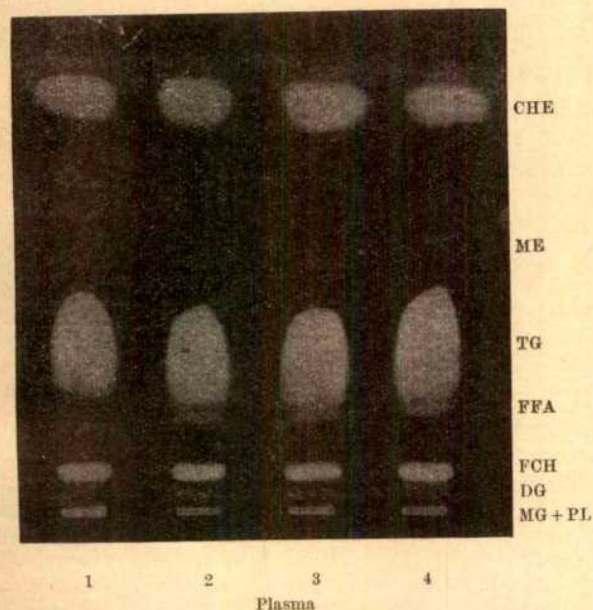


Fig. 1. Negative ultra-violet copy of thin-layer chromatoplate. CHE, cholesterol esters; ME, fatty acid methyl esters; TG, triglycerides; FFA, free fatty acids; FCH, free cholesterol; DG, diglycerides; MG + PL, monoglycerides and phospholipids

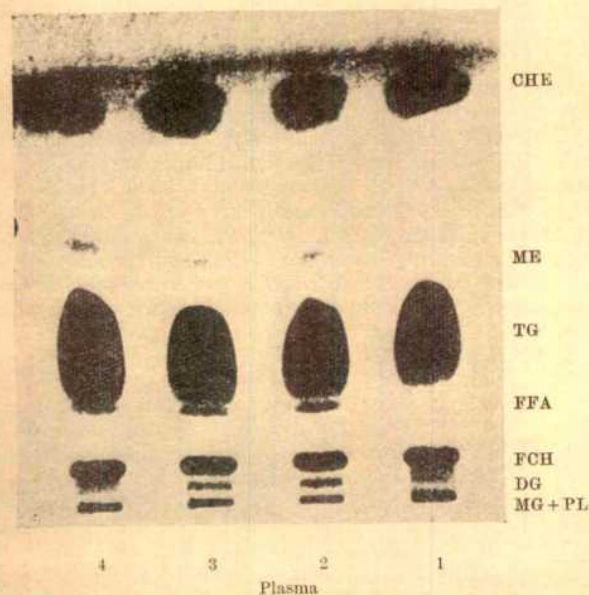


Fig. 2. Positive ultra-violet copy of thin-layer chromatoplate. Same plate as in Fig. 1



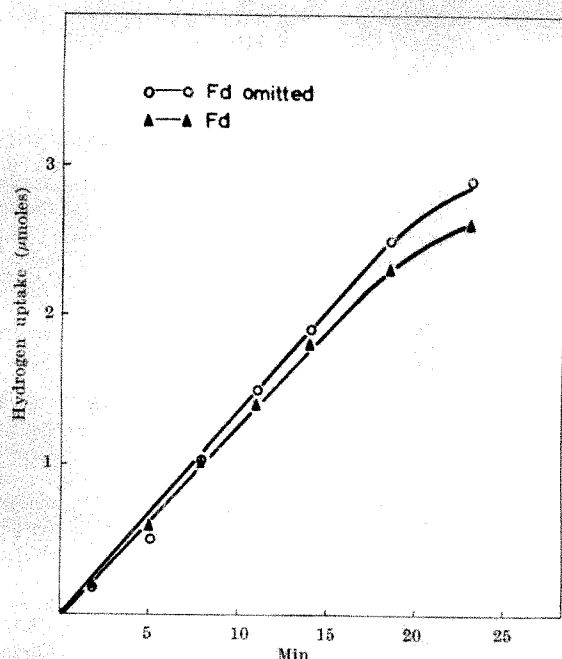


Fig. 1. Reduction of flavin mononucleotide with hydrogen gas in the absence and in the presence of ferredoxin (Fd). The reaction mixture included, in a final volume of 3 ml., sodium phosphate buffer pH 7.0, 200 μmoles; *Clostridium pasteurianum* hydrogenase<sup>10</sup>, 0.9 mg; cysteine, 10 micromoles. The reaction was started by tipping in flavin mononucleotide (4 μmoles) or flavin mononucleotide (4 μmoles) plus purified *Clostridium pasteurianum* ferredoxin<sup>12</sup> (0.3 mg) from the side-arm of the Warburg vessels. Other experimental conditions as in Table 1.

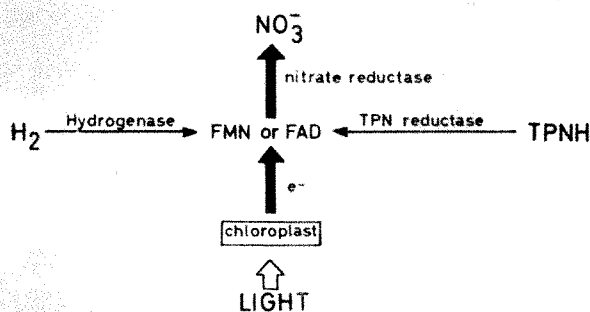


Fig. 2. Diagrammatic representation of the role of flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) in the reduction of nitrate. The transfer of electrons from flavins to nitrate is a dark reaction catalysed by the chloroplast nitrate reductase. Flavins themselves can be reduced either in the light (by grana) or in the dark (by hydrogen gas or reduced triphosphopyridine nucleotide, TPNH).

Whiteley and Woolfolk<sup>9</sup> have reported that the reduction of flavins with hydrogen by *Micrococcus lactylicus* hydrogenase required the presence of ferredoxin isolated from the same micro-organism. We have therefore tested the effect of *Clostridium* ferredoxin addition on the hydrogen uptake catalysed by *Clostridium* hydrogenase using substrate amounts of flavin mononucleotide as the electron acceptor. As Fig. 1 shows, the reduction of this coenzyme was not mediated by ferredoxin.

The role of flavin nucleotides in the photochemical and dark reduction of nitrate catalysed by spinach nitrate

Table 1. REDUCTION OF NITRATE WITH HYDROGEN GAS IN A RECONSTITUTED ENZYME SYSTEM

System	Nitrite formed (μmoles)
Complete	1.24
Nitrate omitted	0.00
Flavin mononucleotide omitted	0.11
Nitrate reductase heated	0.00

The reaction mixture included, in a final volume of 3 ml., ferredoxin-free *Clostridium pasteurianum* hydrogenase<sup>10</sup>, 5.4 mg; spinach nitrate reductase<sup>11</sup>, 4.2 mg; and the following in μmoles: sodium phosphate buffer, pH 7.0, 200; flavin mononucleotide, 0.4; cysteine, 10; potassium nitrate, 20. The reaction was carried out in Warburg manometer flasks at 27° C for 75 min under hydrogen. Nitrite was assayed by the method of Novak and Wilson<sup>14</sup>.

reductase is schematically represented in Fig. 2 (see ref. 1).

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## Cholinesterase Catalysis

CHARACTERISTIC of all known schemes of esterase catalysis<sup>1-5</sup> is the assumption that the reactive form of serine pre-exists in the enzyme molecule before the interaction of enzyme with substrate or inhibitor. This seems to be unlikely, for why this very reactive form is not involved in side-reactions is still very vague. Moreover, the possibility of the preliminary activation of serine aliphatic hydroxyl, by means of such a weak base as imidazole<sup>3-5</sup>, is not convincing.

The scheme outlined here (Fig. 1) suggests that acetylcholine (ACh) participates in activation of the catalytic centre of the cholinesterase (ChE). On approaching inactive ChE (I) the ACh is adsorbed on to the surface of the ChE, this adsorption being orientated by the anionic site of the enzyme. As a result of this process, at first a hydrogen-bond between ACh carbonyl oxygen and imino-nitrogen of a histidine imidazole ring is formed. The formation of this bond increases the basicity of the azolic nitrogen and a re-arrangement of the active centre of ChE occurs. Then the second hydrogen-bond between the activated azolic nitrogen and serine hydroxyl arises. This bond increases the nucleophilic nature of the hydroxyl oxygen and stimulates its interaction with the carbonyl carbon on ACh. This appears to be the essence of the transformation of the enzyme into its active form and of the formation of an enzyme-substrate Michaelis complex (IIa → IIb). The redistribution of the electron density in the 8-member ring results in the formation of the covalent bond between serine oxygen and carbonyl carbon and in the elimination of choline molecule. So the acetyl enzyme is formed (III). Proton transfer from imidazole to choline molecule may be carried out by means of the acidic group of the ester site (the existence of such a group is supposed by some authors<sup>5,6</sup>). The repulsion forces which act between the carbonyl oxygen and the azolic nitrogen seem to change the mutual orientation of the acetyl serine and the imidazole ring. The latter results in the formation of the hydrogen-bond between carbonyl oxygen and imino-nitrogen. The effects connected with this are similar to those inducing the Michaelis complex formation and lead to the appearance of new bonds between the acetyl enzyme and water molecule and to the formation of the intermediate acetyl-enzyme-hydrate complex (IVa → IVb). The redistribution of electron density in the 8-member cycle results in the



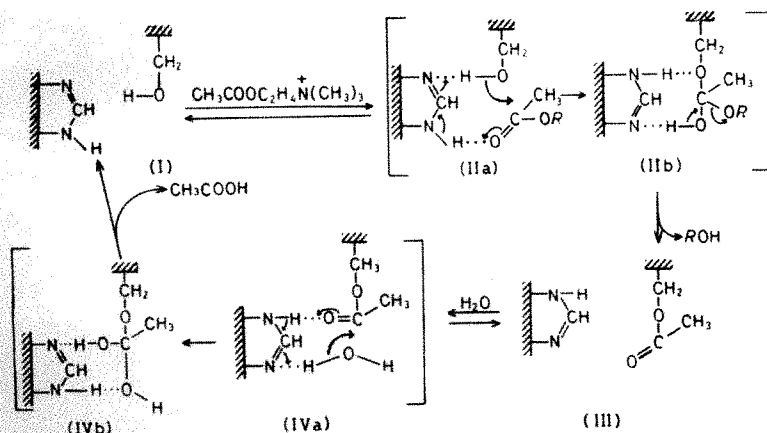


Fig. 1

elimination of the acetic acid molecule and in the transformation of the enzyme in its initial inactive form (I). Proton transfer from the imidazole ring to the serine oxygen is rendered possible by the acidic group of the esteratic site.

The phosphorylation of ChE by organophosphorus inhibitors appears to proceed according to the same scheme with the exception that the phosphorylated enzyme practically does not react with water.

The principal features of the suggested mechanism are:

(1) It assumes the participation of the substrate in the enzyme catalytic centre formation, the process being in good agreement with Koshland's 'induced fit' theory<sup>6</sup>. The conformation alterations which seem to involve also the hydrophobic sites of the active surface of ChE serve as a basis for explanation of the observed phenomena in the interaction of organophosphorus compounds with ChE<sup>7-9</sup>.

(2) The processes of acetylation and deacetylation are based on the same mechanism, which leads to formation of the intermediate enzyme-substrate Michaelis complex in the former process and of the acetylenzyme-hydrate complex in the latter.

(3) The scheme presents a new explanation of the inability of the substrates and inhibitors containing C=S and P=S groups, respectively, to interact with ChE; the sulphur cannot form hydrogen-bonds which are essential for the activation of the catalytic centre of the enzyme.

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## Uranyl Ions and Intestinal Hexose Transfer

It is well established that at least part of the glucose transfer mechanism in the intestinal epithelium lies close to the luminal border of the cell. As Rothstein<sup>1</sup> has shown that uranyl ions exert a surface action in yeast cells in inhibiting hexose transfer, and Ponz<sup>2</sup> and Ponz and Lluch<sup>3</sup> have shown that they interfere with absorption of actively transported sugars, the effect of uranyl nitrate on hexose transfer in the rat small intestine was investigated.

Experiments were carried out on sacs of rat everted small intestine, and the transfers of glucose, galactose and fluid were examined in the presence of uranyl nitrate. Uranyl nitrate was found to have two effects on glucose transfer from the mucosal fluid. In concentrations from  $10^{-5}$  M to  $3 \times 10^{-4}$  M, glucose entry from the mucosal fluid was reduced from 55 mg/h to 40 mg/h, while glucose metabolism was unaffected. These concentrations also reduced the fluid transfer stimulated by glucose initially present in the mucosal fluid but not by glucose initially present in the serosal fluid. This supports the view that uranyl nitrate at these concentrations inhibits the entry of glucose into the epithelial cell from the mucosal fluid. In higher concentrations uranyl nitrate ( $3 \times 10^{-3}$  M) reduced glucose metabolism from 19 mg/h to 9 mg/h.

When galactose was initially present in the mucosal fluid, uranyl nitrate ( $3 \times 10^{-4}$  M) did not inhibit galactose transfer, but in a concentration of  $3 \times 10^{-3}$  M it reduced galactose transfer from 22 mg/h to 12 mg/h. This shows that uranyl nitrate can affect glucose entry from the mucosal fluid in a concentration which does not affect entry of galactose, the latter being inhibited only at concentrations which affect metabolism.

Experiments were also performed in which glucose and galactose were initially present together in the mucosal fluid. Fisher and Parsons<sup>4</sup> have shown that in equimolar concentrations glucose inhibits galactose transfer, and we found also inhibition of galactose transfer when both were present in a concentration of 28 mM. However, when glucose (5.6 mM) and galactose (28 mM) were present together galactose transfer was increased from 21 mg/h to 36 mg/h. Galactose transfer was also stimulated by glucose (111 mM) in the serosal fluid. Although uranyl nitrate ( $3 \times 10^{-4}$  M) did not affect galactose entry when present alone, it inhibited the stimulation of galactose transfer by glucose (5.6 mM) initially present in the mucosal fluid.

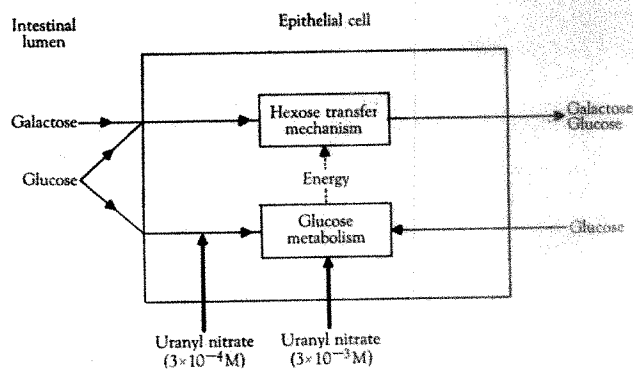


Fig. 1

Various explanations of these findings can be suggested. The most attractive is that illustrated in Fig. 1. This shows two different routes of entry of glucose into the cell from the intestinal lumen. One route is available to glucose only and not to galactose. It channels glucose into metabolism, and is blocked by uranyl ions. The second route is available to both glucose and galactose, and channels these into the mechanism for transfer against a concentration gradient. This route is not blocked by low concentrations of uranyl nitrate. Galactose transfer by this route can use energy from endogenous metabolism, but in addition can use energy from glucose metabolism. When glucose (5.6 mM) and galactose (28 mM) are present together with uranyl nitrate ( $3 \times 10^{-4}$  M) the two hexoses presumably compete for the one

pathway now available, and hence galactose transfer is inhibited.

Other explanations are also possible. There may be one mechanism for entry of hexoses into the cell, and the separation into two pathways—one uranyl sensitive leading into metabolism—may occur inside the cell. In this case it must be postulated that the uranyl sensitive pathway is not available to glucose initially present in the serosal fluid. Another possibility is that uranyl ions may penetrate only into a limited part of the cell and may affect metabolism of part of the cell into which glucose from the serosal side of the cell does not penetrate.

Of considerable interest is the stimulation of galactose transfer by small concentrations of glucose. Fisher and Parsons<sup>4</sup> showed that glucose and galactose compete for entry, and while the present results support this they also show that provided glucose is present in small concentrations in the mucosal fluid or in higher concentrations in the serosal fluid, it may be able to enter the cell without competing with galactose and by its metabolism stimulate galactose transfer. This would be in keeping with the results of Newey and Smyth<sup>5</sup>, who have shown that glucose metabolism can stimulate glycine transfer. It thus appears that glucose metabolism can supply energy for three different transfer systems, that is, galactose, glycine and fluid.

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### Identification of Oestradiol and Oestrone in Avian Plasma

ALTHOUGH it has long been held that the plasma of the laying domestic fowl contains oestrogenic substances no direct identification has been reported. Although oestrone, oestradiol and oestriol have been identified in extracts of ovarian tissue from the laying hen<sup>1</sup> and also in urine<sup>2,3</sup>, attempts to detect oestrogens in blood have been unsuccessful.

As part of an investigation of factors involved in yolk production in the domestic fowl this problem has been investigated using a technique of double isotope derivative analysis<sup>4</sup> using carbon-14-labelled oestrogens and tritiated dimethyl sulphate as identifying agents.

Plasma from laying hens was treated with hydrochloric acid<sup>5</sup> to hydrolyse conjugated oestrogens, and after extraction with chloroform was separated into fractions presumptively containing oestradiol + oestrone and oestriol<sup>6</sup>. The fractions were methylated with dimethyl sulphate-T and, after removal of solvents and decomposition of excess dimethyl sulphate with alkali, were extracted into hexane. Authentic methylated <sup>14</sup>C-oestradiol and oestrone were then added and the methyl oestrogens were separated and purified, first by thin-layer chromatography using solvent systems A, B and C of Lisboa and Diczfaluzy<sup>6</sup>, followed by chromatography on columns of prepared alumina<sup>7</sup>. Samples of each fraction were counted in a liquid scintillation counter.

It was found that the samples of both methyl oestradiol and methyl oestrone contained tritium, the ratios of tritium/<sup>14</sup>C becoming constant after several fractionations (Table 1). As a further check on the chromatographic homogeneity, fractions eluted from the second alumina column (Table 1) were re-chromatographed on alumina and the three fractions comprising the beginning, the

Table 1. RATIOS OF TRITIUM/<sup>14</sup>C IN EXTRACTS OF AVIAN PLASMA TREATED AS DESCRIBED IN THE TEXT AND CHROMATOGRAPHED TOGETHER WITH CARRIER METHYL OESTROGENS

Solvent systems	Methyl oestrone			Methyl oestradiol		
	T (c.p.m.)	<sup>14</sup> C (c.p.m.)	Ratio (T/ <sup>14</sup> C)	T (c.p.m.)	<sup>14</sup> C (c.p.m.)	Ratio (T/ <sup>14</sup> C)
A	2.86 × 10 <sup>5</sup>	460	621.0	1.67 × 10 <sup>5</sup>	324	513.0
B	6.9 × 10 <sup>5</sup>	319	217.0	20.2 × 10 <sup>5</sup>	293	69.0
C	4.71 × 10 <sup>5</sup>	126	37.6	3.28 × 10 <sup>5</sup>	166	19.8
Alumina 1	1.45 × 10 <sup>5</sup>	73	19.8	1.72 × 10 <sup>5</sup>	102	18.1
Alumina 2	1.07 × 10 <sup>5</sup>	57	18.7	1.53 × 10 <sup>5</sup>	75	20.2

Table 2. RATIOS OF TRITIUM/<sup>14</sup>C IN FRACTIONS FROM THE COLUMNS ALUMINA 2, OF TABLE 1, RECHROMATOGRAPHED ON A FRESH ALUMINA COLUMN<sup>8</sup>

Fraction number	Methyl oestrone			Methyl oestradiol		
	T (c.p.m.)	<sup>14</sup> C (c.p.m.)	Ratio (T/ <sup>14</sup> C)	T (c.p.m.)	<sup>14</sup> C (c.p.m.)	Ratio (T/ <sup>14</sup> C)
2	97.1	4.5	21.4	415.0	19	21.9
3	1.07 × 10 <sup>5</sup>	57	18.7	1.53 × 10 <sup>5</sup>	75	20.2
4	336	17.0	19.8	124.2	16	20.7

middle and the end of each elution peak were separately collected. The tritium/<sup>14</sup>C ratios in each were found to be the same within the limits of experimental error (Table 2). At no time was tritium detectable in chromatographic areas corresponding to authentic methyl oestriol.

The results show that materials are present in hydrolysates of avian plasma which after methylation behave in a manner identical with methyl oestrone and methyl oestradiol in several solvent systems. Such behaviour is considered to be proof of identity and to show the existence of oestradiol and oestrone, or their conjugates, in avian plasma.

We thank Cyril Thornber, Ltd., Mytholmroyd, Halifax, for a generous supply of birds.

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### An Extracellular $\alpha$ -L-Arabinofuranosidase secreted by *Sclerotinia fructigena*

AN earlier communication<sup>1</sup> reported the resolution of a maceration factor from endopolygalacturonase (PG) in culture filtrates of *Sclerotinia fructigena* Aderh. and Ruhl., by means of gel filtration on dextran Sephadex 'G 75' and chromatography on 'Ecteola'-cellulose. The biochemical basis of maceration of potato slices by these preparations was not elucidated, but McClendon<sup>2</sup> has demonstrated that, in chromatography on cellulose phosphate of an ultra-filtered and freeze-dried sample of our culture filtrate, maceration of potato disks occurred in two peaks, one associated with a major PG peak and the other with a minor PG peak, with indications that arabanase or galactanase may macerate. We have shown independently that arabinose is liberated from potato fibre by a purified 'maceration factor' preparation free of PG and, subsequent to McClendon's findings, from lupin seed pectate. Incubation of the purified preparation with potato fibre was also accompanied by a release of soluble uronide, as determined by the carbazole method<sup>3</sup> (Fig. 1); the uronide was shown to be of high molecular weight by its failure to pass through a 'Visking' dialysis membrane, suggesting that a partial breakdown of insoluble 'protopectin' had occurred.

'Protopectin' may owe its insolubility to the presence of linkages of uronides with galactans and arabans<sup>4</sup>, which are known to consist of chains of  $\alpha$ -L-arabino-

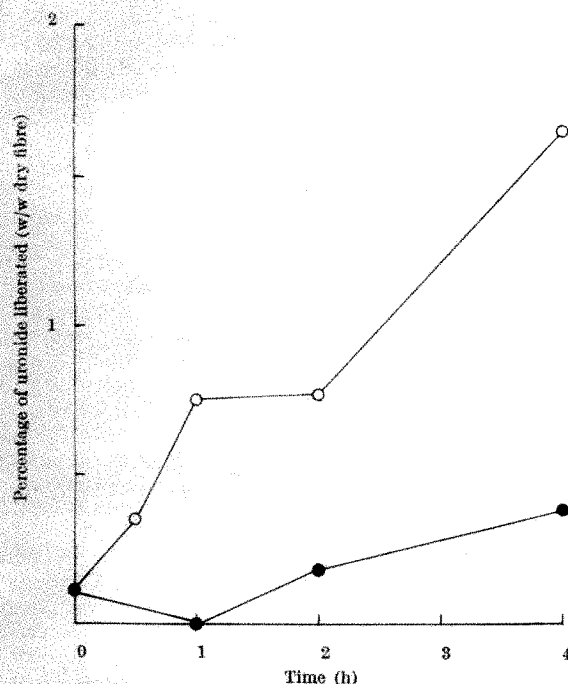


Fig. 1. Liberation of soluble uronide from potato fibre by 'maceration factor'. ●, Heated; ○, unheated

furanoside<sup>5</sup> and  $\beta$ -D-galactopyranoside<sup>6</sup> groups respectively. We have therefore examined the activity of culture filtrates of *S. fructigena* and of the purified 'maceration factor' in hydrolysing phenyl- $\alpha$ -L-arabinofuranoside<sup>7</sup> and *o*-nitrophenyl- $\beta$ -D-galactopyranoside<sup>8</sup> together with *o*-nitrophenyl- $\alpha$ -L-arabinopyranoside<sup>9</sup> and phenyl- $\beta$ -D-galactofuranoside<sup>10</sup> for comparison. The hydrolysis rates were determined at 25° and pH 4.7 by the usual estimation of the intensity of yellow colour due to *o*-nitrophenol liberation or, in the case of the unsubstituted phenyl glycosides, by estimation of liberated phenol<sup>11</sup>. The results given in Table 1 show that, while the culture filtrates hydrolysed all four substrates at varying rates, only the arabinofuranoside was attacked by the purified preparation.

Table 1. HYDROLYSIS RATES AT 25° AND pH 4.7 OF PHENYL- $\alpha$ -L-ARABINOFURANOSIDE (AF), *o*-NITROPHENYL- $\beta$ -D-GALACTOPYRANOSIDE (GP), *o*-NITROPHENYL- $\alpha$ -L-ARABINOPYRANOSIDE (AP) AND PHENYL- $\beta$ -D-GALACTOFURANOSIDE (GF)

Substrate	Initial concn. (mM)	Hydrolysis rate ( $\mu$ h/ml. enzyme) Culture filtrate	Purified 'maceration factor'
AF	1.11	7.02	1.52
GP	1.11	2.56	0.00
AP	1.23	0.59	0.00
GF	0.98	0.10	0.00

In support of the hypothesis that *Sclerotinia* maceration factor is identical with  $\alpha$ -L-arabinofuranosidase, it was found in preliminary experiments that the thermal inactivation pattern of the enzyme appeared to follow that of the maceration of potato disks assessed by Brown's method<sup>12</sup>.

The presence of the enzymes was also demonstrated in filtrates of 11-day-old cultures on pectate media of several other fungi known to secrete macerating enzymes—*Botrytis cinerea*<sup>13</sup>, *Penicillium expansum*<sup>14</sup>, *Rhizoctonia solani*<sup>15</sup>, and *Sclerotinia sclerotiorum*, and in an extract of carrot tissue rotted by the last-named fungus<sup>16</sup>.

These results nevertheless do not preclude the possibility that maceration is due to a substance distinct from  $\alpha$ -L-arabinofuranosidase, but of similar behaviour in the purification procedure adopted and which, if an enzyme, has a substrate not yet biochemically defined.

We thank Prof. B. Lindberg and Dr. Per Jerkeman for samples of phenyl- $\alpha$ -L-arabinofuranoside and phenyl-

$\beta$ -D-galactofuranoside, and Prof. J. H. McClendon for a sample of lupin seed pectate.

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## PHYSIOLOGY

### Influence of External Stimuli on the Secretory Rate of the Avian Nasal Salt Gland

It was noticed during a series of experiments dealing with the functional capacity of the salt gland of sea-gulls that an increase in secretion rate was occasionally associated with 'alertness' caused by distant moving objects or from sudden noises.

In order to prove or disprove this impression, eight sea-gulls were subjected to either photic or auditory stimuli following a minimal salt load<sup>1</sup>, which induced the secretion of nasal fluid from the salt glands. The stimuli were applied shortly after the maximum secretory rate had been reached, at a time when the flow rate was subsiding.

Photic stimulation was produced by repeatedly shining a flashlight into the eye of a gull for periods varying from 2 to 10 min at a distance which did not induce 'escape' movements. In all instances light increased the secretory rate of nasal fluid produced by the salt gland

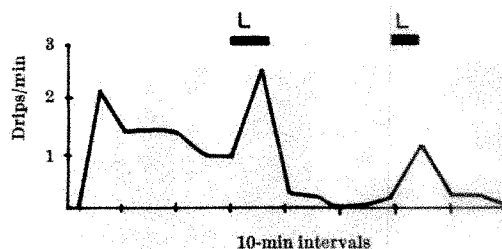


Fig. 1. Typical response of a salt-loaded gull to a light stimulus (L), the duration indicated by the bar. Notice the increased rate of nasal fluid secretion

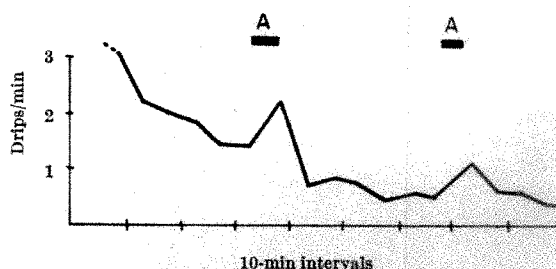


Fig. 2. Typical response of a salt-loaded gull to an auditory stimulus (A), the duration indicated by the bar. Notice the increased rate of nasal fluid secretion



(Fig. 1) 0.5–3 times that noted immediately before applying the stimulus.

The sound stimulus was made with a quitter using individual notes, chords or a melody. Sound increased the secretory rate of nasal fluid (Fig. 2) in the same manner and degree as noticed with light stimulation.

These observations may be explained by assuming that photic or auditory stimuli increase the general excitability of the central neuropile and by facilitation augment the activity of the parasympathetic fibres innervating the gland<sup>2</sup>. These parasympathetic fibres are believed<sup>2</sup> to pass with the facial nerve, and presumably take origin near the motor nucleus of the facial nerve in the medulla oblongata. It is believed<sup>3</sup> that in the central nervous system there are osmoreceptors which react to increased osmolarity of the blood by exciting the parasympathetic fibres that stimulate salt gland secretion. If this is the case, then the finding that light or sound can enhance salt gland activity suggests that the final common parasympathetic pathway is influenced by neurons besides those from the osmoreceptors.

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### Endogenous Shock-inducing Factor in Canine Haemorrhagic Shock

DURING recent work in this laboratory<sup>1,2</sup> it was shown that a transferable 'shock-inducing factor' appears in the plasma of endotoxin-shocked adrenalectomized dogs. This factor was heat-unstable (56° C, 30 min) and seemed to act as a histamine-liberator on the liver and induced a marked hepatosplanchnic blood pooling. Moreover, it was shown that a similar factor can be extracted from the liver slices of the shocked animals by cold physiological saline solution. Thus the extreme susceptibility of adrenalectomized dogs to endotoxin has been attributed to the liberation of the 'shock-inducing factor' from the

liver as the consequence of the disturbed hepatic metabolism after taking up endotoxins in the hepatic reticulo-endothelial cells. Pre-treatment with glucocorticoid could prevent both the hepatic metabolic disturbances, such as acute depletion of glycogen, and the release of the 'shock-inducing factor'.

Here it was intended to examine whether a similar 'shock-inducing factor' takes part in the progression of canine haemorrhagic shock in the absence of glucocorticoids or not. Experiments were made on unanesthetized adrenalectomized dogs well maintained with DCA. The animals were allowed to bleed from a femoral artery into an elevated heparinized reservoir until the blood pressure stabilized at 45 mm mercury. This rather high level of blood pressure was necessary for adrenalectomized dogs in order to prevent an early onset of respiratory failure. After about 3–4 h, when the 'up-take' of the blood (self-transfusion) was more than half the shed blood, the animals were bled and the plasma was tested by transfusing to 'Nembutal'-treated normal dogs. The compatibility of the recipients had been tested by a cross-match blood test beforehand.

As shown in Fig. 1, the transfusion of the plasma (10 c.c./kg) could induce a marked delayed depressor response in the recipient dog. The 'shock-inducing factor' shown here was quite similar to that observed in the endotoxin-shocked adrenalectomized dogs and was found to be heat-unstable; completely inactivated by heating to 56° C for 30 min, but not by heating to 53° C for 15 min which destroys the complement. It was precipitable by 34 per cent saturation with ammonium sulphate.

The depressor response was due to hepatosplanchnic blood pooling similar to that induced by histamine-liberators. The latency of the response was long, and the fall in blood pressure appeared usually a few minutes after the end of the plasma infusion. Moreover, the saline extract of the liver slices of the shocked animals was found to contain a similar factor, indicating its hepatic origin<sup>2</sup>.

The progression of the 'up-take' was accompanied by a progressive hypoglycaemia with marked lactacidaemia. Administration of hypertonic glucose solution could easily recover the hypoglycaemia, but not the progress of the 'up-take'. The marked increase in glucose tolerance characteristic of endotoxin shock in adrenalectomized dogs<sup>2</sup> was not observed. These clearly indicated that the

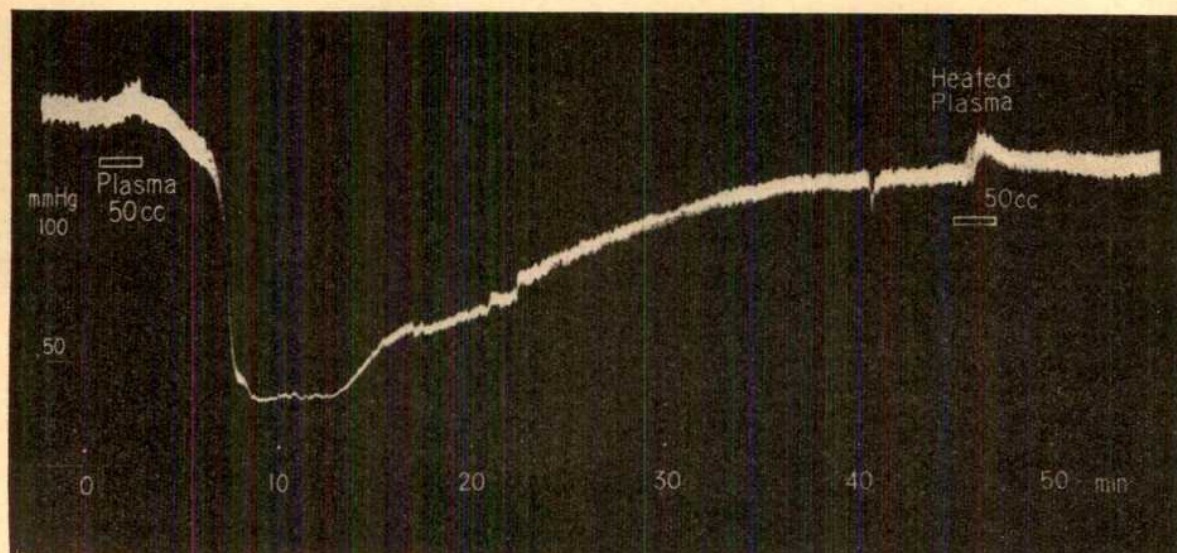


Fig. 1. Effect of transfusion of the plasma of shocked adrenalectomized dog to a 'Nembutal'-treated intact dog. The plasma lost its depressor activity when heated to 56° C for 30 min.



depletion of the liver glycogen and the accompanying hypoglycaemia here observed were not the result of the special derangement of carbohydrate metabolism in endotoxaemia. Moreover, the fact that the characteristic leucopenia in response to shock-dose of endotoxin was not observed indicated that endotoxins or macromolecular tissue substances did not participate in inducing the development of the 'shock-inducing factor'. In this respect the endotoxin hypothesis of Fine *et al.*<sup>3</sup> for the irreversible haemorrhagic shock does not seem acceptable.

The 'shock-inducing factor' was not detected either in plasma or in the liver extract when the animals succumbed to respiratory failure before marked 'up-take' supervened. When the adrenalectomized dogs were treated with glucocorticoids the tendency to hypoglycaemia did not proceed so much within the hypotensive period of 5 h and the 'shock-inducing factor' was not found in detectable amounts in the plasma. In the presence of glucocorticoids, therefore, the anoxic deterioration of the hepatic function leading to the glycogen depletion and to the liberation of the 'shock-inducing factor' seemed to be markedly ameliorated. The significance of glucocorticoids was also obvious in their central analeptic action<sup>4</sup> preventing the early onset of respiratory failure. The fact that glucocorticoids could not prevent the action of the 'shock-inducing factor' once liberated, as was clear in the transfusion experiments on intact dogs, might suggest their applicability to haemorrhagic shock is limited.

In passing, it may be noted that Wiggers<sup>5</sup>, observing the progress of irreversible haemorrhagic shock after restitution of blood volume by re-infusion of all the withdrawn blood (normovolemic shock), mentioned that hepatic resistance contrary to all other organs examined increases markedly and is chiefly responsible for the elevated portal pressure. This might perhaps indicate the participation of the 'shock-inducing factor' shown here, although the amounts liberated are too small to be detected by the passive transfer technique because of the presence of adrenal cortex. Why clear experimental evidence has not been hitherto available that endogenous toxins are concerned as a cause or contributory factor in shock might be due to the fact that the experiments have been exclusively made on intact animals. The significance of the 'shock-inducing factor' in the development of the irreversibility of shock in intact animals will be further investigated.

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### Oral Treatment of Pernicious Anaemia

HEATHCOTE and Mooney<sup>1</sup> reported in this journal their clinical results from treating two cases of pernicious anaemia with a vitamin B<sub>12</sub> glutamic acid mixture, and we published<sup>2</sup> our own results of Schilling tests with and without L-glutamic acid in patients with pernicious anaemia. Our results showed that glutamic acid does not in general increase the absorption of vitamin B<sub>12</sub> measured by the Schilling test. In their reply<sup>3</sup> Mooney and Heathcote disregarded our results, doubting whether the Schilling test has in fact any physiological merit at all. Although we cannot concur in their opinion of the Schilling test, we decided to investigate the problem from another aspect as well.

The essential weakness in the study by Heathcote and Mooney, in our opinion, was the too short control period

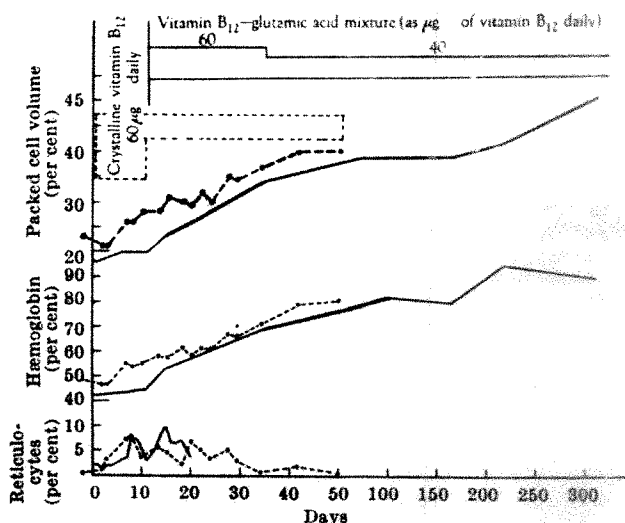


Fig. 1

with oral vitamin B<sub>12</sub> alone. It was just this point which originally made us doubt the significance of glutamic acid. However, as Heathcote and Mooney emphasize the importance of the type of test they used, we treated one pernicious anaemia patient in the same manner, that is, with 60 µg of vitamin B<sub>12</sub> daily orally, except that the glutamic acid additive was omitted. The result is seen in Fig. 1. The development of the blood picture of our case is traced as a dotted line on a copy of Heathcote and Mooney's Fig. 1 (ref. 1). We followed our case for 50 days only, but it is quite apparent that the increase in haemoglobin and packed cell volume is as good as in their case. This we in fact expected, but in addition the reticulocyte curve displayed several successive peaks. We thus obtained a completely similar haematological progress by prolonging the mere vitamin B<sub>12</sub> 'control period' sufficiently.

As, according to Ross *et al.*<sup>4</sup>, about 1 per cent of an oral vitamin B<sub>12</sub> dose is absorbed even without the intrinsic factor, about 0.6 µg daily would be absorbed from 60 µg. This is practically the same as the 1 µg which, given parenterally, suffices in the opinion of Bethel *et al.*<sup>5</sup> to cause remission in pernicious anaemia. It is understandable, then, that the 60 µg dose sufficed, completely independent of the concomitant administration of glutamic acid.

In our opinion, therefore, Heathcote and Mooney's work does not prove that oral administration of vitamin B<sub>12</sub> is more efficacious in cases of pernicious anaemia when administered together with glutamic acid. That the same therapeutic result may in fact be achieved with vitamin B<sub>12</sub> alone, as we have now shown, could have been presupposed from the results of our earlier Schilling tests<sup>2</sup>.

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It will not surprise many workers in the B<sub>12</sub> field to read that Dr. Heinivaara and Dr. Palva have obtained a remission over a period of 50 days in one case of perni-

cious anaemia, which was receiving a daily dosage of 60  $\mu$ g of crystalline B<sub>12</sub> (see above). Indeed, in our original work<sup>1</sup> we chose this dosage because we knew that, being in the sub-optimal range, it should be sufficient to prevent deterioration during the period of test, and thus be ethically justifiable. To draw firm deductions about the significance of glutamic acid additives, however, from a comparison between the blood responses in two selected cases is too ingenuous. The important finding, of course, during this initial period of treatment on sub-optimal dosage is not the haematological response as assessed by haemoglobin (Hb) or packed cell volume (P.C.V.) readings over 50 days (which vary greatly from patient to patient), but the reticulocyte response, obtained from the same patient, on one form of therapy compared with that on another. This test—the double reticulocyte response test—is not our test as Heinivaara and Palva infer. It was originally described in 1935 by Minot and Castle<sup>2</sup>, and is still regarded as the most reliable short-term method for the comparison of the therapeutic effect of two haematinics of unknown potency<sup>3</sup>.

Our control periods were actually within the limits laid down by the authors of the test, but in view of these criticisms we would like to quote another case from our series—which has hitherto remained unpublished—in which the additive was a tripeptide derivative of glutamic acid (glutathione). In this particular case the control period of treatment with vitamin B<sub>12</sub> alone was more than a year. Certainly it will serve just as well as glutamic acid itself in resolving the basic disagreement as to whether an 'intrinsic factor' exists or not. The B<sub>12</sub>/glutathione was prepared by a simple admixture by weight in a 1:1 ratio. Thus the glutathione was considerably less than that used when a mixture of B<sub>12</sub> and glutamic acid was used (1:5).

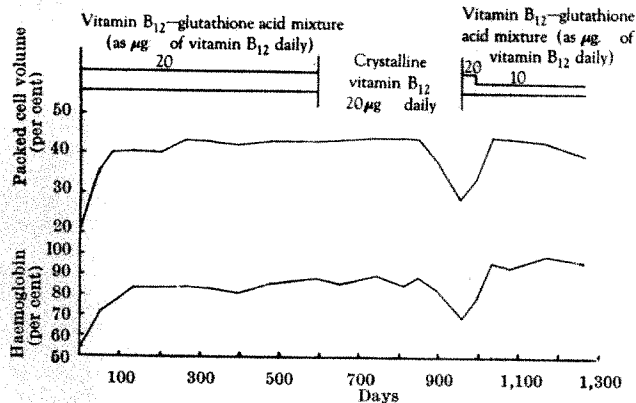


Fig. 2

The patient (case No. 41) was a married woman aged 38 suffering from previously untreated pernicious anaemia which was diagnosed by the methods outlined in our previous report<sup>1</sup>. Her haemoglobin (Hb) at the commencement of treatment was 54 per cent, and the packed cell volume (P.C.V.) was 20.5 per cent (Fig. 2). She received 20  $\mu$ g of B<sub>12</sub> plus 20  $\mu$ g of glutathione orally per day, responded well, and was maintained on this dosage until the 589th day, when the glutathione additive was omitted, and she then received 20  $\mu$ g of crystalline B<sub>12</sub> only each day. At that time the Hb was 89 per cent and the P.C.V. 43 per cent. She remained well—presumably due to accumulation of B<sub>12</sub> previously acquired—until the 894th day when a fall was noticed which continued without arrest, until by the 957th day the Hb was 70 per cent and the P.C.V. was 28 per cent. The glutathione additive (20  $\mu$ g) was then restored to the daily dose of crystalline B<sub>12</sub>. The control period on B<sub>12</sub> alone lasted, therefore, 368 days.

The result of the addition of glutathione in treatment is clearly shown by the satisfactory recovery in the blood picture. Indeed, it was possible on the 993rd day to reduce the daily dosage to 10  $\mu$ g each of B<sub>12</sub> and glutathione, and the patient has remained on this ever since. Currently (1,268th day) the Hb is 97 per cent and the P.C.V. is 40 per cent. This clearly demonstrates, once more, that an enhanced therapeutic effect of B<sub>12</sub> may be achieved by the addition of a relatively simple substance—in this case a tripeptide of glutamic acid.

It is relevant to mention the present state of the two previously reported cases<sup>1-4</sup> which have been maintained on the mixture of glutamic acid and B<sub>12</sub>, should Drs. Heinivaara and Palva wish to follow up our work a little further. Case No. 49 has now been on treatment for 1,361 days and during that time he has received an average daily dose of 25  $\mu$ g of B<sub>12</sub> admixed with glutamic acid in a 1:5 ratio. His Hb is 90 per cent and his P.C.V. is 40 per cent. He has remained on a daily dose of 20  $\mu$ g of B<sub>12</sub> (given with glutamic acid) since the 382nd day of treatment. Case No. 52 has been on treatment for 1,331 days. His Hb is 100 per cent and his P.C.V. is 43 per cent. The average daily dose of B<sub>12</sub> (given with glutamic acid) is 22  $\mu$ g, and he has been on a daily dose of 20  $\mu$ g since the 180th day of treatment.

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### Thalamo-cortical Relations in the Sensory Nuclei of the Cat

THE concept of the control of the somatic afferent pathways by the higher levels of the nervous system, first put forward by Hagbarth and Kerr<sup>1</sup>, has received considerable support from subsequent experiments involving the study of the activity of single neurones in the sensory tracts and nuclei. In particular, the dorsal column nuclei have been studied in detail with anatomical and physiological techniques<sup>2</sup>, and Eccles and Lundberg and their co-workers have demonstrated recently that stimulation of areas S I and S II of the cerebral cortex causes presynaptic inhibition on the afferent fibres relaying with the cells of the dorsal horn and cuneate nucleus<sup>3-7</sup>. Recent experiments have shown that the cerebral cortex can also influence the activity of the higher levels of the sensory pathways, namely the activity of some of the thalamic relay nuclei.

These experiments were performed in cats under chloralose, and gallamine triethiodide. Records were taken by means of concentric electrodes insulated except at the tip, introduced stereotactically into the thalamus in the ventro-basal complex (VB), in the posterior group of nuclei (POn) and other thalamic nuclei. The VB and POn have quite different physiological properties<sup>8-10</sup>.

(i) While the neurones of the VB have a precise somatotopic organization and respond only to appropriate contralateral stimulation, the POn receive afferent impulses



from large bilateral regions of the body, from surface as well as deep sensory nerves, from somatic and visceral (splanchnic) afferents and from the auditory pathways. Activity in any one of the neuronal groups of the PON can be evoked—as a rule—by stimulation of any of the four limbs (Fig. 1).

(ii) The latency of the PON response to contralateral stimulation is only slightly longer than that of the VB. However, the activity evoked by ipsilateral stimulation has a latency which is more variable generally: it is 2–3 msec longer than that of contralateral stimulation, but it may be much longer than can be accounted for by direct transmission of impulses to the PON.

(iii) While the activity evoked in the VB in response to a single shock to a peripheral nerve is brief (for example, 10 msec), the evoked activity in the PON in response to a similar stimulus is a prolonged discharge lasting 30–40 msec.

(iv) In general the activity in the PON evoked by a single stimulus to a peripheral nerve consists of a series of potential waves which are small and fast at the beginning, gradually increasing in voltage and then decreasing smoothly. In other cases, however, the activity consists of two distinct phases: an early phase of small and fast waves followed by a late phase of larger 500–700/sec oscillations. There is no time interval between the two phases, the early one fading into the late phase without any break. But the two phases can be easily distinguished, not only by the type of activity but also by their origin. It has been possible to abolish the

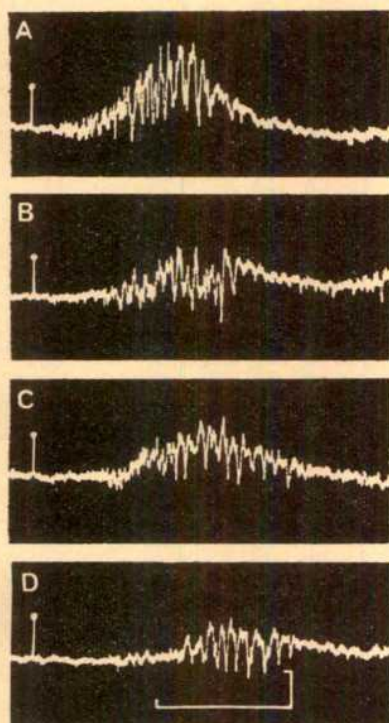


Fig. 1. Potentials recorded in the PON of the thalamus after single shock stimulation of the cutaneous nerves of the limbs. At the signal stimulation of skin nerves of: A, contralateral anterior paw; B, ipsilateral anterior paw; C, contralateral posterior paw; D, ipsilateral posterior paw. Time calibration, 20 msec. Voltage calibration, 100  $\mu$ V

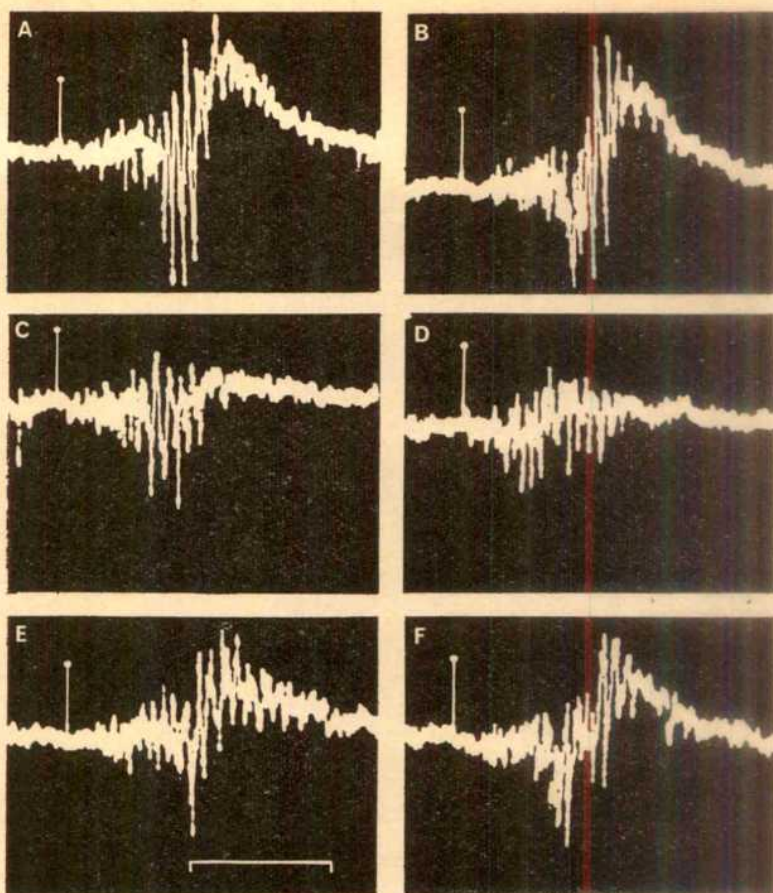


Fig. 2. Potentials recorded in PON of the thalamus on stimulation of cutaneous nerves of anterior contralateral paw; A, B, control; C, D, 90 sec after freezing the sensory-motor area of the cerebral cortex, E, F, after re-warming the cerebral cortex. Time calibration in E, 20 msec

late phase of PON activity by freezing or damaging the sensory cortex, leaving the first phase unaffected (Fig. 2). This shows that the late phase of PON activity is of cortical origin, and probably due to impulses reflexly discharged when afferent impulses reach the cortex. If this is so, it would be expected that stimulation of the sensory areas of the cerebral cortex would cause a similar type of activity in the PON. This has been verified experimentally, and it has been found that a burst of waves very similar to those of the late phase is produced in the PON by stimulation of the cortex. It has been possible to exclude the possibility that this activity is due to antidromic stimulation of the afferent cortical fibres, because a similar type of activity appears in the PON contralateral to the stimulated cortex.

It can be concluded, therefore, that the arrival of afferent impulses to the cortex causes the discharge of impulses which affect the PON bilaterally and that the late phase is due to neurones different from those acting in the first phase or to a different degree of synchronization in their discharge.

In addition to these excitatory effects on some cellular groups of the PON, the sensory areas of the cortex exert an inhibitory effect on other functions of these nuclei. Following 2–5 shocks to the SI or SII areas of the cortex, there occurs a deep depression and even a complete disappearance of the evoked potentials in the PON (Fig. 3). This is not a general effect on all the relay nuclei of the thalamus, because it does not occur in the VB, although there is evidence that it involves not only the PON but also other neighbouring nuclei. We are dealing here with a powerful inhibitory effect, as two or three shocks may be sufficient to prevent the appearance of any sign of evoked activity for 200 msec. It is rather difficult



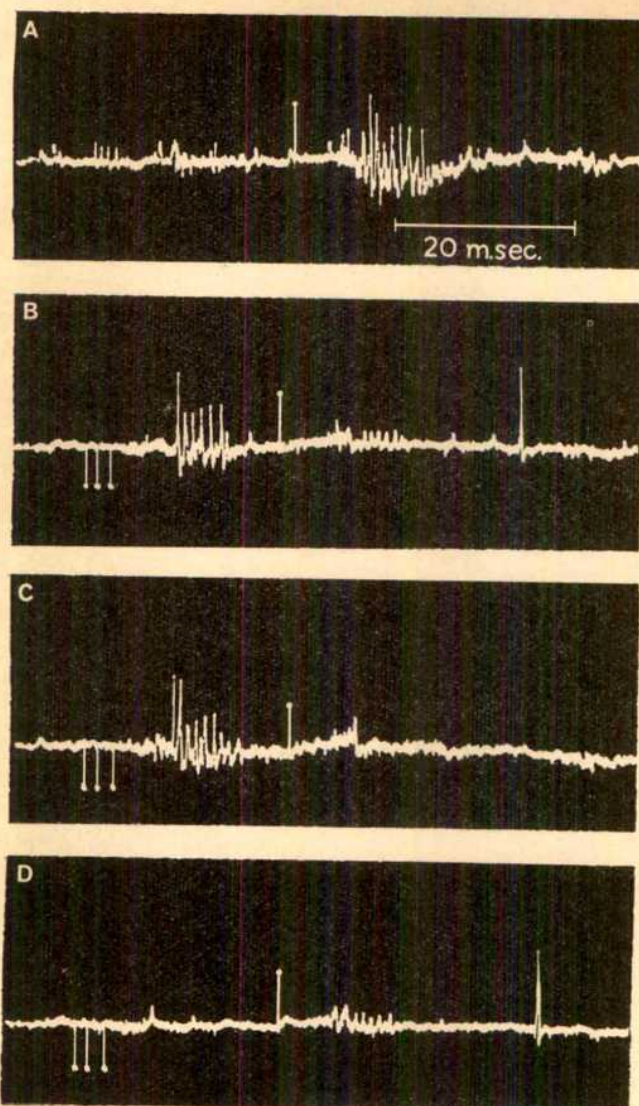


Fig. 3. Cortical inhibition of evoked potentials recorded in the POn of the thalamus. A, potential produced by stimulation of cutaneous nerves of the anterior contralateral paw by single shock at the signal; B, C, D, stimulation of cutaneous nerves of anterior contralateral paw as in A, but cutaneous stimulus is preceded by three shocks applied to the anterior suprasylvian gyrus of the contralateral cerebral cortex. Note the almost total inhibition of the evoked potentials, and the fact that this inhibition occurs both when the cortical stimulation causes activation of the POn (B, C) and when this activation is absent (D). Time calibration in A.

to establish the early course of the inhibitory process, as inevitably cortical stimulation will cause excitation of groups of neurones in the POn, thus masking the inhibition of the evoked potentials. When the cortical shocks and the peripheral stimuli are delivered at the appropriate time interval there is no evidence of peripherally-evoked activity superimposed on the cortically-induced impulses, or of summation of these two types of activity at all the intervals tested. Lack of summation might indicate a complete occlusion, but this is unlikely, as units which are normally excited from the periphery may be completely inhibited by preceding cortical stimulation without being themselves activated by it (Fig. 3D). It would seem, therefore, that cortical stimulation causes a profound inhibition of the responses of the POn.

• The physiological role of these mechanisms is still a matter for speculation. It may be thought that this cortically-induced inhibition of the POn relay nuclei might be part of the effects produced by the cerebral cortex when it

discharges following the arrival of sensory impulses to the cortex. The inhibition of transmission at the POn, but not at the VB, would allow access to the cortex to afferent impulses mediated by the medial lemniscus such as kinesthetic impulses, which are of relevance to motor action, while it would cut out other sensory messages irrelevant to the action performed, and which may be considered at this juncture as interfering sensory noise. Similarly the presynaptic inhibition exerted by the cortex, possibly through the pyramidal tract, on the dorsal horn and cuneate nucleus neurones, affects all afferent fibres but those of I A group. There is no presynaptic inhibition mediated by the cortex on the neurones of the dorsal spino-cerebellar tract activated exclusively by group I afferents<sup>7</sup>. It appears that, as a general rule, the inhibitory action of the cortex affects cutaneous and muscular afferents of high threshold, while the transmission to the cerebral and cerebellar cortex of impulses from kinesthetic and large muscular afferents having an important function in the origin and regulation of motor activity is not influenced by the cerebral cortex inhibitory mechanisms. These mechanisms may be one of the ways in which the cortex can initiate and direct the motor activity into the appropriate channels.

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### A Modified Technique for collecting Mouse Thoracic Duct Lymph

TECHNIQUES for collecting lymph from unanaesthetized mice have been described by Shrewsbury<sup>1</sup> and Gesner and Gowans<sup>2</sup>, who cannulated the duct immediately below the diaphragm in a manner similar to that described by Bollman, Cain and Grindlay<sup>3</sup> for the rat.

In the mouse the abdominal part of the duct is closely adherent to the left postero-lateral aspect of the abdominal aorta, and attempts to encircle the duct with a ligature and tie the cannula in place, as described by Shrewsbury, frequently result in damage to one or both of these structures. Gesner and Gowans modified Shrewsbury's method of fixing and sealing the cannula in the duct by swinging a flap of quadratus lumborum muscle antero-medially and fixing it with a ligature to the emerging part of the cannula. In both methods there is a risk of dislodging the cannula from the duct during the insertion of the ligature; moreover, a second ligature needs to be inserted to provide further fixation of the duct to the posterior abdominal wall, and accurate insertion and tying of ligatures within the small operation field are difficult and time-consuming.

We have developed a simple and rapid method of fixing the cannula using a plastic adhesive, 'Eastman 910'. This substance is a monomer, methyl 2-cyanoacrylate, to which has been added a thickening agent, an inhibitor to stabilize it, and a plasticizing agent to prevent it from becoming brittle on ageing. The material has the appear-



ance and viscosity of water, and readily undergoes a rapid, highly exothermic, anionic polymerization to convert to a solid state forming strong bonds between a variety of materials.

Adhesion results from molecular attraction and mechanical grip action on irregular and porous surfaces<sup>4</sup>, and no heat, excessive pressure, evaporation of a solvent or addition of a catalyst is necessary. A minute amount of moisture on the surfaces to be united is desirable, however, since the polymerization is catalysed by water.

The adhesive has been shown to be inert, non-sensitizing to skin and relatively non-toxic, and evokes only a mild foreign-body reaction comparable in duration and severity to that caused by catgut<sup>5</sup>. It is said to be self-sterilizing<sup>6</sup>, but may if desired be sterilized by X-irradiation.

Reports have been published of the successful use of 'Eastman 910' as an adhesive in a variety of procedures including closure of skin incisions<sup>7</sup>, anastomosis of bowel<sup>8</sup>, and repair of blood vessels<sup>9-12</sup>.

Cannulae are made by threading a fine wire into a piece of 0.5 mm bore nylon tubing (Portland Plastics, Ltd.), bending to form a 'U' and immersing in an oil bath at approximately 130° C for about 30 sec. They are then hardened off at room temperature in methylene chloride, the wire is withdrawn, and one end is bevelled with scissors.

Six hours before operation all food is withdrawn, and 4 h later the mice are given 0.2 ml. olive oil by gastric tube.

The abdomen is opened under 'Avertin' anaesthesia by an incision following the lateral border of the left quadratus lumborum muscle, and the left kidney is mobilized and retracted medially. The duct is exposed by carefully dissecting the fatty retro-peritoneal tissue from the lateral aspect of the aorta and displacing it medially on to the anterior surface of that vessel.

The cannula is passed through the flank of the animal by means of a trocar, and the bevelled end is inserted directly into the duct with a quick stab. The fatty retroperitoneal tissue which was dissected off the lateral surface of the aorta is re-positioned across the junction of cannula and duct, and a small drop (about 0.005 ml.) of 'Eastman 910' is applied with a glass pipette. Within 30 sec polymerization occurs and the cannula cannot readily be dislodged from the duct. A further drop of adhesive is applied to fix the cannula to the posterior abdominal wall, after which the kidney is replaced, the muscle layers are closed with interrupted black silk sutures, and the skin is closed by a further application of 'Eastman 910'.

After operation the mice are warmed on an electric blanket for a few minutes and given 4 I.U. heparin in 1 ml. saline by subcutaneous injection. They are then placed on rotating drums of the type used by Gesner and Gowans<sup>3</sup>, and secured by a loop of adhesive tape around the thorax, and a further strip of tape which fixes their tails to the posterior part of the apparatus. Food and dextrose saline are available *ad lib*. Any small clots which form in the cannula are removed with a piece of fine stainless steel wire—a more effective instrument, in our experience, than the human hair used by Gesner.

Using this technique with adult pure-line (CBA) mice of either sex we have obtained a mean volume of 19 ml. of lymph in 24 h from those animals which drink well post-operatively. The mean total cell count in the first 24 h specimens was 84 million, which is somewhat less than the value reported by Gesner and Gowans. It is felt this difference may be due to the small size of the inbred mice which we have used, or to the fact that these mice, for the purposes of our experiments, had been pre-immunized with A-strain mammary carcinoma cells.

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## PHARMACOLOGY

### Effect of Prostaglandin (PGE<sub>1</sub>) on the Permeability Response of Toad Bladder to Vasopressin, Theophylline and Adenosine 3',5'-monophosphate

THE prostaglandins (PGE), a group of naturally occurring fatty acids, have been isolated from a number of sources including sheep vesicular glands and human vesicular plasma<sup>1,2</sup>. The chemical nature and separation of this series of related analogues have been reported in detail<sup>3-4</sup>. They have profound effects on blood pressure and on smooth muscle contractility, and are also known to interfere with the action of a variety of hormones on adipose tissue<sup>5-7</sup>. Because of the possibility that the prostaglandins may have other physiological effects of a more general nature, and perhaps serve as regulators of hormone action in certain tissues, the effect of one of them, PGE<sub>1</sub>, on the response of the toad bladder to vasopressin, theophylline and adenosine 3',5'-monophosphate (cyclic-AMP) was examined. All three of the latter compounds, when added to the serosal surface of the isolated bladder of *Bufo marinus*, increase the osmotic flow of water across this membrane. It has been suggested that vasopressin exerts its permeability effects here and in the kidney by increasing the concentration of adenosine 3',5'-monophosphate within the tissue<sup>8</sup>. The cyclic-AMP is presumed to be the intracellular mediator of the vasopressin effect. Theophylline mimics vasopressin in toad bladder, since it inhibits the degradation of cyclic-AMP to inactive 5'-adenosine monophosphate<sup>9</sup>.

The increase in permeability to water induced by these agents, as manifested by an augmentation in net water movement along an osmotic gradient, can be estimated by a volumetric technique described by Edelman *et al.*<sup>10</sup>. In the present studies, a 7.5 sq. cm portion of toad bladder was clamped between two glass chambers. The chamber apposed to the blood or serosal surface of the bladder contained a modified Ringer's solution (pH 7.8, 220 mOsm); that apposed to the outer or mucosal surface contained hypotonic Ringer's solution (55 mOsm). Adequate mixing was assured by bubbling air through the serosal chamber. The rate of flow of water along the osmotic gradient from the mucosal solution to the serosal solution was measured by means of a graduated pipette.

Since *Bufo marinus* has a bi-lobed bladder, it was possible to use bladder halves from the same toad in all studies. One hemi-bladder served as the control, the contralateral one as the experimental side. Following



Table 1. EFFECT OF PGE<sub>1</sub> ON TOAD BLADDER

PGE <sub>1</sub>	Vasopressin 1 mU./ml.			Theophylline 10 <sup>-2</sup> M			Cyclic-AMP 10 <sup>-3</sup> M		
	n	$\Delta_{III}^*$ $\mu\text{L}/\text{min} \pm \text{S.E.}$	P†	n	$\Delta_{III}^*$ $\mu\text{L}/\text{min} \pm \text{S.E.}$	P	n	$\Delta_{III}^*$ $\mu\text{L}/\text{min} \pm \text{S.E.}$	P
1.7 × 10 <sup>-6</sup> M	10	-3.7 ± 1.0	<0.005	10	-3.0 ± 1.5	—	6	+0.6 ± 0.3	—
1.7 × 10 <sup>-7</sup> M	8	-7.6 ± 1.3	<0.001	11	-3.8 ± 1.3	<0.02	—	—	—
1.7 × 10 <sup>-8</sup> M	7	-6.2 ± 1.2	<0.005	—	—	—	8	-0.6 ± 0.5	—
1.7 × 10 <sup>-9</sup> M	7	-4.5 ± 1.0	<0.01	8	-3.2 ± 0.8	<0.01	—	—	—
1.7 × 10 <sup>-10</sup> M	5	-0.7 ± 0.5	—	10	-1.9 ± 0.6	<0.02	—	—	—
1.7 × 10 <sup>-11</sup> M	—	—	—	5	+0.8 ± 0.8	—	—	—	—

\*  $\Delta_{III}$  is the mean of the differences between  $\Delta_I$  and  $\Delta_{II}$  as described in the text and is an index of the effect of PGE<sub>1</sub> on the permeability response to the three agents tested.

† P is the significance of  $\Delta_{III}$ .

n, number of paired experiments.

In the control periods the mean response to vasopressin was 6.1  $\mu\text{L}/\text{min}$ , to theophylline 6.1  $\mu\text{L}/\text{min}$ , and to cyclic-AMP 4.1  $\mu\text{L}/\text{min}$ .

a preliminary period in plain Ringer's solution in which there was negligible osmotic flow of water across the bladder, the test agent, vasopressin (1 mU./ml.  $\approx 2 \times 10^{-9}$  M), theophylline (10<sup>-2</sup> M) or cyclic-AMP (10<sup>-3</sup> M) was added to the serosal bathing medium of the two hemi-bladder preparations. The concentrations of test agents were selected to yield a submaximal increase in water movement. The difference in response of the two sides with respect to water movement ( $\Delta_I$ ) was measured over a 20-min period. The bladders were then reintroduced into fresh Ringer's solution until the initial impermeability was re-established. Following this the same concentration of test agent as originally used was added to both bladder halves, and PGE<sub>1</sub> in concentrations varying from 1.7 × 10<sup>-6</sup> M to 1.7 × 10<sup>-11</sup> M was added to the serosal solution bathing the experimental side only. The difference in response of the two sides was again observed ( $\Delta_{II}$ ) for 20 min. The means of the differences between  $\Delta_{II}$  and  $\Delta_I$  are noted in Table 1 and are an index of the effect of PGE<sub>1</sub>.

It is apparent that PGE<sub>1</sub> significantly diminishes the permeability response of the toad bladder to vasopressin and to theophylline, but not to cyclic-AMP. The inhibitory effect is reversible and is not induced by the addition of PGE<sub>1</sub> to the mucosal bathing medium. Although 1.7 × 10<sup>-7</sup> M–1.7 × 10<sup>-10</sup> M PGE<sub>1</sub> effectively inhibited the theophylline response in the present studies, whereas 1.7 × 10<sup>-6</sup>–1.7 × 10<sup>-9</sup> was effective for vasopressin, a somewhat different result was obtained in earlier experiments. In these, whole bladder sacs were used according to the method of Bentley<sup>11</sup> in which net water movement is estimated by measuring the weight loss of the toad bladder sac over an appropriate period of time. 10<sup>-8</sup> M PGE<sub>1</sub> interfered with the effect of vasopressin in the earlier studies as in the present experiments, but did not inhibit the response to theophylline. In the latter instance, an apparent enhancement of the theophylline effect was observed. The significance of this difference in results with two different techniques and concentrations of PGE<sub>1</sub> is obscure. However, in the earlier experiments as well as those reported herein the effect of cyclic-AMP on water movement was uninfluenced by PGE. It should be noted that PGE<sub>1</sub> alone has no effect on permeability and that ricinoleic acid, a fatty acid which in some studies served as a control for PGE, did not influence the response to vasopressin, theophylline, and cyclic-AMP, attesting to the relative specificity of the inhibition by prostaglandin.

No other naturally occurring compound in these concentrations is known to alter the response of this tissue to vasopressin. Cysteine, in considerably higher concentrations, 10<sup>-3</sup> M, limits the response to vasopressin and theophylline but not to cyclic-AMP<sup>12</sup>. It is conceivable that PGE may, in fact serve as a regulator of hormone action with respect to antidiuretic hormone. Vasopressin increases cyclic-AMP concentration in toad bladder<sup>13</sup> and also activates the enzyme phosphorylase in this tissue and in kidney<sup>14</sup>. The effect of PGE on the

concentration of cyclic-AMP and on phosphorylase activation in this tissue has not as yet been examined, although it has been reported that phosphorylase activation induced by epinephrine in adipose tissue is inhibited by pre-incubation of the tissue with prostaglandin<sup>7</sup>.

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### Effect of Anaesthetics on the Duration of Embolization of Platelet Thrombi formed in Injured Blood Vessels

WHEN an artery of the cerebral cortex of an anaesthetized rabbit is pinched with a pair of fine forceps so that it bleeds briefly, some platelets rapidly form a haemostatic plug outside the vessel and others adhere to its endothelial lining at the site of injury to form platelet clumps or 'white bodies' which embolize intermittently in the blood stream. The formation and embolization of white bodies may go on for several hours<sup>1-3</sup>.

These observations were made on rabbits anaesthetized with urethane. In continuing an investigation designed to discover substances capable of inhibiting the formation of white bodies<sup>4,5</sup>, we wanted to use animals smaller than rabbits in order to save material. We found that, in rats, the response of cortical vessels to mechanical injury was similar to that in rabbits except that, under apparently similar conditions, the embolization of white bodies appeared to stop sooner. In rats, both arteries and veins

were injured and embolization in both lasted for about the same periods of time. In the course of the experiments an interesting difference in the duration of embolization was observed depending on whether the rats were anaesthetized with sodium pentobarbital or ether instead of with urethane. The experiments which showed this difference were as follows:

Male and female albino rats of the Wistar strain, weighing 190–250 g, were anaesthetized and the skull and dura carefully removed from one cortex to expose an area measuring about  $15 \times 7$  mm. This area was kept moist by a gentle flow of warmed physiological saline and the temperature of the cortical surface was maintained at about  $30^\circ\text{C}$ . The exposed vessels were observed under a Zeiss model II dissecting stereomicroscope at a magnification of 42 times. A vein, about  $100\mu$  in diameter, was injured by pinching it with fine ophthalmic forceps so that it bled for 10–40 sec (under ether anaesthesia the bleeding usually went on for 90 sec or even longer). White bodies began to form after 2–5 min and the time from the embolization of the first white body to that of the last was recorded. Several veins in each rat were injured and timed in this manner and the longest period of embolization was used for statistical comparisons.

Table 1 shows that injured cortical vessels produced embolizing white bodies for significantly longer periods of time under urethane anaesthesia than under anaesthesia with either sodium pentobarbital or ether. It seems, therefore, that urethane in anaesthetic concentrations greatly prolongs the production of platelet thrombi in injured vessels.

Anaesthetic	No. of rats	Range	Duration of embolization (min) Mean	S.E.M.	P
Sodium pentobarbital	11	4–61	$18.9 \pm$	$5.79$	} 0.001
Urethane	10	47–240+	$106.7 \pm$	$19.64$	
Ether	8	4–43.5	$20.4 \pm$	$4.91$	} 0.001

This observation is presumably related to the irritant and cytotoxic properties of urethane. Urethane has been used as a sclerosing agent in the treatment of varicose veins<sup>4–10</sup> and as a cytotoxic agent in the treatment of leukaemia and other malignancies<sup>11–18</sup>. Urethane increases capillary permeability<sup>17,18</sup>, and it causes severe damage to portal veins and sinusoidal capillaries in rats<sup>19</sup>. These experiments suggest that urethane, in anaesthetic concentrations, somehow irritates vascular endothelium sufficiently to prolong the effects of mechanical trauma as indicated by the intravascular adhesion and aggregation of platelets.

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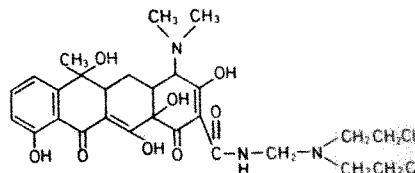
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## Antitumour Activity of *N*-( $\beta,\beta'$ -dichlorodiethylaminomethyl)-tetracycline, 'Tetracycline-mustard'

LOCALIZATION of tetracycline in tumour tissue has been reported with both human and animal neoplasms<sup>1</sup>. Though the tetracycline class antibiotics have no antitumour effect by themselves, the possibility of using them as carriers of more potent agents seemed worthy of investigation. As a model system a nitrogen mustard (HN2) derivative was considered most practical because of simplicity of structure, availability of starting materials and general knowledge concerning the antitumour activity of mustard type alkylating agents.

Preparation of *N*-( $\beta,\beta'$ -dichlorodiethylaminomethyl)-tetracycline was carried out according to the carboxamido-Einhorn derivative method of Gottstein, Minor and Cheney<sup>2</sup>. Fifty g of tetracycline base, 20.6 g of  $\beta,\beta'$ -dichlorodiethylamine hydrochloride (*nor* HN2), and 7 g of formaldehyde (14 ml. of 55 per cent methylformcel) were dissolved in 250 ml. of methanol. This solution was held at  $22^\circ\text{C}$  for 1.5 h and then added with rapid stirring to 3 l. of ethyl ether. The resulting precipitate was removed by filtration, washed with 300 ml. of ethyl ether and dried at  $50^\circ\text{C}$  *in vacuo* for 24 h. Fifty-five g of tetracycline-mustard Einhorn derivative (TCM) were obtained. This amorphous material was tested without further treatment since extensive hydrolysis was noted with various crystallization techniques. Analytical data for TCM as *N*-( $\beta,\beta'$ -dichlorodiethylaminomethyl)-tetracycline were: theory—(for  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_8\text{Cl}_2$ ) per cent chlorine, 11.85; per cent nitrogen, 7.02; antibacterial assay potency, 760 tetracycline units/mg; found—per cent chlorine, 11.6; per cent nitrogen, 6.3; assay potency 730 tetracycline units/mg. On the assumption of an Einhorn reaction a tentative structure is proposed here.



- (1) *N*-( $\beta,\beta'$ -dichlorodiethylaminomethyl)-tetracycline.  
(2) Epithets: tetracycline mustard, TCM.

Tetracycline mustard was tested under the auspices of the Cancer Chemotherapy National Service Center at Hazleton Laboratories against the *in vivo* mouse tumours sarcoma 180 (S-180), adenocarcinoma 755 (Ca755) and leukaemia 1210 (L-1210). Inhibition of Ca755 was observed at doses of 80 and 60 mg/kg/day (intraperitoneal injections) whereas S-180 was not reproducibly inhibited. Significant prolongation of survival time of mice with L-1210 was noted over a dose range of 80–5 mg/kg/day. Tests against the same tumour types at Bristol Laboratories confirmed these results in every respect. Therapy of mice with L-1210 caused a significant increase in median survival-time over controls (25 per cent or greater) at doses ranging from 80 to 3 mg/kg/day (Fig. 1). These experiments used L-1210 carried in the ascitic form. TCM was not effective against solid trocar implants of L-1210 nor was it effective against ascitic L-1210 if the drug was administered orally at doses up to 180 mg/kg/day. As a control procedure the possibility of carry-over of, or breakdown to, starting reactants was checked biologically. None of the starting materials or formaldehyde reaction products of *nor*HN2 or tetracycline had anti-L-1210 activity at doses within the stoichiometrically equivalent range of effective TCM doses (Table 1). For comparative purposes  $\beta,\beta'$ -dichlorodiethyl-*N*-methylamine (HN2) was tested against L-1210 and found effective at doses of

Table 1. EFFECT OF TCM STARTING MATERIALS ON LEUKEMIA 1210

Preparations	Dose (mg/kg/day)	Theoretically equiv. TCM dose	Response test/control		
			Median survival time (days)	Effect (%)	Survivors (day 5)
Tetracycline HCl	176.0	200.0	8.00/7.00	114	6/6
Tetracycline HCl	88.0	100.0	7.50/7.00	107	6/6
Tetracycline HCl	58.0	73.0	7.50/7.00	107	6/6
Formaldehyde	8.0	160.0	7.00/7.00	100	6/6
Formaldehyde	2.0	40.0	7.50/7.00	107	6/6
Formaldehyde	0.5	10.0	6.50/7.00	93	6/6
Formaldehyde	0.125	2.5	7.00/7.00	100	6/6
$\beta,\beta'$ -Dichlorodiethylamine (nor HN2)	80.0	338.0	11.25/7.00	161	12/12
$\beta,\beta'$ -Dichlorodiethylamine (nor HN2)	40.0	164.0	8.00/7.00	114	6/6
$\beta,\beta'$ -Dichlorodiethylamine (nor HN2)	20.0	82.0	7.00/7.00	100	6/6
Formaldehyde- $\beta,\beta'$ -dichlorodiethylamine reaction product	120.0	419.0	12.00/10.00	120	6/6
Formaldehyde- $\beta,\beta'$ -dichlorodiethylamine reaction product	80.0	280.0	10.00/10.00	100	6/6
Tetracycline-formaldehyde reaction product	130.0	164.0	8.00/7.00	114	6/6
Tetracycline-formaldehyde reaction product	57.0	73.0	7.00/7.00	100	6/6
Tetracycline-formaldehyde reaction product	26.0	32.8	7.00/7.00	100	6/6
Tetracycline-formaldehyde reaction product	11.0	13.8	6.00/7.00	86	6/6

See legend, Fig. 1. 125 per cent or greater considered 'active'.

1-0.03 mg/kg/day with a maximum increase in survival time of 47 per cent (broken line in Fig. 1). Thus HN2 was almost 100 times more potent than TCM. However, with the curves adjusted so as to be superimposed, the striking similarity of the dose-response is apparent. As a check on stability, tests against *Ca755* were performed with TCM: (1) made up in a single solution which was then refrigerated for two weeks during the period of its use for therapy; (2) prepared fresh daily from the dry powder. The standing preparation seemed to be somewhat more potent than the fresh daily preparation regarding tumour inhibition (for example, *Ca755* was inhibited 77 per cent at 53 mg/kg/day by the standing preparation compared to 42 per cent for a fresh daily preparation).

Since activity of TCM could not be accounted for by its starting materials the remaining possibilities are that either the intact molecule is the active agent or that TCM splits at the amide bond and releases an HN2-like compound. Though no direct chemical evidence is available at present, the following biological data and theoretical

considerations tend to support the latter hypotheses. First, the standing preparation, that is, the material made up once for multiple injection, was more potent than the fresh daily preparation, suggesting instability and breakdown into a more active agent. Second, the dose-response curves of TCM and HN2 were very similar to each other. Third, others have shown that when Einhorn derivatives of tetracycline are subjected to reductive degradation under appropriate conditions, the bridging methylene group remains attached to the amino nitrogen<sup>3,4</sup>.

Obviously the results presented here provide no evidence that TCM accumulates on neoplastic tissue as shown for other tetracyclines. However, if splitting of TCM does occur as described here, it would be of interest to determine whether the hypothesized HN2-like substance is released mostly in the injection solution, the animal's blood stream, or at the neoplastic cell.

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### Inhibition of 5-Hydroxytryptamine Liberation from Blood Platelets by Reserpine

RESERPINE causes a release of monoamines from various tissues including isolated blood platelets<sup>1,2</sup>. This effect is generally attributed to an interference of the drug with the storage mechanisms for the amines. Furthermore, some authors report a protective action of reserpine against the spontaneous liberation of norepinephrine from adrenal medullary and splenic nerve granules<sup>3-5</sup>. The mechanism is not fully understood. It might be partly connected with the lack of glucose in the incubation medium, because this source of energy seems to be necessary for retaining the 5-hydroxytryptamine (5HT) within the platelets<sup>6</sup>. We have investigated the effect of reserpine on the metabolism of 5HT of isolated platelets in the absence and presence of glucose.

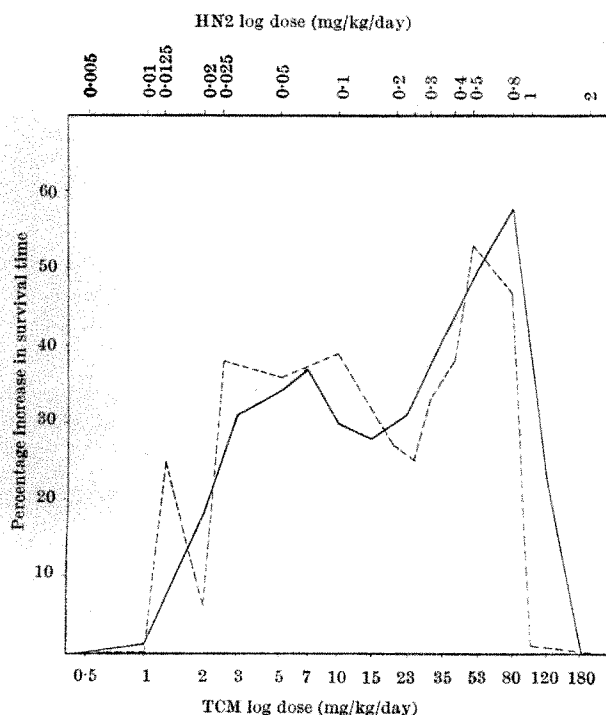


Fig. 1. *BDF<sub>1</sub>* mice inoculated day 0 with  $1 \times 10^6$  ascitic *L-1210* cells intraperitoneally. Therapy started day 1 intraperitoneally and continued once daily 13  $\times$  or until death of the animal. Survival increase of 25 per cent or greater above control ( $T/C \geq 125$ ) considered effective for inhibition of leukemia growth. All effective doses comprise an average of two or more experiments with at least 6 mice per treatment dose and 10 controls for each experiment. —, TCM; ---, HN2



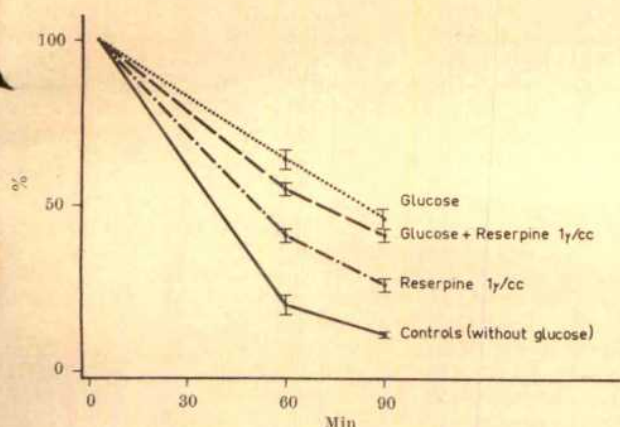


Fig. 1. Effect of reserpine on the spontaneous 5-hydroxytryptamine (5HT) liberation of platelets pre-incubated and re-suspended in isotonic potassium phosphate, pH 7.5, with or without glucose. Ordinate, 5HT of platelets in per cent of their 5HT content immediately after re-suspension; abscissa, time after re-suspension. The points represent averages  $\pm$  S.E. of 6 experiments. Significance: reserpine 60 and 90 min versus controls:  $P < 0.01$ ; glucose 60 and 90 min versus controls:  $P < 0.01$ ; glucose + reserpine versus reserpine { 60 min  $P < 0.01$ ; 90 min  $P > 0.01$ .

Thrombocytes of rabbits were isolated as previously described<sup>7</sup> and pre-incubated at 37°C for 60 min in isotonic, glucose-free potassium phosphate, pH 7.5, used by previous authors<sup>3</sup>. The centrifuged platelets were then re-suspended in fresh buffer of the same composition and in part supplemented with glucose, reserpine, or glucose + reserpine. Spectrophotofluorimetric measurements of the 5HT of the platelets were carried out during an incubation period of 90 min<sup>8</sup>, and the 5HT metabolites present in the incubation medium were identified by paper chromatography<sup>9</sup>.

In platelets pre-incubated in a glucose-free potassium phosphate medium, the 5HT is rapidly liberated on re-suspension in new buffer of the same composition. This behaviour differs from that under more physiological conditions. Thus, without glucose, platelets lose about 80 and 90 per cent of their 5HT in 60 and 90 min respec-

tively, and no appreciable amounts of 5HT metabolites are present in the buffer (Figs. 1 and 2). The physiological 5HT release, on the contrary, is slow (maximum loss of 5 per cent 5HT within 2 h), and appreciable amounts of 5HT metabolites, especially 5-hydroxytryptophol, appear in the incubation medium<sup>7,9</sup>. The unphysiological liberation of 5HT in glucose-free buffer might be explained by an impairment of the active storage or transport of 5HT in the platelets which seems to depend on supply of energy<sup>6</sup>. Accordingly, supplementation of the buffer with glucose results in a more physiological 5HT liberation, because the diminution of the platelet 5HT is attenuated and 5-hydroxytryptophol reappears in the medium (Fig. 2).

Addition of 1  $\gamma$ /c.c. reserpine to the glucose-free buffer significantly counteracts the spontaneous decrease of the platelet 5HT. After 60 and 90 min respectively, the platelets have lost only about 60 and 75 per cent of their 5HT (Fig. 1). This protective action is dependent on the reserpine concentration, 0.1  $\gamma$ /c.c. causing less, 10  $\gamma$ /c.c. more marked inhibition of the 5HT liberation than 1  $\gamma$ /c.c. In contrast to glucose, reserpine does not restore the metabolism of the 5HT by platelets, because practically no 5-hydroxytryptophol and 5-hydroxyindoleacetic acid are present in the incubation medium (Figs. 1 and 2).

In buffer supplemented with glucose, reserpine no longer inhibits but rather enhances the spontaneous 5HT liberation from blood platelets. In addition, appreciable amounts of 5-hydroxytryptophol are found in the incubation medium (Figs. 1 and 2). This essentially represents the typical pattern of activity of reserpine on platelets suspended in more physiological media<sup>3,7</sup>.

The protective effect of reserpine on the platelet 5HT is thus not observed under relatively physiological conditions. It appears, however, if the active storage mechanism for 5HT is impaired; for example, in a glucose-free incubation medium. Inhibition of the 5HT release from platelets by reserpine does not seem, therefore, to be due to interference with the active storage of the amine. It might rather be the consequence of an action of the drug on the outflux of 5HT through the platelet membrane.

In conclusion, reserpine has probably two separate actions on the platelet 5HT, that is, a releasing effect by interfering with active storage and a protective effect, possibly by decreasing the 5HT outflux through the platelet membrane. In normal platelets with intact storage mechanism, the first action might predominate and conceal the second.

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### Anti-viral Activity of Two Guanidine Derivatives in vitro

THE inhibitory properties of guanidine on enterovirus replication have been fully demonstrated<sup>1,2</sup>.

Structural modifications in the guanidine molecule are assumed to cause a marked decrease or a loss of virus inhibiting activity<sup>1,3</sup>. However, in previous publications from these laboratories, we have shown that two guanidine-

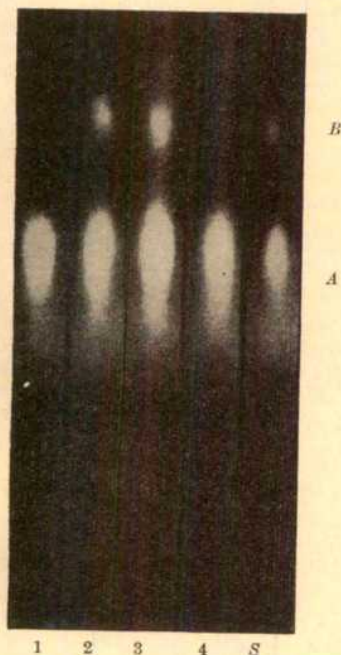
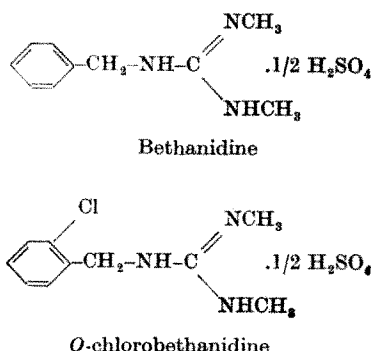


Fig. 2. Paper chromatogram of basic extracts of the incubation medium 90 min after re-incubation of isolated blood platelets of rabbits in potassium phosphate buffer, pH 7.5. Paper: Schleicher and Schnell, No. 2043. Solvent system: *N*-propanol-NH<sub>3</sub>, 1 N (5:1 v/v). 1, without glucose; 2, +0.011 M glucose; 3, +0.011 M glucose + 10  $\gamma$ /c.c. reserpine; 4, +10  $\gamma$ /c.c. reserpine; S, standards: A, 5-hydroxytryptamine 3 $\gamma$ ; B, 5-hydroxytryptophol 3 $\gamma$ .



pyrimidines (*N*-2-guanidine-pyrimidine sulphate and *N*-4-guanidine-2,6-pyrimidine sulphate) maintain a high guanidine-like anti-polio activity. Indeed, not only is their antiviral spectrum identical to that of guanidine, but they are also ineffective against guanidine-resistant poliovirus and can replace guanidine in supporting the growth of guanidine-dependent polioviruses<sup>4,5</sup>.

During our investigations on antiviral activity of guanidine-derivatives in order to find some structure-activity relationships, we were able to find that *BW 467C60* (Bethanidine) and its derivative *BW 392C60* were endowed with anti-polio and anti-vaccinia activity *in vitro*.



The viral strains and the procedures used for the culture of human amnion cells (Mascoli's line) were as described previously<sup>6</sup>. The assays of poliovirus cytopathic effect (CPE) were performed by the plaque technique of Dulbecco<sup>7</sup>; those of vaccinia virus CPE were described previously<sup>8</sup>. The inhibition of polio- and vaccinia-virus replication was evaluated by counting the cytopathic units (CPU) present at various intervals in the cell cultures. Since *BW 467C60* and *BW 392C60* lower the pH of the medium, this was kept at 7.3 by adding a few drops of 1 per cent  $\text{NaHCO}_3$  solution.

The data in Tables 1 and 2 demonstrate that the two compounds exert a clear inhibition of the CPE and multiplication of polio and vaccinia viruses.

Table 1. INHIBITION BY BETHANIDINE (467C60) AND ITS O-CHLORODERIVATIVE (392C60) OF POLIO I AND VACCINIA VIRUS CYTOPATHIC EFFECT

Viral strain	Compound tested ( $\mu\text{g/ml.}$ )*	Percentage inhibition (mean and range)
Polio 1	Bethanidine 125.0 (5)	92.2 (75-100)
Polio 1	Bethanidine 62.5 (4)	81.0 (57-96)
Polio 1	Bethanidine 41.0 (2)	3.5 (0-7)
Polio 1	O-Chl. Beth. 125.0 (2)	81.0 (62-100)
Polio 1	O-Chl. Beth. 62.5 (2)	50.0 (24-76)
Polio 1	O-Chl. Beth. 41.0 (2)	0.0 —
Vaccinia	Bethanidine 125.0 (3)	89.0 (72-98)
Vaccinia	Bethanidine 62.5 (3)	64.0 (50-72)
Vaccinia	Bethanidine 41.0 (2)	13.0 (6-20)
Vaccinia	O-Chl. Beth. 125.0 (2)	90.0 (85-95)
Vaccinia	O-Chl. Beth. 62.5 (2)	48.0 (40-56)
Vaccinia	O-Chl. Beth. 41.0 (2)	2.0 (0-4)

\* No. trials in parentheses.

Table 2. INHIBITION BY BETHANIDINE (467C60) OF POLIO I AND VACCINIA VIRUS REPLICATION

Viral strain	Inoculum (CPU)	Bethanidine ( $\mu\text{g/ml.}$ )*	CPU detected after 36 h (mean and range)
Polio 1	$10^4$	— (4)	$5.8 \times 10^7$ ( $6.8 \times 10^6$ – $9.7 \times 10^8$ )
Polio 1	$10^4$	125 (4)	$6.1 \times 10^5$ ( $4 \times 10^5$ – $6.8 \times 10^6$ )
Vaccinia	$5 \times 10^3$	— (4)	$1.5 \times 10^8$ ( $10^8$ – $3 \times 10^8$ )
Vaccinia	$5 \times 10^3$	125 (4)	$1.9 \times 10^4$ ( $6 \times 10^3$ – $3 \times 10^4$ )

\* No. trials in parentheses.

*BW 392C60* and bethanidine seem to act through a different mechanism from that of guanidine.

In fact, (a) the spectra of their virus-inhibiting action differ from that of guanidine, (b) they are fully effective in inhibiting guanidine-resistant poliovirus strains, and (c) they cannot replace guanidine in supporting the growth of guanidine-dependent strains: on the contrary, they inhibit their guanidine-conditioned multiplication.

It appears that antiviral activity is present in several guanidine derivatives, but they seem to exert their inhibiting properties by different mechanisms.

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### Bronchoconstrictor Action of Bradykinin, Kallidin and Eledoisin

COLLIER *et al.*<sup>1</sup> reported in 1959 that partially purified natural bradykinin, made by incubating trypsin with ox globulin, causes bronchoconstriction when injected into guinea-pigs. This action was confirmed for the synthetic nonapeptide<sup>2,3</sup> as well as for the decapeptide kallidin<sup>4</sup> and the endecapeptide eledoisin<sup>5</sup>. In contrast to the bronchoconstrictor action of serotonin and histamine, the effect of bradykinin and kallidin was completely abolished by pretreatment with acetylsalicylic acid and phenylbutazone<sup>1,4</sup>.

These experiments were carried out in artificially respired guinea-pigs using the sensitive overflow method of Konzett and Rössler<sup>6</sup>. In spontaneously respiring guinea-pigs, however, we found that doses of bradykinin and kallidin up to 16  $\mu\text{g/kg}$  (intravenous) did not decrease but rather increased the respiratory minute volume by more than 100 per cent<sup>7,8</sup>, while higher doses (20–60  $\mu\text{g/kg}$ ; intravenous) produced apnoea which is probably of central origin<sup>9</sup>.

In the experiments to be described the actions of synthetic bradykinin and kallidin on tidal volume and on inflation volume were examined in guinea-pigs under urethane anaesthesia by means of body-plethysmography according to the method of Koller<sup>10</sup>; the effects were compared with those of eledoisin and serotonin. All injections were made through a cannula in the external jugular vein.

In spontaneously breathing guinea-pigs graded doses of serotonin and eledoisin produced a dose-dependent reduction of the tidal volume (Fig. 1). Furthermore, a tachypnoic response of short duration was regularly observed. Following the injection of high doses of serotonin (10  $\mu\text{g/kg}$ ) and of eledoisin (5  $\mu\text{g/kg}$ ), the tidal volume became virtually zero although the movements of the respiratory muscles (Fig. 1, bottom trace)<sup>11</sup> had increased. Bradykinin, however, reduced the tidal volume only slightly in doses up to 20  $\mu\text{g/kg}$  and caused a long-lasting tachypnoea, while higher doses (25  $\mu\text{g/kg}$ ) produced an arrest of respiration in expiratory position. Similar results were obtained with kallidin.

In artificially respired guinea-pigs serotonin and eledoisin reduced the inflation volume by an amount depending on the given dose (Fig. 2). To reduce the inflation volume by 50 per cent ( $ED_{50}$ ) approximately 1  $\mu\text{g/kg}$  of eledoisin and 9  $\mu\text{g/kg}$  of serotonin were required. The dose-response curve of bradykinin differed from those of eledoisin and serotonin by its slope. 1  $\mu\text{g/kg}$  of bradykinin had nearly



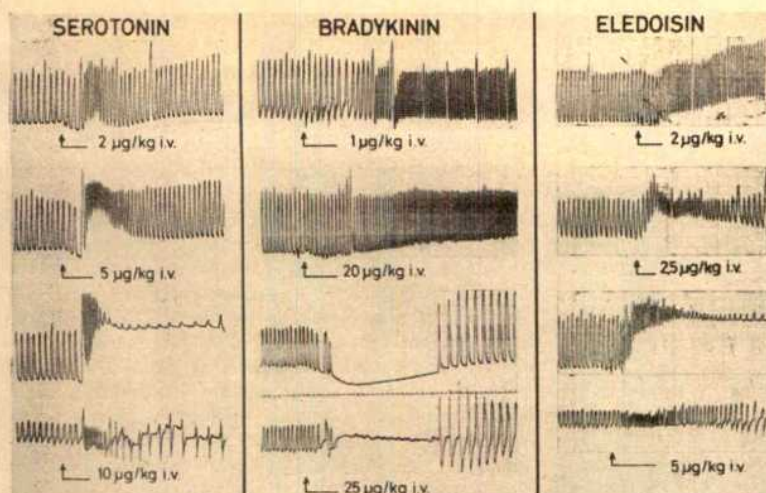


Fig. 1. Effect of bradykinin, eleodoin and serotonin on the tidal volume in the guinea-pig. Guinea-pig (420 g) under urethane anaesthesia (1.5 g/kg; subcutaneous). Body-plethysmography; for details of method see Gjuriš *et al.*<sup>4</sup>. Bottom trace: movements of respiratory active muscle groups

maximal effect, and even extremely high doses (up to 200 µg/kg) were unable to reduce the inflation volume by more than 50 per cent. The dose response curve of the less-active kallidin closely resembled that of bradykinin.

The reduction of inflation volume by small doses of bradykinin and kallidin (5 µg/kg) was completely prevented by the broncholytic agents isoprenaline (1–10 µg/kg) or epinephrine (0.5–10 µg/kg). High doses of the plasma kinins (50 µg/kg), although not significantly more effective, remained unaffected or were only slightly reduced by the broncholytics which, on the other hand, readily abolished even the maximal effects of eleodoin or serotonin.

Acetylsalicylic acid (5–10 mg/kg) and phenylbutazone (5–10 mg/kg), which did not prevent the reduction of inflation volume caused by eleodoin or serotonin, abolished only the action of small doses of bradykinin and kallidin (5 µg/kg), but did not antagonize the action of higher doses of the plasma kinins (50 µg/kg). Even pretreatment with 100 mg/kg of acetylsalicylic acid failed to prevent the action of 50 µg/kg of bradykinin or kallidin.

The different slopes of the dose-response curves of bradykinin and kallidin as compared with those of eleodoin and serotonin suggest that the action of the plasma

kinins cannot be explained simply by bronchoconstriction. Since the pleura of the guinea-pig's lung contains an exceptionally large number of smooth muscle fibres<sup>11</sup>, it seems possible that the reduction of inflation volume caused by the plasma kinins is at least partly due to the contraction of pleural muscle fibres. This view is supported by the finding that bradykinin is much more effective in reducing the inflation volume when applied locally to the pleural surface instead of being administered by inhalation or by intravenous injection, while the reverse holds true for histamine<sup>4,12</sup>.

Recently, it has been reported<sup>13,14</sup> that in guinea-pigs small doses of the plasma kinins produce constriction of pulmonary vessels (possibly of the pulmonary veins) which was also prevented by salicylates. Thus, an increased blood volume of the lungs as caused by the constriction of pulmonary veins might be an additional factor in the action of the plasma kinins.

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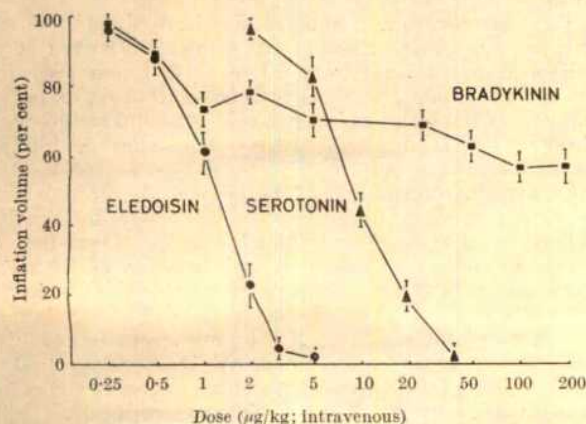


Fig. 2. Effect of bradykinin, eleodoin and serotonin on the inflation volume in guinea-pigs. Body-plethysmography in guinea-pigs (350–520 g) under urethane anaesthesia (1.5 g/kg; subcutaneous). Artificial respiration with a Starling pump (40 strokes per min) at constant pressure (100 mm H<sub>2</sub>O). Paralysis of the respiratory muscles with succinylcholine (0.2 mg/kg; intravenous) 1 min before the intravenous injection of bradykinin, eleodoin and serotonin resp. Inflation volume before the injection of the bronchoconstrictor agents was assumed to be 100 per cent. Each point represents the mean  $\pm$  S.E. of 6–13 experiments

THE interesting observation of Gjuriš and Westermann that bradykinin differs from 5-hydroxytryptamine in its action on the tracheobronchial muscle of the guinea-pig accords with previous findings. For example, Jänkäälä and Virtama<sup>1</sup> concluded from radiography that 5-hydroxytryptamine "causes vigorous constriction of all the constrictable bronchial tree of the guinea-pig", whereas "bradykinin apparently only affects the respiratory bronchioles". I am glad that Gjuriš and Westermann support the suggestion we made some time ago<sup>2</sup>, that bradykinin may also affect the smooth muscles of the pleural surface of the lung.

Since bradykinin probably acts on a smaller part of the bronchial tree than does 5-hydroxytryptamine, it cannot be expected to reduce to the same extent the volume of air entering the lungs. In our hands, intravenous doses of 8 or more µg/kg of bradykinin usually reduce by > 50 per cent the inflation volume of a guinea-pig, which is artificially ventilated after suppression of spontaneous respiration with urethane. The difference from the 40 per cent reduction obtained with 50–200 µg/kg of bradykinin by Gjuriš and Westermann (Fig. 2) may perhaps arise from the fact that repeated doses of bradykinin readily cause refractoriness of the bronchoconstrictor response<sup>3</sup>. Repeated doses of 5-hydroxytryptamine, on the contrary, often elicit successively larger responses.



Such changes of sensitivity are particularly likely to intrude when many doses are given in obtaining a dose-response curve.

The failure of Gjuriš and Westermann to 'antagonize' the bronchoconstrictor action of a large intravenous dose of bradykinin (50 µg/kg) with a still larger dose of aspirin (100 µg/kg) only partly accords with our observations<sup>4</sup>. We found that, within the intravenous dosage range of 0.5–8 mg/kg of aspirin, the ratio of this drug to bradykinin required to yield a constant response is roughly constant. Increasing the dose of aspirin above 8 mg/kg does not proportionately increase the antagonism. An intravenous dose of 8–16 mg/kg of aspirin, however, greatly reduces the bronchoconstrictor response to 32 µg/kg of bradykinin and makes the response to 100 µg/kg of bradykinin about the same as that to 1 µg/kg without aspirin. This action of aspirin is local, since it occurs after destroying the spinal cord and cutting the autonomic nerves to the lungs<sup>5</sup>.

The finding of Gjuriš and Westermann that isoprenaline fails to antagonize bronchoconstriction induced by large doses of bradykinin agrees neither with our experience nor with their own previous statement<sup>6</sup>.

Since, in at least some of their experiments<sup>7,8</sup>, Gjuriš and Westermann have used bradykinin supplied in ampoules, a further possible source of difference from those who have derived their bradykinin solutions directly from the pure synthetic solid should be considered. Chlorbutol, which is sometimes present in ampoules of bradykinin at a concentration of 5 mg/ml., diminishes the amplitude of inspiration in both dog and rabbit, according to Impens<sup>9</sup>. Although the doses he used were relatively large, they were given by mouth, and the possibility must therefore be considered whether smaller doses of chlorbutol, injected into blood vessels along with bradykinin, might themselves affect respiration, especially if respiratory depressants such as urethane had previously been given.

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## HAEMATOLOGY

### Detection of Catalase after Electrophoresis of Hæmolysates on Starch Gel

CATALASE, a hæmoprotein normally present in erythrocytes, can be separated from hæmoglobin by electrophoresis of hæmolysates on starch gel. The production of gas bubbles with hydrogen peroxide, and enzyme assays of eluates from segments of the gel, have shown that, after electrophoresis, erythrocytic catalase is confined to one discrete zone<sup>1</sup>. This zone stained weakly with naphthalene black, but, with benzidine or *o*-tolidine, showed a strong colour reaction, similar to the colour produced in the hæmoglobin zones. Owen, Silberman and Got<sup>2</sup> found that *o*-dianisidine was the most satisfactory reagent for the detection of hæmoglobin and of hæmoglobin-haptoglobin complexes on starch gel. The use of *o*-dianisidine as a stain for catalase on starch gel has been investigated, and it has been found that, under certain conditions,

a differential colour staining can be obtained, catalase being green and hæmoglobin brown.

After separation of plasma from heparinized blood, erythrocytes were washed three times with cold isotonic saline, and hæmolyzed by freezing and thawing twice. The hæmolysate was diluted by the addition of 0.5 volume of water and then was shaken up with 0.5 volume of carbon tetrachloride. The aqueous layer was removed and centrifuged at 4° C and 55,000*g* for 60 min. Finally, the hæmoglobin content was adjusted to 15 g/100 ml. by the addition of water. Starch-gel electrophoresis was based on the method of Smithies<sup>3</sup>. Hydrolysed starch (Connaught Medical Research Laboratories, Toronto) was used in a concentration of 14 g/100 ml. buffer. A *tris*-citrate-borate buffer system was used at pH 9.5, as these conditions were found to provide the best separation of the non-hæmoglobin erythrocytic proteins<sup>1</sup>.

The reagents for the *o*-dianisidine stain were: *o*-dianisidine, 0.3 g in 100 ml. methanol; acetate buffer, pH 4.6, prepared by mixing 1.5 M acetic acid solution with 1.5 M sodium acetate solution; hydrogen peroxide solution, 100 vols; 'Teepol' (Shell Chemical Company, Ltd.), 1:250 dilution in water. The most satisfactory method was found to be as follows. Fifty ml. of the dianisidine solution and 50 ml. of the acetate/acetic acid buffer were mixed and 0.4 ml. H<sub>2</sub>O<sub>2</sub> (100 vol.) added just before use. The gel was flooded with this mixture until the hæmoglobin stained dark brown (5–10 min). After pouring off the staining solution and washing with 1:250 'Teepol' solution the gel was then allowed to stand for several hours in the 'Teepol' solution. Within a few minutes of the addition of the 'Teepol', a faint green colour was apparent in the solution, and this colour gradually increased in intensity. The green staining of the catalase zone did not appear immediately, but only after standing in the 'Teepol' solution for a few hours. The hæmoglobin zones were then purplish-brown, and no area of the gel showed any green stain except the catalase zone (Fig. 1). The differential staining did not depend on the different concentrations of peroxidatic substance, as hæmoglobin did not stain green by this method after electrophoresis of a series of dilutions of hæmolysates with hæmoglobin concentrations ranging from 15 g/100 ml. to 0.015 g/100 ml.

Best results were obtained with 1:250 'Teepol' solution, but green colouring of the catalase zone also occurred, more slowly, and less intensely, with other dilutions of 'Teepol' within the range 1:25 to 1:2,500. With weaker 'Teepol' solutions or when distilled water was used alone following staining with *o*-dianisidine, no green colour developed. Also, green did not appear with a stronger 'Teepol' solution (1:10).

The concentration of *o*-dianisidine in methanol was not critical and green staining of catalase occurred with *o*-dianisidine solutions from 0.1 to 1.0 per cent provided the time of staining was adjusted so as to avoid excessive staining of the hæmoglobin zones. Optimum results were obtained by staining with 0.3 g *o*-dianisidine in 100 ml. methanol for 5–10 min.

On varying the molarity of the acetic acid/acetate buffer, at pH 4.6, similar differential staining was produced using buffers in the range of 0.75 M–1.5 M. Less distinct colour differentiation occurred with molarity of 3.0 and a green colour was not consistently produced with buffer more dilute than 0.75 M. When acetic acid/acetate buffer, 0.75 M, was used in a range of pH values from 3.25 to 5.90, similar green colouring occurred with buffer of pH 4.80 or less but no green appeared with buffer of higher pH. The most satisfactory results were obtained with acetic acid/acetate buffer, 1.5 M and pH 4.6.

After electrophoresis of an aqueous solution of bovine liver catalase (Mann Research Laboratories, New York) (20 mg/ml.), on starch gel, at pH 9.5 under the same conditions as for hæmolysates, the main catalase zone (corresponding to the area of gas bubbles when hydrogen peroxide was applied to the gel) stained green with



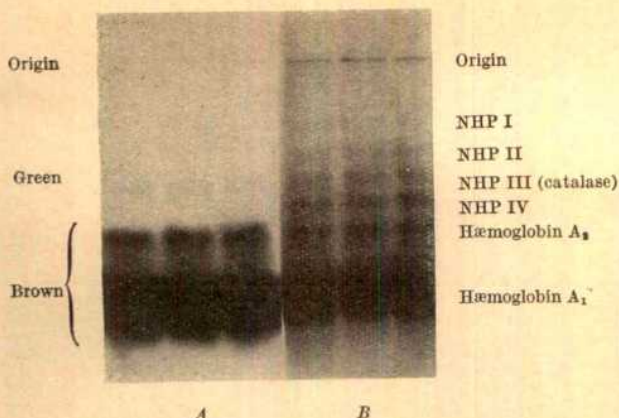


Fig. 1. Starch-gel electrophoresis of a normal hemolysate. A, Stained with dianisidine. The hemoglobins ( $A_1$  and  $A_2$ ) stained brown, whereas catalase (non-hemoglobin protein zone III) stained green; B, stained with amido-black, showing the hemoglobins and the non-hemoglobin proteins (NHP zones I-IV, following the nomenclature of Haut *et al.*)

*o*-dianisidine. Horseradish peroxidase (Mann Research Laboratories, New York), on electrophoresis of an aqueous solution (0.4 mg/ml.) at pH 9.5, showed separation into at least eight zones—two anodal and six cathodal to the origin. When the gel was stained with *o*-dianisidine these zones transiently became bright green, and within a few minutes the colour had changed to chocolate-brown.

A method for the localization of catalase on starch gel, based on the oxidation of iodide to iodine by hydrogen peroxide with subsequent staining of the starch gel except in the area of  $H_2O_2$  destruction by catalase, has been described<sup>4</sup>. This method distinguishes catalase from other hydro-peroxidases, but the area showing the catalase reaction is more diffuse than with the *o*-dianisidine stain and overlaps several non-hemoglobin protein zones in the electrophoretic pattern of normal hemolysates<sup>1</sup>.

The *o*-dianisidine method produces the staining reaction as a result of the peroxidatic activity of catalase, and of hemoglobin, catalysing the oxidation of *o*-dianisidine to a coloured compound. When the hemoglobin concentration in hemolysates is greatly reduced by dilution, electrophoresis and staining with *o*-dianisidine results in the same colour of the hemoglobin zones as with the more concentrated solutions, so that the green colour of the catalase zone is not due solely to reduced concentration of peroxidatic material compared with the hemoglobin zone.

The appearance of green colour in the supernatant 'Teepol' solution before the catalase zone itself stains green and the relatively slow development of the green catalase zone suggest that the green pigment is formed from the *o*-dianisidine by the action of 'Teepol' and then is selectively absorbed into the gel in the position of the catalase. The explanation of the different colours produced with the catalase and hemoglobin with *o*-dianisidine is not clear, but it affords a convenient method of localizing catalase on starch gel, and of distinguishing it from hemoglobins.

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## Presence of Two $Ph^1$ Chromosomes in Cells from a Patient with Chronic Granulocytic Leukaemia

THE 'Philadelphia' chromosome first described by Nowell and Hungerford<sup>1</sup> is characteristic of chronic granulocytic leukaemia and can be demonstrated in the myelocytic cells of most cases.

In typical cells only one  $Ph^1$  chromosome is present. Recently a number of cases with cells containing two  $Ph^1$  chromosomes have been described<sup>2,3</sup>. In most of these the finding has been made in the acute blast stage of the disease or shortly before this change has occurred. This might suggest that the finding, like that of aneuploidy with more than one cell line, is a sign of impending if not actual 'blastic crisis'.

We report here a case of chronic granulocytic leukaemia with cells showing two  $Ph^1$  chromosomes in direct bone marrow preparations. Five months later the patient still shows the features of chronic granulocytic leukaemia with no evidence of morphological or clinical change in the disorder.

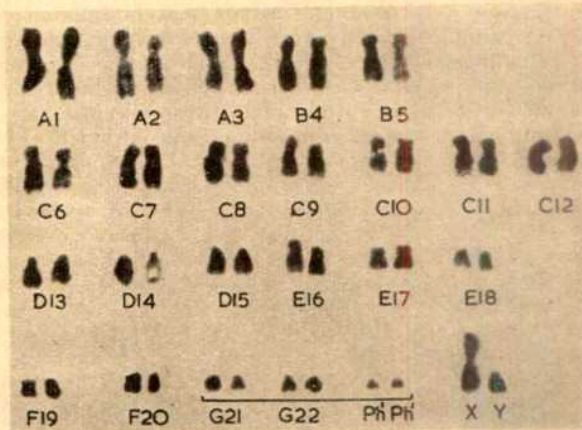


Fig. 1. Karyotype of cell from bone marrow of J. V., with 48 chromosomes. Group G contains six chromosomes of which two are  $Ph^1$  chromosomes

J. V., a male born in 1900, was diagnosed as having chronic granulocytic leukaemia in January 1963. He was initially treated with busulphan. In May 1963 thrombocytopenia developed and he was referred to one of us for an opinion as to further therapy. Busulphan therapy was ceased and steroids were given with a good platelet response. In February 1964 he was seen again with a rising white cell count. The haemoglobin was at a satisfactory level and, as he was well, no therapy was advised. A bone marrow specimen was taken at this time and showed the features of chronic granulocytic leukaemia. Cytogenetic material was prepared directly from this specimen of marrow.

Results from investigation of these preparations are shown in Table 1. A karyotype of a bone marrow cell showing two  $Ph^1$  chromosomes is illustrated in Fig. 1.

In June 1964 the patient was seen again. The spleen was enlarged and the white cell count had risen further. A course of splenic irradiation was given with a good therapeutic result.

The presence of two  $Ph^1$  chromosomes in this case suggests that the finding is a variant of the usual picture in chronic granulocytic leukaemia. Since it can be found

Table 1

Type of preparation	Date	Haematological findings	Treatment	Total No. cells	46	47	48	Tetra-ploid
Direct bone marrow	3.2.64	Hypercellular marrow with characteristic features of chronic granulocytic leukaemia	Busulphan from 11.1.63 to 22.5.63	50	15 (14 $Ph^1$ +)	34 (32 $Ph^1$ +)	1	1

Of the 15 metaphases with 46 chromosomes, 14 showed the  $Ph^1$  chromosome. Of the 34 metaphases with 48 chromosomes, 32 showed 2  $Ph^1$  chromosomes, and the remaining 2 showed a single  $Ph^1$  chromosome.



in the chronic phase of the disease it is of no prognostic significance.

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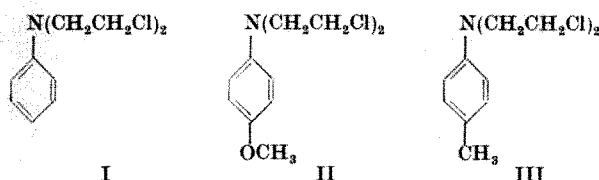
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## PATHOLOGY

### Drug-induced Regression of Large Plasma Cell Tumours

WHILE many transplanted animal tumours may be cured if treated with chemotherapeutic agents the day after implantation, there are few reports of the regular cure of such tumours if treatment is delayed until the tumours are advanced and large (that is, more than 20 per cent of the body-weight). This communication reports the regular cure of large, well-established mouse plasma cell tumours (*ADJ-PC5*) (ref. 1) up to 21 days old and 5 g in weight with a single intraperitoneal dose of *N,N*-di-2-chloroethyl aniline (aniline mustard, *CB 1074*) (I).



The high activity of aniline mustard against this tumour was first observed in an examination of the tumour as a screening test for chemotherapeutic agents likely to be of value in the treatment of multiple myeloma in man<sup>2</sup>. Tested against 10-day implants weighing about 0.1 g, aniline mustard proved to have a more selective inhibitory effect than a number of agents in clinical use, such as *cyclophosphamide* and *melfalan*. When more advanced tumours were used, namely, 17–21 days after implant, aniline mustard still gave complete regression of the tumours at a dose of 40 mg/kg, that is, one-third the *LD*<sub>50</sub>. None of the early symptoms usually associated with nitrogen mustard toxicity was seen at this dose but some late deaths occurred after regression was complete. A dose of 60 mg/kg, however, which did not harm normal mice, killed 30 per cent of mice bearing 21-day tumours and 90 per cent of those bearing 25-day tumours. The results of some typical experiments are shown in Table 1. Tumour-weight was estimated from caliper measurements using a standard curve obtained experimentally<sup>3</sup>. Tumour-weights thus obtained ranged from 1.1 g to 5.1 g on the day of treatment. Regression occurred very rapidly during the first week, then much more slowly (Fig. 1). Mice were usually killed at the 50th day, but those of

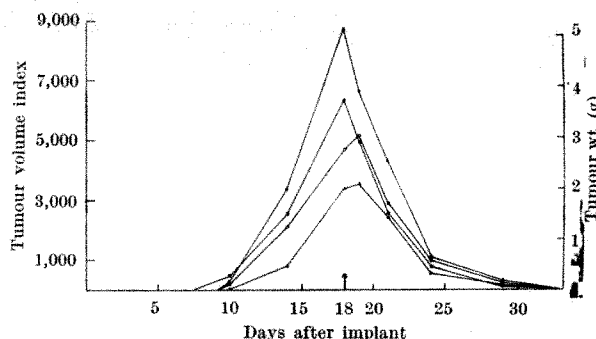


Fig. 1. Representative growth and regression curves of *ADJ-PC5* implants given *CB 1074* 18 days after implantation

experiment IV have now been kept for 140 days and no recurrence can be found by palpation.

Sugiura and Stock<sup>3</sup> have reported the cure of a number of 7-day-old mouse and rat tumours, and complete regression of 21-day-old Flexner-Jobling carcinomas in rats was obtained with a single dose of OPSPA by Heidelberger and Baumann<sup>4</sup>. Long survival of another mouse plasma cell tumour, *YPC-1*, given courses of *cyclophosphamide*, beginning day 16, has recently been reported<sup>5</sup>.

The reason for the high selectivity of aniline mustard requires further investigation. It is certainly closely related to the structure of the alkylating agent since the *p*-methyl derivative (III, *CB 1044*) caused little inhibition of the growth even of 10-day *ADJ-PC5* implants, while *p*-methoxy aniline mustard (II, *CB 1045*) appears to be somewhat more effective than the unsubstituted compound, causing, in a recent experiment, complete regression in three out of four 27-day tumours having a mean weight of 7 g at the time of treatment. It is possible that *p*-hydroxylation by the tumour is required for selective action, and investigations of this possibility are in progress.

The mechanism of cure of mice bearing large tumours has been investigated, notably by Martin<sup>6</sup>. This and other work<sup>7,8</sup> suggests that a specific host response may be necessary for cure to occur. The *ADJ-PC5* tumour is carried in the isologous host, and in more than 300 implants allowed to grow for 20 days no spontaneous regressions were observed. That an immune mechanism is active in our tumour system was, however, revealed by re-implantation of 12 of the mice of experiment IV, 100 days after the original treatment with aniline mustard. Only one-third of the second implants were palpable at 23 days, and the mean size was only 0.25 g compared with 3.3 g in the control group.

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Table 1

Exp.	No. of mice	Day of treatment	Mean tumour weight (g)	Dose (mg/kg)	No. of complete regressions	No. of tumour free survivors day 50
I	6 (9)	20	3.0 (1.3-4.1)	30	4	4
II	12 (9)	21	2.1 (1.3-3.4)	40	11	10
III	7 (9)	17	2.6 (1.5-5.1)	40	7	6
IV	15 (5)	18	2.7 (1.1-5.1)	40	15	14*

\* Twelve tumour-free survivors day 120.



### Gamma-glutamyl Transpeptidase in Cancers of Different Human Organs

In 1961 we published<sup>1</sup> a method for the histochemical localization of  $\gamma$ -glutamyl transpeptidase (GGTP) in the organs of experimental rodents and in man. Independently, a few months later, similar results were reported by Glenner, Folk and McMillan<sup>2,3</sup>, who in contrast to our findings<sup>1,4</sup> also discovered GGTP activity in the bronchi and bronchioles of the rat.

In 1962, at the seventh International Cancer Congress in Moscow, we exhibited photomicrographs indicating the GGTP distribution in renal cancer in man<sup>5</sup>. The activity of the enzyme was found in the cells of solid cancer and of that consisting of glandular tubules. Using the biochemical method<sup>6</sup> we were able to establish that the GGTP content in renal cancer is about 10 times less than in the normal kidney<sup>5</sup>. Recently we realized that in Wilms's tumour (nephroblastoma) the cells lining the canalicular lumen showed GGTP activity.

Then we proceeded to the examination of GGTP activity in other tumours in man: these included sarcomas and cancers of various organs. We found that only cancers originating from organs exhibiting normal GGTP activity contained the enzyme; thus, GGTP activity could be detected in cancers of the hepatic cells, of the mucous membrane of the uterus, of the pancreas, breast or prostate, whereas none was exhibited by cancers taking their origin from the epidermis, the multilayered flat epithelium of the larynx, or the vaginal part of the uterus and bladder; it was also absent in sarcomas.

Table 1. GAMMA-GLUTAMYL TRANSPEPTIDASE ACTIVITY IN PRECANCEROUS LESIONS, BENIGN AND MALIGNANT TUMOURS OF THE BREAST

Histological diagnosis	Mean activity in units	
	Per 1 g of tissue	per 1 g of protein
Normal woman breast	0.290 $\pm$ 0.081	0.0028 $\pm$ 0.0003
Breast cancer	0.259 $\pm$ 0.049	0.00200 $\pm$ 0.0003
Mastopathia	0.321 $\pm$ 0.197	0.0024 $\pm$ 0.0016
Fibroadenoma	0.357 $\pm$ 0.036	0.00305 $\pm$ 0.0004

An observation, related to GGTP activity, might help in the histological diagnosis of some cancer cases. Post-mortem histological examination in a case of pancreatic cancer showed in the omentum the presence of cell infiltrates which could not be identified as being of cancerous or inflammatory nature. As the cells of pancreatic cancer contain a clear-cut GGTP activity the omentum infiltration was examined from this point of view. The omentum cells showed a conspicuous GGTP activity, thus pointing to the existence of metastatic lesions and not to the inflammatory reaction. This method supplements staining techniques for the detection of mucus in the diagnosis of cancer originating from the glandular epithelium.

As to renal tumours, GGTP activity of the cancers was lower than that of organs from which they came. Using bioptic intraoperative specimens, we have investigated cancers, fibro-adenomas and mastopathia cystica of the breast in women. It has been found that GGTP values in normal breast exceeded those found in cancers, but in fibro-adenomatous mammae and in cases of mastopathia cystica they were still higher than in the normal organ (Table 1).

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### Kinetics of Cellular Growth after Repeated Applications of Methylcholanthrene

EARLIER I reported my examinations on variations in the mitotic duration, mitotic count, and mitotic rate after a single surface application of methylcholanthrene to mouse skin<sup>1</sup>. It was found desirable to continue these studies after repeated paintings inasmuch as continuous painting is followed by hyperplasia and tumour formation, and the present communication concerns an investigation of the mitotic rate during a period of additional and excessive growth. Hairless mice (*hr/hr*) were painted 3 times weekly with 0.6 per cent solution of methylcholanthrene in benzene. Each week one group of eight mice was killed, the last group 13 weeks after the first application. Mitoses were counted, the mitotic duration was estimated by the 'Colcemid' method, and the rate calculated according to the equation: (mitotic duration)  $\times$  (mitotic rate) = mitotic count (ref. 2).

The first tumours appeared after some 6 weeks, and after 10 weeks all the remaining animals had one or several manifest carcinomas in the treated area. The results are shown in Fig. 1 and Table 1.

Table 1. CHANGES IN EPIDERMAL MITOTIC COUNT, MITOTIC DURATION, AND MITOTIC RATE AFTER REPEATED APPLICATION OF 0.6 PER CENT METHYLCHOLANTHRENE

Time (weeks)	Mitotic count (per 6 mm)	Mitotic duration (h)	Mitotic rate (per 6 mm/h)
1	15.8 $\pm$ 4.6	3.1	5.2
2	19.0 $\pm$ 2.2	2.6	7.5
3	15.5 $\pm$ 1.7	1.9	8.3
4	16.5 $\pm$ 3.0	1.6	10.4
5	18.3 $\pm$ 1.7	1.8	10.0
6	26.0 $\pm$ 3.5	1.9	13.7
7	22.3 $\pm$ 4.1	2.3	9.9
8	38.0 $\pm$ 7.7	1.8	21.1
9	28.5 $\pm$ 2.6	2.6	10.9
10	40.3 $\pm$ 7.1	2.9	13.9
11	30.3 $\pm$ 7.1	2.8	10.7
12	29.0 $\pm$ 8.5	2.2	13.1
13	22.8 $\pm$ 4.2	3.0	7.6

The mitotic counts represent the means of groups of four mice. The mitoses in 6 mm epidermis were counted in each specimen. The mitotic duration and rate represent the means of observations on 8 mice; four killed before, and four after a delay of 4 h after injection of colcemid.

The experiment shows that the highly increased mitotic count during the first weeks is exclusively due to prolonged mitotic duration. The mitotic rate is lower than normal in this period and only from about the 5th week is the rate slightly increased. Incidentally, the mitotic duration after 13 weeks, in the skin between the tumours, was the

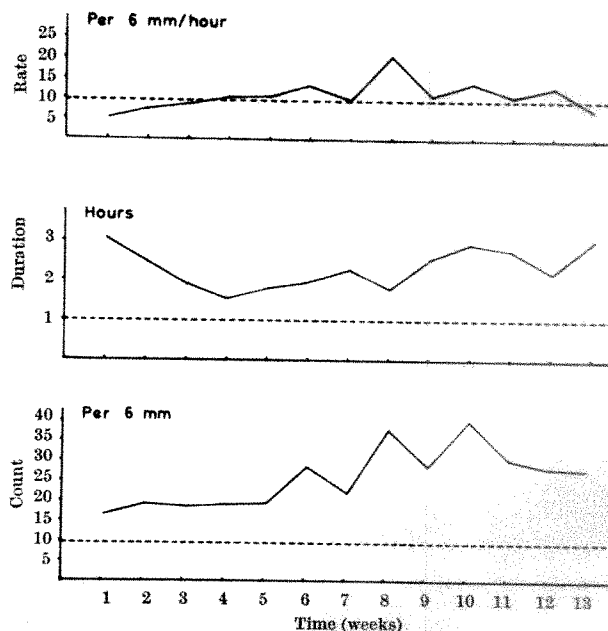


Fig. 1. Variations in mitotic count, mitotic duration, and mitotic rate after repeated applications of 0.6 per cent methylcholanthrene



same as in the spinocellular induced carcinoma in a hairless mouse reported by Evensen and Iversen<sup>3</sup>.

Again it is seen that an increased number of mitoses is not synonymous with a high rate (defined as the number of mitoses completed per unit time). The findings are fully in accordance with those observed immediately after application of different carcinogens to the epidermis of mice<sup>4</sup>. The results also offer an explanation for the discrepancy between the high number of mitoses and the moderate growth rate of some tumours observed by earlier authors<sup>5,6</sup>.

According to the findings reported here the carcinogens, as represented by methylcholanthrene in the present experiment, do not as such 'stimulate' the mitotic rate. The explanation of the hyperplasia thus has to be sought in a different way.

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### Causative Agent of a Spontaneously Originating Visceral Tumour in the Newt, *Triturus*

A SPONTANEOUS visceral tumour was found about ten years ago by one of us in the Japanese newt, *Triturus pyrrhogaster*<sup>1</sup>. The original animal showed a number of tumorous nodules in the liver and spleen ranging in size from tiny ones to the largest measuring 1.1 cm × 0.7 cm × 0.4 cm. The tumour was transplanted through many generations to other *pyrrhogaster*; and it was classified as a lymphosarcoma on the basis of its histogenesis after transmission<sup>2</sup>. It may be transmitted not only to *pyrrhogaster* but also to other species of newts and salamanders<sup>3</sup> and, as we have found recently, to the adult anuran, *Xenopus laevis* (unpublished). Moreover, the supernatant of tumour homogenates retained the capacity to produce tumour even after the homogenates were frozen and thawed and dried *in vacuo*. Evidence presented here points to a bacterium as the likely agent.

Electron microscopical observations were made on excised tissue fixed in 1 per cent osmic acid or osmic acid-sucrose solution buffered at pH 7.5 for about 1.5 h and embedded in butyl methyl methacrylate mixture (7:3) or in 'Epon'. They showed numerous intracytoplasmic oval or rod-shaped bodies the structure of which is consistent with that of bacteria<sup>4-6</sup>. The inclusions were approximately 0.22–0.45  $\mu$  wide and up to 2.0  $\mu$  long; they were provided with a cell wall of two concentric layers surrounded by an amorphous, presumably capsular material of variable thickness (Fig. 1). Internal to the cell walls is an area, presumably cytoplasmic, showing low-density inclusions and, centrally placed, a light region containing a meshwork of fine threads, presumably the nucleus.

Histological and cytological investigations were made of tumour tissue and of smeared filtrates of tumour and normal liver homogenates passed through a 3.0  $\mu$  'Millipore' filter. Smear preparations—dried, heat-fixed and stained with Giemsa, periodic acid-Schiff or iron haematoxylin—showed, in the case of tumour homogenates, bacterial-like bodies of about the size seen in electron microscopic preparations. They were oval or rod-shaped and occurred singly, in pairs, clusters, or sometimes in chains. They were not found in similarly prepared smears of normal liver.

Histological sections (3  $\mu$ ) of tumorous liver after fixation in osmic acid and paraffin embedding showed a faint

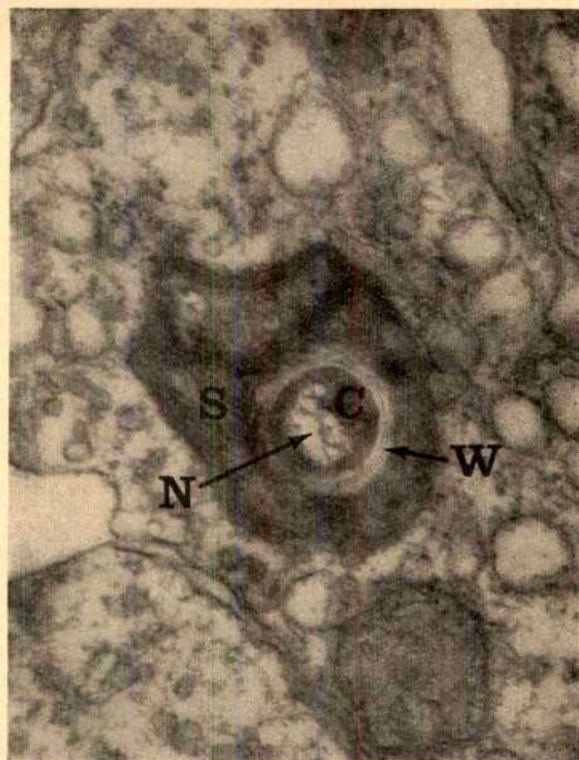


Fig. 1. Electronmicrograph showing bacterial organism in cytoplasm of tumour cell of *T. pyrrhogaster*. Note voluminous capsular substance (S), typical bacterial cell wall (W) composed of two layers, cytoplasmic area (C) and inclusions, and light nuclear area (N) with a meshwork structure ( $\times 48,800$ ).

Feulgen reaction for deoxyribose nucleic acid in the cytoplasmic granules, which also stained with the Giemsa dye and with iron haematoxylin. The granules also were acid-fast as demonstrated in sections (5  $\mu$ ) of tumorous liver fixed in Helly's fluid, stained with carbolfuchsin, decolorized in 95 per cent ethyl alcohol containing hydrofluoric acid (3 per cent), and counter-stained with methylene blue.

*T. pyrrhogaster* carrying the tumour were injected subcutaneously with four successive doses of 16  $\mu$ c. of tritiated thymidine in 0.15 ml. of saline solution at intervals of 2 h. Four hours after the final injection, the liver was excised and fixed in 1 per cent osmic acid-sucrose solution. After sectioning at 3  $\mu$ , the specimens were stained with Feulgen reagent and coated with Kodak nuclear track emulsion, 'NTB3'. The coated slides were developed after 21 days at 2° C. The sections showed incorporation of radioactive thymidine in the cytoplasm as well as in the nucleus of tumour cells. Liver parenchyma and non-tumorous areas of the capsule showed no cytoplasmic incorporation.

Bacteriological investigations were undertaken in which the tumorous liver and spleen of two newts, one *T. pyrrhogaster* and one *T. viridescens*, were removed under sterile conditions, and ground in sterile mortars and pestles containing 4 ml. tryptose phosphate broth; 0.01 ml. of the homogenates was planted on the following media and then incubated aerobically or anaerobically at 22° C: tryptose blood agar base containing 5 per cent sheep blood; Mueller-Hinton agar; and Endo agar. Within two days 5–20 colonies of several species of Gram-negative bacilli were isolated which, because of their variety and low numbers, were regarded as autopsy contaminants. Numerous tiny colonies appeared after 10 days aerobically at both temperatures on blood agar and Mueller-Hinton agar. Microscopic observations showed slender, irregularly staining Gram-positive bacilli reminiscent of similarly stained human tubercle bacilli. The



organisms were solidly acid fast after the cold acid fast technique using 'Tergitol 7' and 3 per cent acid alcohol. Sub-culture in 'Tb' broth (Difco) medium containing 'Tween 80' gave in each case good growth in 4 days at 22° C, but growth in Lowenstein-Jensen's medium required about 3 weeks. The procedures of plating and sub-culturing were repeated at least four times for each of the cultures. The liver and spleens of four uninoculated control newts, two of each species, were similarly removed, ground, and planted in 'Dubos Oleic' agar (Difco) at both temperatures; the cultures were negative after six weeks of incubation.

Four *T. pyrrhogaster* and four *T. viridescens* were respectively injected intraperitoneally with 0.3 and 0.2 ml. each of a heavy serially cultured 'Tb' broth of the acid-fast bacterium. Gross observations after 5-14 weeks showed disseminated nodules of the liver and spleen resembling those induced by intraperitoneal tumour transplants. Smears of the organ or of ground samples revealed large numbers of acid-fast bacilli. When cultured, these organisms were identical with those isolated originally. Histological examinations of these bacterially induced growths showed cell masses in the liver and spleen with the same histological characteristics of the original tumour, namely, masses of mononuclear cells of the capsule invading the parenchyma of the liver. Bacterial cultures which yielded tumours in 3 *T. pyrrhogaster* and 3 *T. viridescens* were also made on Sabouraud's dextrose agar; but these cultures were not pursued through serial passage.

The histopathological response to the injected culture is being investigated further and assessed against the background of opinions now prevailing on granulomatous and sarcomatous diseases. The ensemble of evidence at the moment shows that the tumour cells, first found in the Japanese salamander and transmitted through many generations, contain a bacterium; that cultures of the organism yield a response which resembles closely the response induced by tumour transplants or by injections of tumour homogenates; and, therefore, that the bacterium or some causative factor associated with the bacterium may be the agent of the tumour. Organisms, similar to that which we have described, have been isolated and cultured from tumours and blood of tumorous mammals including man<sup>7,8</sup>, and injection into mice and guinea-pigs has been reported to yield a chronic granulomatous disease or neoplasms<sup>7,8</sup>. In the case of plants, ample proof and experimentation have been published to show that the crown gall is bacterial in origin<sup>9</sup>.

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## IMMUNOLOGY

### Comparison of Tumour Antigens in AKR and Gross Virus (Passage A)-induced Leukaemias

EARLIER experiments have shown that it is possible to characterize the group of lymphomas elicited by Gross Passage A virus using immunological methods. It was shown that a resistance to tumour transplantation could be developed in mice by administering low doses of tumour cells; this effect was obtained in both the syngeneic and the allogeneic tumour-host system<sup>1</sup>. Serum from immunized mice exerted a cytotoxic effect on transplanted Gross tumour cells *in vitro* in the presence of complement<sup>2</sup>. Individually induced Passage A tumours were found to cross-react, whereas tumours induced by other means did not. Cross-reactivity was demonstrated for Gross tumour cells by (a) ability to induce and sensitivity to transplantation resistance, (b) ability to induce and sensitivity to cytotoxic antibodies, (c) specific absorption of cytotoxic antibodies by tumour cells, and (d) specific tumour cell neutralization by immune lymph node cells.

It was concluded that the group of tumours produced by Passage A virus possessed a specific antigen different from normal tissue antigens and other tumour antigens. Using the cytotoxic technique, this antigen could be detected *in vitro*.

The Gross Passage A virus was originally isolated from a spontaneous leukaemia of an AKR female<sup>3</sup>. The AKR mouse strain has been inbred with the aim of producing a high leukaemia mouse strain. They develop a lymphatic leukaemia spontaneously and at a high rate at 6-8 months of age.

The isolated agent was found to produce leukaemias in certain low-leukaemia mouse strains also, for example, when inoculated into new-born animals of C3H/BI or C57BR/cd origin. After several passages in new-born mice, the potent Passage A virus was obtained, which produced lymphatic leukaemias at a high frequency and after a relatively short latency period (3-4 months) in those low-leukaemia strains<sup>4</sup>.

In view of the fact that Passage A virus was originally derived from AKR mice, we found it promising to compare AKR leukaemias with Passage A virus-induced leukaemias, with regard to specific antigenicity. Leukaemias which had arisen spontaneously in 6-8 months old AKR mice were accordingly investigated for the possible presence of tumour antigen(s). Preliminary attempts to obtain cytotoxic sera from AKR mice inoculated with low doses of AKR lymphoma tissue were not rewarding. Therefore, Passage A tumours induced in C3H/BI or C57BR mice were used for pretreatment.

Groups of 2-month-old AKR or C3H mice were inoculated with cells from Gross tumours with a previously known tumour antigenicity (GHA, GBA, GBE) and according to procedures previously published<sup>1,2</sup>. Serum from these animals was then tested for cytotoxic activity against six different AKR leukaemias, primary or iso-transplanted (Table 1). The mean values given in the tables are obtained from several experiments with different serum pools. The figures given in parenthesis show the range of variation between these different serum pools. Of the six AKR tumours tested, three were found to be sensitive to cytotoxic action in the great majority of experiments (26 out of 39), while one of the other three was sensitive in only one case (out of 15). Lymph-node tissue from apparently normal 2-month-old AKR mice and 4-6-month-old mice in the preleukaemic stage did not reveal any cytotoxic sensitivity (0 out of 30). A cytotoxic effect of these sera was registered on Passage A-induced tumour cells as previously demonstrated<sup>2</sup>. Normal lymph-node cells from syngeneic donors were not affected; some of the data are not included in Table 1.



Table 1. CYTOTOXIC EFFECT\* ON SPONTANEOUS (*AKR*) AND GROSS VIRUS-INDUCED LEUKAEMIAS  
Cytotoxic index, mean and range

Serum donor	Passage A tumour inoculation	<i>AKR</i> preleukaemic lymph-node cells		<i>AKR</i> leukaemias		Syngeneic Passage A leukaemias
		2 months	6 months	Sensitive	Non-sensitive	
<i>AKR</i>	<i>GHA</i>	0.12	0.02 (0-0.08)	0.36 (0.16-0.68)	0.06 (0-0.11)	—
<i>AKR</i>	<i>GBB</i>	0.01 (0-0.3)	0 (0)	0.34 (0.16-0.69)	—	—
<i>C3H</i>	<i>GHA</i>	0 (0)	0.04 (0-0.17)	0.26 (0.16-0.41)	0.04 (0-0.16)	0.37 (0.18-0.74)
<i>C3H</i>	<i>GBA</i>	—	0.01 (0-0.04)	0.34 (0.16-0.51)	0.11 (0.08-0.14)	0.32 (0.27-0.42)

\* Cytotoxic index was defined as: percentage of living cells in control minus percentage of living cells in test, all divided by the former figure. A cytotoxic index exceeding 0.15 was considered to signify a positive effect<sup>2</sup>.

In *C57BR* mice, immunization was also performed with two of the sensitive *AKR* leukaemias, A2 and A6. Although only sera without a cytotoxic effect on *AKR* lymphocytes were used, a reaction was found against both *AKR* leukaemias and syngeneic Passage A tumours (Table 2).

Some of the spontaneous *AKR* leukaemias thus seem to possess the same tumour antigen as that found in the leukaemias induced by Passage A virus. In other *AKR* leukaemias, which were morphologically identical, this antigen could not be detected by the direct cytotoxic test.

The fact that some *AKR* leukaemias were sensitive to a cytotoxic serum, while others were not, may reflect a quantitative difference in the number of antigenic receptors on the tumour cells. Absorption experiments indicated that this, rather than a qualitative difference, was probably the case. Crude suspensions of both sensitive and non-sensitive *AKR* lymphoma cells were effective in absorbing cytotoxic sera, while lymph node cells from apparently healthy *AKR* mice, 2 or 6 months old, were ineffective. Quantitative work is now in progress, since the absorption capacity should compare with the sensitivity<sup>5</sup>.

It is, however, not possible to rule out the possibility that the virus is the stimulating antigen and that the sensitivity to cytotoxic action is due to a difference in the amount of virus produced per tumour cell. A third possibility is that a qualitative difference exists between the spontaneous leukaemias<sup>6</sup>. The Passage A virus strain may represent a selection and concentration of one of several agents responsible for leukaemias in *AKR*. The experiments reported here, however, make this last hypothesis less likely. The cross-reactivity which could be demonstrated by cytotoxic tests and absorption experiments between Passage A-induced tumours and several of the tested *AKR* leukaemias indicates that the Gross virus plays the most important part in induction also of the *AKR* leukaemias. It should be mentioned that another recently discovered antigen, the *TL* antigen<sup>7</sup>, has not been found in *AKR* leukaemias or in leukaemias induced by Passage A Gross virus.

The subsequent step was to examine the properties of sera from untreated *AKR* mice. Serum was therefore taken from untreated mice of different ages and tested on Passage A leukaemias and sensitive *AKR* leukaemias. No cytotoxic effect was demonstrated in serum from 2-month-old or from leukaemic *AKR* mice. When sera from mice 4-6 months old were tested, a weak and varying cytotoxic effect (C.I. = 0.13-0.31) was found. A similar increasing cytotoxic activity in sera from preleukaemic mice has been found in *C3H* mice injected with Passage A virus when newly born<sup>8</sup>. The serum titre was generally low during the first months after virus inoculation. After several months mice with negative and positive sera were found (C.I. = 0.12-0.56) before macroscopical tumours had developed.

The absence of detectable serum antibodies in 2-month-old *AKR* mice confirms the results of Axelrad<sup>9</sup>. He found no resistance to colony formation by lymphoma cells in young adult *AKR* or *C3H* mice inoculated with Gross virus when newly born. Instead there was an increase of tumour cell proliferation, which was interpreted as a

state of tolerance in the animals exposed to or carrying leukaemogenic virus at birth.

It is not yet clear why there should be a low production of specific antibodies in the preleukaemic mice. It is known that the presence of thymus tissue is essential to tumour genesis in this type of lymphatic leukaemia<sup>10,11</sup>. Malignant cells appear first in the thymus, from where they spread to other parts of the lymphatic system, giving rise to a generalization of the disease. As long as lymphoma cells stay in the thymus, they should be protected from the humoral antibodies by the immunological barrier of the thymus.

Table 2. CYTOTOXIC EFFECT OF *C57BR* SERA ON SPONTANEOUS AND GROSS VIRUS-INDUCED LEUKAEMIAS

Tumour inoculation	<i>AKR</i> preleukaemic lymph-node cells		<i>AKR</i> sensitive leukaemias	Syngeneic passage A leukaemias
	2 months	6 months		
<i>GHA</i>	0.06 (0.05-0.07)	0.05 (0-0.13)	0.27 (0.16-0.42)	0.40 (0.28-0.52)
<i>GBB</i>	0 (0)	—	0.22 (0.05-0.40)	0.31
A2, A6	0 (0)	—	0.39 (0.38-0.41)	0.37 (0.17-0.42)

It is possible that the first tumour cells that spread from the thymus stimulate the production of specific antibodies in the host. A parallel may be drawn with the stimulation that occurs when low doses of syngeneic Passage A tumour cells give rise to a specific resistance to transplantation and to cytotoxic antibodies<sup>1,2</sup>. The relatively small amounts of antibodies found in preleukaemic *AKR* mice are probably insufficient to prevent outgrowth if a large number of tumour cells erupt from the thymus. The same destruction of transplantation resistance is found when a large number of Passage A lymphoma cells is administered to animals immunized with Passage A-induced tumour cells.

The results reported here are indicative of serologically detectable properties, common for some of the spontaneously occurring *AKR* leukaemias and the Gross virus Passage A-induced leukaemias.

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## RADIOBIOLOGY

### Evidence for the Existence of Non-exchangeable Calcium in Human Urine

It is customary to interpret the results of kinetic metabolic studies with radioactive tracers in terms of compartmental models. In the case of calcium, it is usual to assume the existence of a central pool of exchangeable calcium the specific activity of which is equal to that of

the plasma. Several workers have found discrepancies between specific activities of plasma and urine samples in various species<sup>1-5</sup> though others have not<sup>6,7</sup>. As far back as 1930 Brull<sup>8</sup> suggested that "what is excreted and concentrated by the kidney is the very part of the calcium which is diffusible but inactive and useless". If this were the case, it would seem likely that any differences between urinary and plasma calcium specific activities might be attributable to the presence of calcium in the central pool in chemical forms which do not exchange, or exchange only slowly, with ionized calcium. Recently, Giese and Comar<sup>5</sup> have demonstrated that there is, in fact, a non-exchangeable calcium fraction in sheep plasma. In the present work, it is shown that a similar non-exchangeable fraction occurs in normal human urine and presumably in the plasma.

The urine samples studied were either freshly collected specimens from normal persons or were taken from 24-h pooled collections from patients; the latter specimens were stored at 4° C with toluene as a preservative. The pH of the urine was not altered. About 0.1 µc. of calcium-47 solution in the form of chloride (Radiochemical Centre, Amersham) was added to 50–70 ml. of urine which was then incubated in a closed container for approximately 24 h at 37° C. After incubation, a portion of the urine was passed through a cation exchange resin ('Zeokarb 225' in the sodium cycle); using a column 7-mm diameter and 10-cm long. Chemical calcium in the treated and untreated urine was estimated by titration with EDTA using murexide as indicator and also by absorption flame photometry as described by Baker<sup>9</sup>; good agreement between the two methods was obtained. Specimens, the chemical concentration of calcium of which was too low for accurate measurement, were concentrated by evaporating to dryness in a platinum dish, ashing over a bunsen burner flame and reconstituting to a smaller volume in N/10 HCl. The calcium-47 content of each treated urine was compared with that of the corresponding untreated specimen in a well-type scintillation counter with the discriminator level set to eliminate scandium-47. Usually only a small fraction of the calcium-47 passed through the resin unabsorbed, but a greater proportion of the chemical calcium was recovered. It is convenient to define the effective non-exchangeable calcium ( $C_{NE}$ ) as that amount of unlabelled calcium which would have to be present in the processed urine to account for any difference in its specific activity compared with that of the original specimen; though this almost certainly represents an oversimplification. This is calculated using the expression:

$$C_{NE} = C_2 - A_2(C_1 - C_2)/(A_1 - A_2) \text{ mg per cent}$$

where  $A_1$  and  $A_2$  are the original and final count rates (c.p.m.), and  $C_1$  and  $C_2$  the initial and final concentrations of chemical calcium (mg per cent).

It can be seen from the results in Table 1 that the urines examined contained variable amounts of effectively

non-exchangeable calcium, in two cases exceeding 40 per cent. It is evident that this fraction is firmly bound since it is not removed by a strongly acidic unfunctional resin; increasing the incubation period to 48 h has little effect, and in several experiments increasing the pH to 10 or more also had little effect. Further investigations are in progress on the chemical nature of the non-exchangeable calcium, but it is already clear that this fraction is only a part of the complexed calcium fraction since calcium as citrate is readily extracted by the resin used. The demonstration of non-exchangeable calcium in plasma is necessarily difficult, since its preferential excretion would result in it being only a small part of the central pool, though it would have a very large turnover rate. Giese and Comar found it necessary to use starved sheep in order to show the existence of such a pool in plasma; and it is noteworthy that the highest non-exchangeable fraction in the present series was found in a patient with malabsorption syndrome who had hypocalcaemia as well as hypocalcaemia. Conversely it was not possible to derive a figure for the non-exchangeable calcium in urines with a high calcium content; the negative values shown in Table 1 for such urines may be due to experimental errors.

It has been pointed out by Veall and Parsons<sup>10</sup> that the use of a model which does not distinguish between ionized and complexed calcium for the interpretation of radio-calcium tracer data is likely to lead to questionable conclusions, at least so far as the quantitative aspects are concerned. Furthermore, the existence of an appreciable non-exchangeable calcium fraction in urine may account for some of the difficulties which have been encountered by many workers in determining accurately the chemical calcium content of urine samples<sup>11</sup>.

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Table 1

Subject	Urine calcium $C_1$ (mg per cent)	<sup>47</sup> Ca extracted by resin (per cent)	Effective non- exchange- able calcium $C_{NE}$ (mg per cent)	Non- exchangeable (per cent)	$C_{NE}$ mg/day*
BD†	2.0	99.2	0.87	43.8	29
JG	6.3	99.0	2.72	43.2	—
NV	7.2	99.3	0.33	4.6	—
FH	7.4	96.1	1.90	25.7	—
NV	8.4	94.2	0.90	10.7	29
AF	10.0	92.6	0.50	5.0	—
JP	9.0	99.4	0.44	4.9	6
BC	11.0	90.4	2.50	22.7	—
JB	11.3	96.7	0.05	4.4	—
MJ	14.0	89.8	1.75	12.5	—
KB	14.0	95.5	2.95	21.3	—
FO†	22.8	97.5	0.90	2.5	—7
JB	23.4	75.3	0.10	0.04	—
PK	42.0	90.8	1.00	2.4	—

\* 24-h urine specimens.

† Malabsorption syndrome plasma calcium 6.5 mg per cent.

‡ Hyperparathyroidism, plasma calcium 13.5 mg per cent.

## BIOLOGY

### *Mansonia crassipes* as the Natural Vector of Filarioids, *Plasmodium gallinaceum* and Other Plasmodia of Fowls in Ceylon

NILES<sup>1,2</sup> recently reported an infective larva of a filarioid and an unidentified *Plasmodium* from *Mansonia crassipes* in Ceylon. Since then a number of *M. crassipes* from the same locality (Hokkandara, in the suburbs of Colombo) were found infected, the mosquitoes frequently having both oocysts and sporozoites as well as filarial infections. Two types of oocysts and two types of infective filarial larvae were seen.

As *M. crassipes* was found to be ornithophilic, the parasites were considered to be of avian origin. In view

Table 1

Para- site(s)	<i>Plasmodium</i> , microfilariae and trypanosome	<i>Plasmodium</i> and micro- filariae	<i>Plas- modium</i> only	Micro- filariae only	Trypano- some only	Nega- tive
No. of fowls	1	5	4	1	1	6

of this, blood films of country fowls from the locality were examined. Eighteen fowls have been investigated since August 1964 and the results are summarized in Table 1.

These results strongly suggested that the fowl is the vertebrate host of the parasites in *M. crassipes*. Accordingly, the following preliminary experiments were carried out: (a) Sporozoites from wild-caught *M. crassipes* were inoculated subcutaneously into day-old chicks. (b) Infective filarial larvae from wild-caught *M. crassipes* were inoculated subcutaneously into day-old chicks. (c) Blood from *Plasmodium*- and trypanosome-infected fowls was inoculated intraperitoneally into day-old chicks.

The results obtained were as follows:

(a) Sporozoite-inoculated chicks became infected with three different species of *Plasmodium*. The incubation period for two of these was about 15 days. One of the latter was indistinguishable from *P. gallinaceum*, while the other produced mostly elongate gametocytes which tended to grow round the host cell nucleus rather than to displace it. This appeared to be a new parasite. Prof. P. C. C. Garnham and Prof. A. Coradetti, who examined the parasites, were of the same opinion. The third species appeared after an incubation period of 30 days and the parasite was very much like *P. lophurae* with the characteristic vacuolation of the cytoplasm. Of all three species this was the least pathogenic to the chickens, which lost the infection in seven days.

(b) Infective filarial larvae with three caudal papillae and approximately 1,800 $\mu$  in length (described by Niles<sup>1</sup> as being closely similar to those of *Wuchereria bancrofti*) were inoculated into two day-old chicks, one receiving 38 and the other 14 larvae. The first chick became microfilaria-positive after 29 days and the second after 25 days. The microfilariae were 280–300 $\mu$  in length and appeared to be similar to those of *W. bancrofti*, but lacked a sheath. The development in the mosquito took place in the thoracic muscles. The first chick was killed 24 days after it began to show microfilariae, and 6 adult female and 12 adult male worms were recovered from its peritoneal cavity. These and the other adult filarioids from fowls are now being examined.

The second type of filarial larva found in *M. crassipes* developed in the fat body. The mature larva was short (920–1,020 $\mu$ ) and devoid of any distinct caudal papillae. Fourteen infective larvae of this type were inoculated into a day-old chick which died 24 days later without showing microfilariae. No adult worms were recovered from it.

(c) Blood from five of the *Plasmodium*-infected fowls was inoculated intraperitoneally into day-old chicks. The incubation period was 8–10 days, and the parasites were mostly of the *P. gallinaceum* type. The various strains isolated showed different degrees of virulence to the chickens. The trypanosomes did not show up in the chickens after blood inoculation.

It would appear from these observations that *M. crassipes* is the natural vector of *P. gallinaceum* of fowls in Ceylon and possibly of other species of *Plasmodium* of fowls or of related avian hosts. If one of the other plasmodia from *M. crassipes* is in fact *P. lophurae* then this will be the first record of *P. lophurae* from this part of the world. We are, however, not certain whether the fowl is the natural vertebrate host of *P. lophurae* in Ceylon.

We have seen crithidial forms in the midgut of engorged wild-caught *M. crassipes*. This mosquito may, therefore, prove to be the vector of the trypanosome as well. The

trypanosome differs from *Trypanosoma gallinarum* and *T. calmettei* which have previously been reported from fowls.

So far five different types of microfilariae have been found by us in fowls. One of these is similar to that obtained in the chick experimentally infected with infective larvae from wild-caught *M. crassipes*. Perhaps the most interesting feature here is that the pre-patent period of at least one of the filarioids of this easily available laboratory animal is only 25–30 days. This parasite will therefore prove most valuable in teaching and research.

Because the infective larva of one of the filarioids resembled that of *W. bancrofti* we tested the susceptibility of laboratory-bred *Culex pipiens fatigans* to infection with four of the five types of microfilariae seen in fowls. This mosquito fed avidly on the fowls but was not receptive to any of the microfilariae.

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### Age Determination in Baleen Whales (Mystacoceti)

THE determination of the age of cetaceans is of importance for the organization of a rational exploitation of their stock. Age determination of baleen whales by the layers of ear plugs<sup>1,2</sup> does not always give accurate results and raises several technical difficulties<sup>3</sup>.

When staining the transverse section of baleen plate with haematoxylin and eosin, multiple layers in the walls of large and small tubules composing the medullar layer of plates are clearly seen. An investigation of the structure of baleen plates of two finwhales (*Balaenoptera physalus*), the age of which was determined with the accuracy of  $\pm 0.5$  years by means of the analysis of ear plug layers, showed a certain correlation to exist between the maximal number of layers in small tubules and the age of the animal (Fig. 1a). An examination of a large number of such tubules revealed that in some of them (about 10 per cent of the total number) no layers could be seen at all, the wall of the tubules being smoothly or spottily stained. A less clear manifestation of stratification was found in large tubules of the baleen plate.

In the tubules of the baleen plate of a considerably older animal the number of layers is much greater than that in three to four year old ones (Fig. 1d).

The character of the stratification is not related to the location of the section either at the base, in the middle or in the upper part of the baleen plate. There are specimens which show the stratification of large external tubules to be more pronounced at the apex of the baleen plate.

We have examined the histological structure of the baleen plates in several *Mystacoceti* species caught by coastal whale trading stations on the Kuril Islands and by the inhabitants of the Chukotsk Peninsula. In sections of *Balaenoptera musculus*, *B. borealis*, *B. acutorostrata*, *Megaptera nodosa*, and *Balaena glacialis* the layers of tubules in the medullar layer of the baleen plate were clearly seen.

The material at our disposal has been insufficient for firm conclusions to be drawn concerning the relation of tubule stratification of the baleen plate to the age of



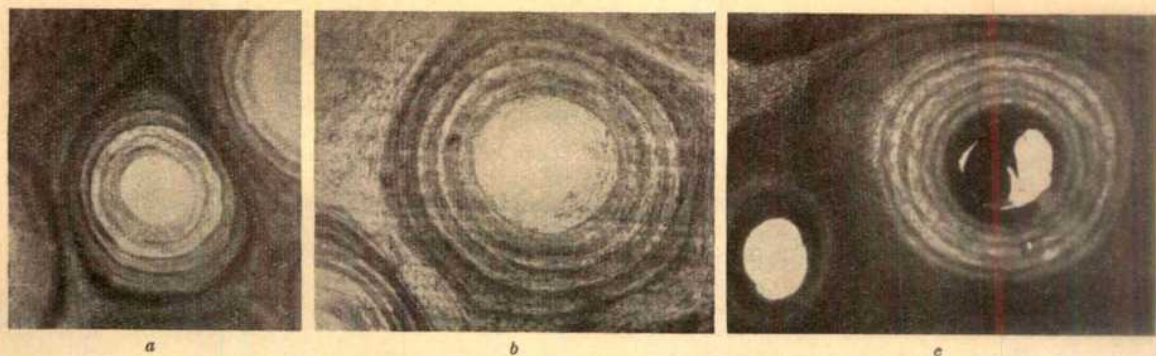


Fig. 1. Transverse sections through a small tubule of the fin-whale (*Balaenoptera physalus*) baleen plate (haematoxylin and eosin), a, age of the animal  $3 \pm 0.5$  years; b, age  $4 \pm 0.5$  years; c, an older animal ( $\times c. 75$ )

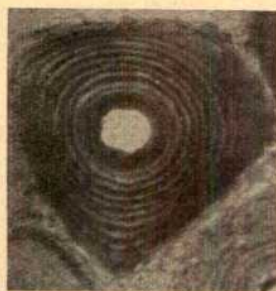


Fig. 2. Transverse section through a small tube of the baleen plate of a seiwhale (*B. borealis*) of an unknown age

the animal. However, the results obtained show that there is a possibility that such a relation exists. By examination of further specimens it may be possible to develop a new and simpler method for the determination of the age of baleen whales.

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### A Triennial Copepod (Crustacea) in the Temperate Zone

BOTH marine and freshwater copepods have, with one exception from the high arctic<sup>1</sup>, generally been regarded as having a life-cycle of one year or less. A study of the freshwater planktonic copepod *Cyclops scutifer* Sars now in progress in Scandinavia and in North America has shown, however, that this species has a 3-year cycle in a small lake near Oslo, Norway.

The lake, Store Tryvann, is situated at 60° N and at an elevation of 395 m above sea-level, has an area of 0.0437 km<sup>2</sup>, and a maximum depth of 16 m, is mesohumic, and surrounded by dense coniferous forests. The surface temperatures rise to about 20° C in the summer, and bottom temperatures go only slightly below 4° C. The mean air temperature of the surroundings (1931–60) for July is 14.3° C.

The seasonal cycle of *C. scutifer* is rather complex, its main outlines being as follows: the period of reproduction occurs in the summer, and nauplii (Fig. 1, 0) develop to the penultimate naupliar stage during the autumn and remain at this stage throughout the winter. During the following spring the nauplii develop to small copepodids (Fig. 1, 1) most of which, in the course of the summer, go

into diapause<sup>2,3</sup> as copepodids II and III in the bottom mud, where they stay in a dormant condition until the next spring. During the spring of the second year the small copepodids develop into large copepodids of stages IV and V, and most of them descend to the bottom mud for a second period of diapause (Fig. 1, 2). After having spent the autumn and winter in diapause these large copepodids develop into reproducing adults in the course of the spring and early summer of the third year. The adults die off during the summer. There is, however, some evidence that a fraction of the population of large copepodids may develop into adults in the second summer without a second diapause, thus producing a secondary maximum of adults and a new wave of reproduction later in the same summer. The maximum of adults in Fig. 1 (3) is therefore probably composed of some 2-year and some 3-year-old individuals. It is of course also possible that a few individuals survive more than 3 years.

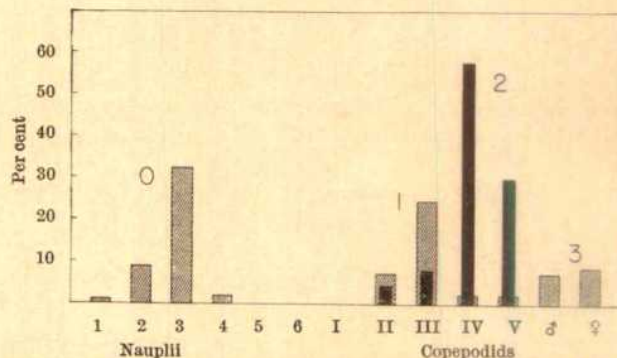


Fig. 1. Relative distribution of the population of *Cyclops scutifer* in Lake Store Tryvann on July 25, 1962. Cross-hatched bars, in the plankton; black bars, in diapause in the bottom deposits. Figures indicate the different year classes referred to in the text

The 'dualism' previously observed in Scandinavian populations of *C. scutifer*<sup>4-6</sup> can now most simply be explained by the existence of different year classes in the population.

The prolongation of the seasonal cycle in this species may be considered as an adaptation to arctic conditions which is maintained also in the temperate zone.

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### A Model of Tumour Growth in Irradiated Hosts

It has been found that local irradiation of the transplantation site prior to grafting experimental animal tumours influences the subsequent growth rate of the tumours and the survival time of the tumour-bearing hosts<sup>1,2</sup>. While the results of the experiments indicated that irradiation of the host tissue slowed the growth of the tumours, more precise interpretation of the data seemed desirable. The following simple model was thus developed. In the light of this model, experimental data can be interpreted in a more meaningful fashion. In addition, the approach utilized in the model may be useful in other problems of host-tumour interaction.

Assume that a tumour consisting of  $n$  cells will double as soon as it has been exposed to a given quantity per cell ( $m$ ) of some limiting metabolite. If  $A$  is the effective area of the capillary bed and  $J$  is the transcapillary flux of this limiting metabolite, then the doubling time,  $t_d$ , is given by:

$$t_d = \frac{mn}{AJ} \quad (1)$$

Now irradiation of the host changes  $A$  by  $\Delta A$  and  $J$  by  $\Delta J$ . Call these new values  $A'$  and  $J'$ . The doubling time of the tumour in an irradiated host,  $t_d'$ , is  $mn/A'J'$  and:

$$\frac{t_d}{t_d'} = \frac{A'J'}{AJ} = 1 - \frac{\Delta A}{A} - \frac{\Delta J}{J} + \frac{\Delta A \Delta J}{AJ}$$

The magnitudes of  $\Delta A/A$  and  $\Delta J/J$  are likely to be rather small,  $\Delta A \Delta J/AJ$  is much smaller, so as an approximation:

$$\frac{t_d}{t_d'} = 1 - \frac{\Delta A}{A} - \frac{\Delta J}{J} \quad (2)$$

Since  $\Delta A/A$  and  $\Delta J/J$  are properties of the host animal, they may change as the host tissue recovers from the effects of irradiation, and hence are functions of time. For a slowly growing tumour the repair per generation of tumour cell will be greater than for a rapidly growing tumour, so as approximations:

$$\frac{\Delta A}{A} = R + Kt_d \text{ and } \frac{\Delta J}{J} = M + Nt_d \text{ (K and N negative)}$$

On substitution into equation (2):

$$\frac{t_d}{t_d'} = F + Gt_d, \text{ where } F = 1 - R - M, \text{ and } G = -K - N \quad (3)$$

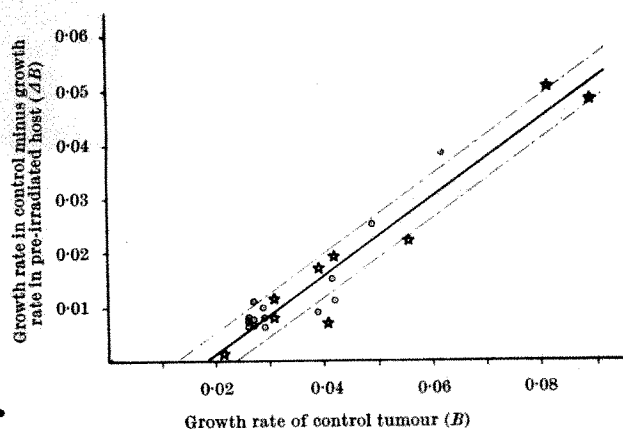


Fig. 1. Growth rate depression induced by irradiation (3,000 r.) of the tumour bed plotted against the growth rate of the same tumour in an unirradiated bed. Standard error of estimate is indicated. Data are from ref. 1 (stars) and ref. 2 (dots).

Expressed in terms of the growth rate,  $B$  ( $B = (\log 2)/t_d$ ):

$$\Delta B = PB - Q, \text{ where } B - B' = \Delta B, P = 1 - F, Q = G \log 2 \quad (4)$$

This equation predicts the expected relationship between the growth rate ( $B$ ) of the control tumour and the growth rate depression ( $\Delta B$ ) induced by prior irradiation of the host. As can be seen in Fig. 1, the experimental data verify this prediction ( $P = 0.72$  and  $Q = 0.013$ ).

The magnitude of the effect of pre-transplantation irradiation of the tumour bed appears to be related to the normal growth rate of the tumour. The relationship that exists can be predicted by assuming quantitative alterations in the effective capillary bed and in the transcapillary flux. The nature and relative importances of these changes are not immediately apparent; experimental work based on this model is in progress.

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### In vitro Conversion of Neutral Gibberellin-like Substances from Potato Tubers

RECENTLY, a chemically neutral substance, separated chromatographically from a methanol extract of potato tubers, was reported to stimulate elongation of 'Morse's Progress' dwarf pea plants grown under red light<sup>1,2</sup>. This bioassay is particularly sensitive to certain gibberellins, and the neutral substance, tentatively termed potato factor I, was therefore considered gibberellin-like. The substance was separated under well-controlled conditions to avoid the possibility of contamination by acid substances. Its discovery was of considerable interest since gibberellin-like substances that exhibit activity on the dwarf pea bioassay were previously found only in the acid fraction of plant extracts. The growth promotion by potato factor I became of even greater interest when, after two weeks standing and rechromatography, its activity had disappeared from its original place on the chromatogram and a new zone, now giving an acid test and showing much higher biological activity, appeared at  $R_F$  0.5-0.6.

To prepare the neutral fraction, 1 kg of peelings and buds from well-sprouted 'Red Pontiac' potato (*Solanum tuberosum* L.) tubers was extracted twice with methyl alcohol at 0°C. The alcoholic extracts were evaporated to the water phase which was extracted with ethyl acetate at pH 7.5. Basic substances were removed by shaking the ethyl acetate phase with 1 per cent sulphuric acid, leaving only neutral substances. The neutral fraction was concentrated and streaked on Whatman No. 3 MM paper and developed in isopropyl alcohol/ammonium hydroxide/

Table 1. The response of 'Morse's Progress' dwarf pea plants to eluates of the original neutral fraction and to the rechromatographed  $R_F$  0.3-0.4 eluate after standing two weeks at room temperature

Fraction	$R_F$	Dwarf maize $d_1$	Dwarf pea $d_1$
		Per cent of control	
Original	0.3-0.4	100.2	122.1
Neutral	0.4-0.5	84.9	112.7
	0.5-0.6	82.9	115.8
Rechromatographed	0.3-0.4	89.7	107.2
	0.4-0.5	89.7	143.2
$R_F$ 0.3-0.4	0.5-0.6	109.3	256.2



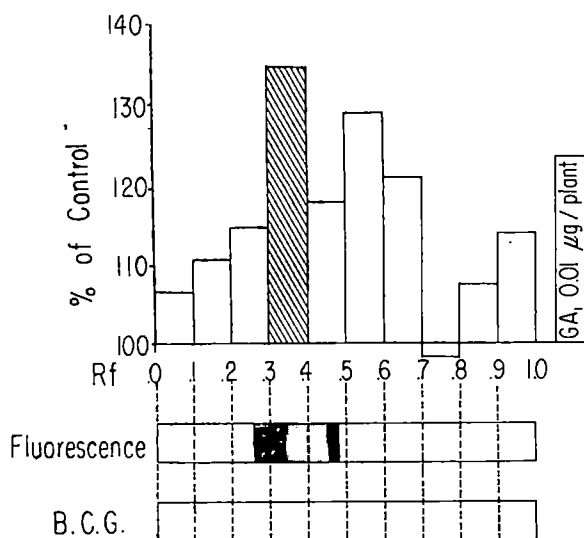


Fig. 1. The response of dwarf pea plants to the original neutral fraction from potato peelings and buds. Shaded area is potato factor I. Developed in isopropyl alcohol : ammonium hydroxide : water (10 : 1 : 1 v/v). Note the absence of colour development when chromatogram strips were sprayed with bromocresol green (BCG)

water (10 : 1 : 1 v/v). In separate tests, methyl alcohol extraction in the presence of nitrogen gas increased the yield of the neutral substance.

Potato factor I showed activity only on dwarf pea plants and affected neither dwarf maize  $d_1$  nor  $d_8$  (Table 1). The  $R_F$  0.3–0.4 eluate was dried and left standing for two weeks under aerobic conditions at room temperature. It was then dissolved in methyl alcohol and again chromatographed as described previously. Surprisingly, the  $R_F$  0.3–0.4 activity had disappeared completely and a new promoting zone had appeared at  $R_F$  0.5–0.6. This zone now gave an acid test with bromocresol green (BCG) (Fig. 2). Moreover, the activity of the new zone on dwarf pea plants was doubled; it also showed slight activity on dwarf maize  $d_8$ . Thus it appears that a substance(s) of the  $R_F$  0.3–0.4 eluate, chemically neutral and of relatively low activity, may have changed to a substance(s) that migrates to a higher  $R_F$ , is acid in nature, and shows qualitatively and quantitatively different biological effects. This kind of change was also noted in a 50 per cent alcoholic polyoxyethylene sorbitan monolaurate ("Tween-20") solution

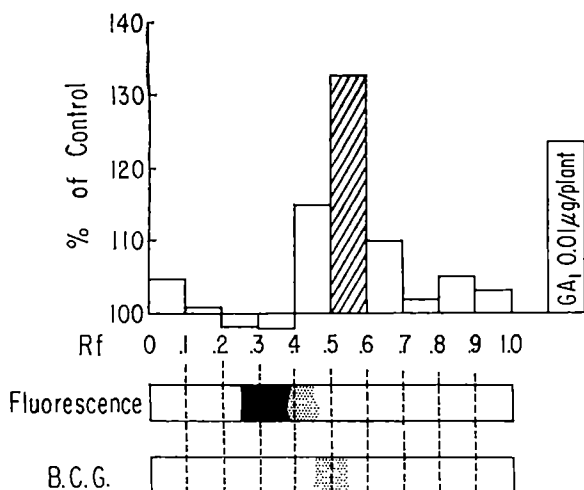


Fig. 2. The response of dwarf pea plants to the rechromatographed  $R_F$  0.3–0.4 eluate from the neutral fraction of potato peelings and buds. Note the appearance of colour after a chromatogram strip was sprayed with bromocresol green (BCG)

of the extract which was kept at 5° C for four weeks and which also increased dramatically in biological activity.

In an attempt to learn something of the chemical nature of the new zone it was separated using thin layer chromatography with silica gel-G ('Kieselguhr G') as stationary phase and benzene/propionic acid/water 8:3:5 (v/v) as developing solvent. The plate was sprayed with 5 per cent sulphuric acid in 98 per cent alcohol and a blue-green fluorescent spot was detected at  $R_F$  0.52 in ultra-violet light. This  $R_F$  corresponds to that of gibberellin  $A_1$ . Of course, additional information must be obtained before identification of the new form is assured. Lack of pure crystalline potato factor I prevented a direct attack on the mechanism of the reaction and the *in vitro* nature of the experiments permits only the most cautious speculation.

This may serve to highlight the significant aspects of this report.

The low activity of potato factor I and the much higher activity of the new active acid region suggest that potato factor I is a comparatively inactive, conceivably bound or precursor form of gibberellin. This speculation is supported by the very high activity of the 0.5–0.6 zone on dwarf peas and the new, although slight, activity on dwarf maize  $d_8$ .

The manner in which the apparent change occurred and the higher yields of 0.3–0.4 under anaerobic extraction conditions indicate a very simple, possibly oxidative, reaction. The response of dwarf pea, but not of dwarf maize, to potato factor I is of interest since the former might have the ability to convert the neutral to the acid form of the compound. The possibility of related changes occurring *in vivo* is suggested in the experiments of Hashimoto and Rappaport<sup>3</sup> in which incubation of bean seeds with gibberellin  $A_1$  resulted in a definite increase in activity of the neutral fraction.

Additional evidence for bound forms of gibberellins is presented by McComb<sup>4</sup> in which a homogenate of runner bean seeds yielded several times the original gibberellin-like activity after treatment with ficin, a proteolytic enzyme. Moreover, Murakami<sup>5</sup> reported the formation of a gibberellin  $A_1$  glycoside after incubation of cucumber leaf disks in the growth substance. The glycoside could be hydrolysed into gibberellin and sugar moieties with either dilute hydrochloric acid or the enzyme emulsin.

A better understanding of potato factor I must await its isolation, crystallization and use in physiological experiments.

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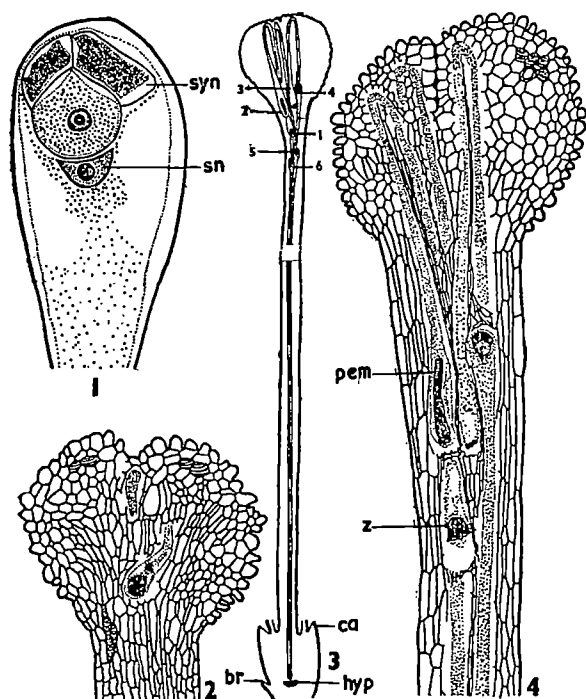
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### Embryo Sac Development in *Moquiniella*

THE Loranaceae are characterized by extremely long embryo sacs the tips of which reach up to various lengths in the style<sup>1</sup>. In four genera—*Barathranthus*<sup>2</sup>, *Helicanthera*<sup>3</sup>, *Tapinostemma*<sup>4</sup> and *Tupeia*<sup>5</sup>—they invade the stigma ascending as far as its apex. While working on *Moquiniella rubra* (Spreng. f.) Balle, a species endemic to South Africa, we observed that the tip of the embryo sac not only ascends





Figs. 1-4. Fig. 1. Tip of organized embryo sac ( $\times c. 360$ ). Fig. 2. Longitudinal section of stigma showing the curved position of an embryo sac ( $\times c. 40$ ). Fig. 3. Longitudinal section of gynoecium with six embryo sacs of which three (1, 2 and 3) have curved tips (diagrammatic) ( $\times c. 2-4$ ). Fig. 4. Magnified view of the stigmatic region from Fig. 3 showing four embryo sacs, three with curved tips ( $\times c. 40$ ). *br*, bract; *ca*, calyculus; *hyp*, hypostase; *pem*, pro-embryo; *sn*, secondary nucleus; *syn*, synergid; *z*, zygote

up to the apex of the stigma but also elongates further and curves downward.

*Moquiniella* is a stem parasite which grows on various species of *Acacia* and *Rhus*. It is a glabrous shrub with opposite leaves and brown flowers borne in umbellets. The flowers are 42-44 mm long, sub-sessile, pentamerous, hermaphrodite, and subtended by a small bract. The calyculus forms a 5-lobed rim and is devoid of any vascular supply. The corolla lobes are initially straight but become strongly reflexed at anthesis; the stamens are epipetalous and basifixed; and the fruit is an ellipsoidal pseudoberry.

The stylar canal is continuous with the central ovarian cavity and a plate of 5-8 hypodermal archesporial cells differentiates at its base. These cells elongate considerably and divide mitotically, forming a large mass of sporogenous cells which function directly as megaspore mother cells. After meiosis linear tetrads are formed, and the development of the embryo sac conforms to the Polygonum type. During megasporogenesis, several mother cells, dyads and tetrads degenerate so that only a few gametophytes develop. Two and 4-nucleate stages are gone through as usual, and at the 4-nucleate stage the tips of embryo sacs elongate into the style. Owing to their unusual length the gametophytes could not be dissected out in entirety, and it could not be established if a 6-nucleate stage (formed due to an earlier division of the two lower nuclei of 4-nucleate embryo sac) precedes the 8-nucleate condition<sup>1</sup>. The basal end of the embryo sac (situated in the ovary) forms a caecum leaving the antipodal cells *in situ*. The lower polar nucleus migrates into the upper part of the embryo sac, fuses with the upper polar nucleus, and the secondary nucleus becomes closely appressed to the egg (Fig. 1).

Four to nine embryo sacs extend into the style and stigma (Fig. 3). A very unusual feature, hitherto unreported, is the bending downward of the tips of some of the embryo sacs for 2-4 mm (Figs. 2-4). Therefore, the final shape of the curved embryo sac resembles an inverted J.

The maximum length of the straight arm of the embryo sac is 44 mm and of the curved portion 4 mm. To the best of our knowledge *Moquiniella* shows the longest embryo sac in angiosperms with a close approach to another loranthaceous genus, *Tapinostemma*<sup>2</sup>, which has a gametophyte 42 mm long.

The inverted tips of some of the embryo sacs in a style showed an egg apparatus while others had pro-embryos (Fig. 4), which indicates that fertilization occurs in this position. Whether such embryos reach maturity could not be ascertained as we had very little material of the developing fruits.

We thank Prof. P. Maheshwari for his advice, Prof. E. S. Twyman, Rhodes University, Grahamstown, South Africa, for providing the material; and the Ministry of Education, Government of India, for the award of a research training scholarship to one of us (B. R.).

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## ENTOMOLOGY

### Effect of Hormones on the Respiration of Body Fragments of Adult *Pyrrhocoris apterus* L. (Hemiptera)

PREVIOUS investigations of normal adult female *Pyrrhocoris* have demonstrated cyclic changes in the rate of oxygen consumption closely correlated with cycles of ovarian development and oviposition. These cycles disappear in females deprived of corpus allatum; when the corpora cardiaca were also removed, the absolute level of oxygen consumption decreased to low and steady levels similar to those encountered in normal adult males or diapausing adult females. The hormones seem to affect the total metabolism indirectly. For example, the activation hormone (AH) secreted by the brain neurosecretory cells—corpora cardiaca system—is a trophic factor which stimulates metabolic activity in tissues engaged in digestion and food utilization. The corpus allatum hormone (CAH) is thought to regulate metabolic activity in reproductive system<sup>1-3</sup>.

The high respiratory metabolism of normal adult females was shown to be composed of three main components; namely: (1) the reproduction metabolism, dependent on the CAH; (2) the digestive metabolism, dependent on the AH but independent of the CAH; (3) muscle metabolism and the basal cellular metabolism, identical with diapause metabolism, which are essentially independent of both hormones. The first of these metabolic components is absent in adult females deprived of their corpora allata. The first and the second are lacking in females deprived of both the CAH and the AH. As in normal males the total body respiration is then dependent solely on the third component<sup>1</sup>.

In the work recorded here these suggestions have been subjected to a further experimental analysis, using body fragments. As is well known, the tracheal system of insects ensures the oxygen supply to each tissue or cell independently of the circulatory system<sup>4,5</sup>. The isolated body fragments with uninjured tracheation retain their metabolic activity for a long time, until the reserve substrate material is completely exhausted. Thus, for example, it is not uncommon for an isolated pupal abdo-

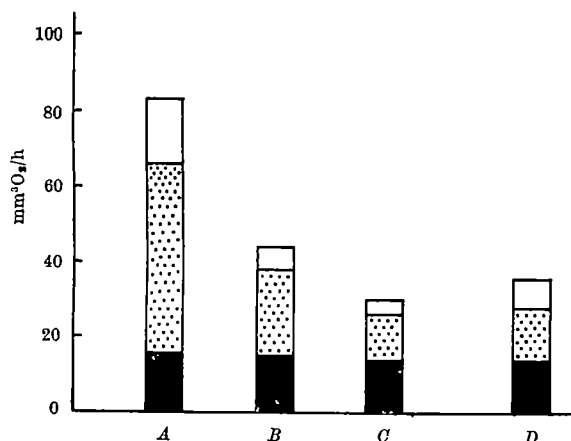


Fig. 1. Oxygen consumption of the whole-body (whole columns), thoracic fragments (black areas) and abdominal fragments (stippled areas) in 15 days old *Pyrrhocoris* adults. A, normal active females; B, females deprived of the corpus allatum soon after adult emergence; C, females with diapause induced by short photoperiod; D, normal males

men to develop and even to reach the adult form if the necessary hormones are supplied<sup>4</sup>. In our laboratory we have succeeded in keeping alive the abdomens isolated from the sawfly prepupae for more than three years.

Oxygen consumption was determined by the Warburg technique. The experiments were initiated by measuring oxygen consumption of the individual intact specimens. The specimens were then removed from the manometers and asphyxiated by submerging them under water for 15–20 min. Each individual was then decapitated and dissected in two separate fragments. The thoracic fragment contained the three thoracic segments with the legs and the complete thoracic somatic musculature; the thoracic part of the intestine, the fat body, and the salivary glands were taken out. The abdominal fragment contained the whole reproductive system, most of the intestine, and a great part of the fat body, etc. The separate fragments were quickly transferred into the respiratory vessels and the oxygen consumption was determined four times at 30-min intervals. The oxygen consumption of the fragments was found to be constant for 5–10 h.

The experiments were performed on the following groups of 15–30 specimens of 15-day-old *Pyrrhocoris* adults: (a) the normal active females at the middle of the reproduction cycle, with maximal respiration rate, with intestine full of food and with ripening eggs in the ovaries; (b) females deprived of their corpus allatum, with intermediate respiration rate and suppressed ovarian development; (c) diapausing females (obtained by rearing under conditions of 8 h photoperiodic illumination) with low respiratory rate, suppressed hormonal activity, arrested ovarian development and reduced digestion; (d) normal males with low respiratory rate, reduced digestion, etc.<sup>2</sup>. The weight of the thoracic fragments was about 22–24 mg in all groups. That of the abdominal fragments was about 48–52 mg in groups (a) and (b), and about 25 mg in groups (c) and (d) respectively.

The results are summarized in Fig. 1. They show that the thoracic fragments (containing most of the somatic muscles present in the body) maintain a similar rate of oxygen consumption in all these adults. This confirms the earlier suggestions<sup>1</sup> that metabolism of these somatic muscles is independent of hormonal activity.

A different situation occurs in the respiration of isolated abdominal fragments (Fig. 1). These fragments have a very low respiratory rate in diapausing females, where the organs of digestion and reproduction are functionally inactive. The same is true of the abdominal fragments of the normal males. The fragments isolated from the allatectomized females have a relatively higher respiration

rate, which seems to be connected with the functional activity of the digestive tube. The abdominal fragments isolated from the normal active females have the most intensive respiration rate. This can probably be explained by an enormously increased metabolic activity in connexion with the intensive digestion and egg maturation processes occurring in these insects<sup>7</sup>. Moreover, it is evident that the single abdominal fragment taken from an active female utilizes nearly twice as much oxygen as does the whole body of a diapausing female. These results indicate that the decisive part of the hormone-stimulated respiration results from increased metabolism in some tissues located in the abdomen, that is, the digestive tube, ovaries, fat body cells, etc.

These findings are consistent with the earlier suggestions<sup>1–3</sup> that insect hormones control the body metabolism indirectly by stimulating the growth processes in special target cells or tissues. The stimulated cells would themselves be responsible for the various biochemical reactions.

In the complete absence of the metamorphosis hormones the tissues that require hormonal stimulation do not grow and function. They maintain only a low, basal metabolism necessary for their preservation. This is probably the case with the abdominal tissues mentioned here. However, some of the tissues do not require hormonal stimulation. They can perform their metabolic functions in the absence of hormonal activity. As demonstrated here, these are mainly the somatic muscles of adult *Pyrrhocoris*; they have completed their differentiation during metamorphosis and their function in the adult stage is independent of growth. This suggests again that insect hormones regulate physiological processes by inducing growth in some special target cells or target tissues<sup>8,9</sup>.

I thank Prof. V. B. Wigglesworth and Prof. C. M. Williams for their advice.

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### 'Gynandromorphic Effect' and the Optical Nature of Hidden Wing-pattern in *Gonepteryx rhamni* L. (Lepidoptera, Pieridae)

G. A. MAZOKHIN-PORSHNYAKOV<sup>1</sup>, examining the optical characters of insect wings in connexion with the visual perception of insects, and taking photographs using ultra-violet rays, found that the smooth, yellow surface of the *Gonepteryx rhamni* wing has a real pattern which is invisible to the human eye. He also found that such a pattern in the full sense is presented in males only. The wing of the female absorbs ultra-violet rays by the entire surface and then looks dark. Mazokhin-Porshnyakov supposed that this hidden pattern may be considered as a character of taxonomic value. This assumption has been partly supported by my work<sup>2</sup>.

It is known that wing-patterns of Lepidoptera may be conditionally divided in two groups: pigmental and optic. I say conditionally because of the absence in Nature of any pure representative of any of this group. Real patterns are usually of a combined nature. Pigmental pattern

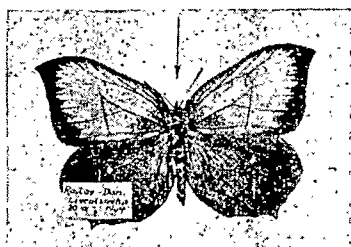


Fig. 1

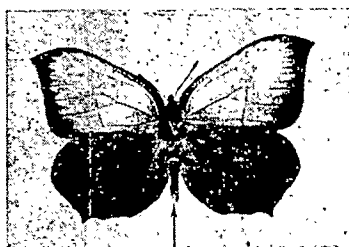


Fig. 2

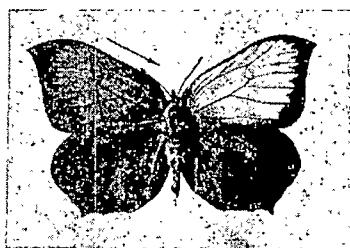


Fig. 3

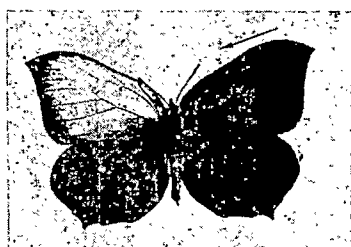


Fig. 4

peculiarities may be explained by the ability of a given pigment to absorb and (or) reflect the ray of a given wavelength. The optical pattern depends on effects of interference (genus *Haetera*) and diffraction (*Urania*, *Morpho*, *Apatura*, *Chlorippe*, etc.), and the visible appearance of wing-patterns of this group depends on the dip angle of light and the angle of observation. Now, naturally, the question arises: to which group does the hidden wing-pattern belong?

Once, taking photographs of *G. rhamni* in ultra-violet light and developing the film, I suddenly recognized the fact that all butterflies photographed turned out gynandromorphous: on one side the wings were like male, on the other, female. Then I remembered that the source of light (a mercury vapour lamp) had been placed so that light came from a side (the normal position was as shown in Fig. 1) and thus the observed effect may be explained only by the assumption that here we deal with the pattern of the optical group. If it were a pigmental one, the dip angle would not affect the appearance of pattern. The clear evidence of it is that in the lateral lighting the wing patterns of right and left sides are not identical ('gynandro-

morphic effect'). The hidden wing-pattern comes out exactly when the light comes toward the wing surface at an acute angle and from a distance. Figs. 1-4 depict the variation of the hidden wing-pattern depending on the position of the source of light. The arrow on each figure shows the direction of rays.

The luminescent observations of wings of these butterflies also supported the assumption concerning the optical nature of this pattern. Bright central field (for terminology see ref. 2) of the forewing luminesces in the dark-room when lighting with ultra-violet light in violet-purple colour (the range of this colour corresponds to the bright central field of the forewing in Figs. 1-4), and when a butterfly is turned, one can observe an effect much like that in *Apatura* in visible light. It is also interesting to note such an irisation in visible light in a North-American relative of *Gonepteryx*—*Zerene eurydice* Boisd.—and in some palaearctic representatives of the genus *Colias*.

Perhaps in all these cases we deal with the same effect, but in various ranges of wave-lengths. It is possible to explain this phenomenon by the presence on the *G. rhamni* wing of scales of the *Morpho* type<sup>3</sup>.

All the aforementioned may also be of importance when one seeks for a better mutual arrangement of the subject and the source of light in the photographic investigations in the ultra-violet part of the spectra.

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## MICROBIOLOGY

### Isolation and Characterization of an Actinophage (Phage A) active against a Strain of *Streptomyces* producing Novobiocin

AN actinophage (phage A), active against *Streptomyces*  $C_1V_{23}$  obtained from strain S-800<sup>1,2</sup>, a producer of novobiocin, has been isolated from industrial fermentation broth cultures with scanty or abnormal mycelial growth and no antibiotic production. So far as we know, this is the first time that an actinophage destructive to a *Streptomyces* strain producing novobiocin has been isolated.

The multiplication of the phage was easily obtained by contaminating, with a single plaque or 1-2 ml. of phage suspension, a 24-30 h culture of vegetative mycelium of  $C_1V_{23}$  in  $V_{10}$  modified broth (beef extract, 5.0 g; peptone, 5.0 g; autolysed yeast, 5.0 g; pancreatic digest of casein, 3.0 g; dextrin, 25.0 g;  $K_2HPO_4$ , 1.0 g;  $MgSO_4 \cdot 7H_2O$ , 0.25 g; NaCl 0.5 g; distilled water to 1,000 ml.) and incubating on a reciprocal shaker for 24-30 h at 28° C. Under these conditions lysis of the mycelium is complete with a titre of  $10^8$ - $10^9$  phage particles per ml.

The morphological characteristics of the plaques on the  $C_1V_{23}$  strain after different periods of time at 28° C were studied, using the double-layer technique of Gratia<sup>3</sup> as follows: After 24 h, circular shaded plaques,  $\phi$  1.0-2.0 mm, with slightly clearer centre. After 48 h, circular plaques,  $\phi$  2.0-3.0 mm, with very clear centre and turbid halo at the border. After 72-96 h, circular plaques,  $\phi$  4.0-5.0 mm, very similar to the 48 h plaques (Fig. 1). After 120-144 h, the distinction between the clear centre and the turbid halo at the border tends to diminish and the plaques ( $\phi$  4.0-5.0 mm) appear more uniformly turbid. The clear part is reduced to a small central dot-like zone.





Fig. 1

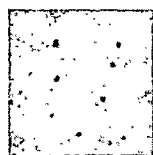


Fig. 2

From the phage-lysed cultures were also isolated sensitive colonies on which the phage, instead of forming plaques, as described for the  $C_1V_{23}$  strain, formed much smaller dot-like plaques (Fig. 2) and much fewer in number:  $10^3$  on  $10^7$  phage particles employed. These results seem to indicate that, keeping the plating conditions constant, the plaque size and its morphology are characteristic of the specific host-phage system and that the host and not the phage is the more active determinant of the plaque size. They also agree with the results obtained by St. Clair and McCoy<sup>4</sup> for other actinophages and by Thiernann *et al.*<sup>5</sup> for phages active on *Streptomyces mediterranei*.

Resistant colonies were easily obtained by plating aliquots of the lysed culture broths. The criteria adopted to test the resistance of the isolated colonies were those of van Alstyne *et al.*<sup>6</sup>. In flask fermentation, the novobiocin-producing capacity of the resistant colonies was of 600–700 µg/ml. (microbial titre), practically the same as that of the sensitive parent strain.

The host-range of phage A has been determined, in a preliminary way, using the following strains of the genera *Streptomyces* and *Nocardia*: *S. griseus* 'NRRL B-150', *S. griseus* 'Waksman WC 3475', *S. lavendulae* 'ATCC 8664', *S. fradiae* 'NRRL B-1195', *S. erythraeus* 'C.B.S.', *S. viridifaciens* 'ATCC 11989', *S. antibioticus* 'Ist. Genetica, Università di Pavia—Italy', *S. diastaticus* 'ATCC 3315', *S. aureofaciens* Duggar 'NRRL 2209', *S. psammoticus* 'C.B.S. 17561', *S. hygroscopicus* 'NRRL B-1865', *S. rimosus* 'NRRL 2234', *S. niveus* 'NRRL 2466', *S. sphaeroides* 'NRRL 2449', *N. blackwellii* 'C.B.S.', *N. asteroides* 'C.B.S.', *N. erithropolis* 'C.B.S.', *N. polychromogenes* 'C.B.S.', *N. brasiliensis* 'C.B.S.', *N. tenuis* 'C.B.S.', and *N. lutea* 'C.B.S.'.

The test has been performed with the double-layer technique of Gratia and with the drops method described by Weindling *et al.*<sup>7</sup>. Under these conditions, all species and strains of *Streptomyces* and *Nocardia* tested, with the exception of *S. sphaeroides* 'NRRL 2449', have proved to be resistant to phage A. The host-range will be further investigated, using other species of *Streptomyces*, in order to define it more completely.

Phage A proved to be Ca-dependent since the addition to the broth of the Ca-sequesterant agent EDTA inhibited the lysis of the mycelium almost completely. EDTA, at concentrations inhibiting the phage lysis, was shown to be partly toxic to the mycelium while no toxic effect on the phage was observed at the same concentrations.

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## Pure Culture of *Anabaena flos-aquae* A-37

SEVERAL methods have been proposed for the isolation of bacteria-free cultures of algae<sup>1-6</sup>. Methods reported to provide some measure of success, using antibiotics, ultra-violet light, chlorine compounds, etc., are fairly drastic and, in some cases, mutagenic. The problem of separating algae from bacteria may be related to a binomial distribution function in which the probability of choosing a bacteria-free algal colony in any trial varies in an unknown fashion from one trial to another. Successful, practical use of the proposed method may be expected only when the chance of choosing a bacteria-free algal colony exceeds about one in twenty.

Since the skill of the operator doubtless contributes to success, the method succeeds in proportion to the amount of operator bias employed in choosing uncontaminated colonies. This method has as its aim the separation of algae from the contaminating bacteria by the simple techniques of 'positive operator bias' coupled with numerous replication.

Accordingly, the method consists of (a) creating artificial predominance of the chosen alga by accumulation with a micropipette, (b) culture of the accumulated algal cells in liquid HGZ medium, a *tris*-buffered modification of the medium employed by Hughes, Gorham and Zehnder<sup>7</sup>, (c) culture on HGZ agar dilution plates, (d) repetition of (b) and (c) alternately to obtain uni-algal cultures and to show which is the highest dilution in which algal colonies grow well separated and in small numbers, (e) preparation of 20 replicate plates of the plate dilution which affords good colony separation, (f) daily microscopic observation of plates from which approximately 20 sub-cultures are made into liquid HGZ, (g) after incubation and growth, inoculation of 1 ml. of the HGZ liquid cultures into nutrient broth. Observation at 48 h indicates the presence or absence of bacteria.

All the algae employed have been isolated from either pond water or sewage, using micropipettes to collect several units of a species for culture. Isolates were cultured alternately in agar dilution plates and in liquid medium to produce uni-algal cultures. Cultures were incubated in the light at 40° C on HGZ. Serial dilutions of isolates in HGZ agar were observed at least once each 24 h. A low-power, wide-field microscope was employed, using magnifications of the order of 90×.

It was soon found that several replatings reduced the apparent number of types of bacterial contaminants which would grow on HGZ medium from as many as 10 to one or two types. These plating trials also indicated the limits of dilution for any given culture, beyond which growth of the alga could not be expected with useful frequency. It also suggested that, contrary to the opinions of some research workers, the bacteria often outnumber the algae by more than ten to one in what might appear to be a fairly 'clean' culture of algae grown in mineral salts medium.

Having determined the limiting dilution for the growth of a culture of algae, 20 poured plates were prepared at the limiting dilution less one. Growth was meticulously observed daily, using the wide-field microscope, to find the algal colonies growing at maximal distances from each other and from bacterial colonies. All the colonies which appeared to afford good separation from the surrounding bacteria were cut out of the agar with an inoculating needle or a micro-scalpel and placed in liquid HGZ medium for incubation. If possible, 20 cultures were made. As soon as growth was observed, sub-cultures were made in nutrient broth. Absence of growth in nutrient broth after 48 h of incubation was accepted as proof of the absence of bacteria.

When this procedure was employed, *Anabaena flos-aquae* A-37, which was isolated in this laboratory from an oxidation pond located in Mississippi, grew through the  $10^7$  dilution. Of 16 sub-cultures on liquid HGZ, 11, or almost

70 per cent, were free of bacteria as judged by sub-culture on nutrient broth. Frequent sub-culture of these bacteria-free isolates continues to prove the absence of bacteria which will grow either on HGZ medium or in nutrient broth.

While it is admittedly impossible to provide complete proof of the purity of any algal culture from all bacteria, the 'positive operator bias' technique appears to increase the chances of success by at least one order of magnitude without engendering serious metabolic disturbance. It has been employed successfully in this laboratory, to provide bacteria-free cultures of two *Anabaena*, two *Polycystis*, two brown *Oscillatoria* and four green *Oscillatoria*. It is being used routinely in this laboratory for the bacteria-free isolation of other types of algae which may be of interest. This work was done in co-operation with the National Aeronautics and Space Administration.

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### Inhibition of Growth of *Aerobacter aerogenes* by Sodium Chloride

SODIUM chloride is usually added to a culture medium to make the medium isotonic, so that any blood added as an enrichment is not haemolysed. It is conceivable that any specific requirements a non-marine bacterium might possess for the Na<sup>+</sup> or the Cl<sup>-</sup> ion is so small it can be supplied by the culture medium itself. Thus, the addition of sodium chloride, at a concentration of 0.5 per cent (w/v), to any medium intended for the growth of non-marine bacteria seems arbitrary unless it has previously been shown to be necessary to promote optimal growth or to demonstrate some particular metabolic activity (for example, haemolysis).

Certain bacteria can tolerate relatively high concentrations of sodium chloride. For such salt-tolerant bacteria the term 'halophilic' has been adopted. This characteristic is not an expression of a specific requirement for sodium chloride, but of an acquired ability to tolerate high salt concentrations. Marine bacteria, particularly *Halobacterium* species, require a concentration of 1.0 per cent (w/v) of sodium chloride before growth can take place in a liquid medium<sup>1</sup>. Agar-liquefying pseudomonads do not exhibit agarase activity until 2.4 per cent (w/v) sodium chloride is present in a nutrient agar<sup>2</sup>.

Few reports of the 'inhibitory effects' of 0.5 per cent (w/v) sodium chloride appear to have been made. Indeed, the widespread use of this concentration of this electrolyte would lead one to believe that its inclusion in a medium is essential or even beneficial to the growth of bacteria. During some growth experiments on *Proteus*<sup>3</sup> (in a medium containing 0.5 per cent (w/v) peptone 0.3 per cent (w/v) 'Lab-Lemco' meat extract), it was noted that *Aerobacter aerogenes* produced less growth when 0.5 per cent (w/v) sodium chloride was added to the medium than when grown in the absence of sodium chloride. As a result of this it was decided to examine the effect more closely. The methods and experimental techniques used were those described previously<sup>3</sup>.

From the growth curve (Fig. 1) over a 24-h incubation period at 37° C it will be seen that sodium chloride exerts a growth depressant effect on *A. aerogenes* N.C.T.C.

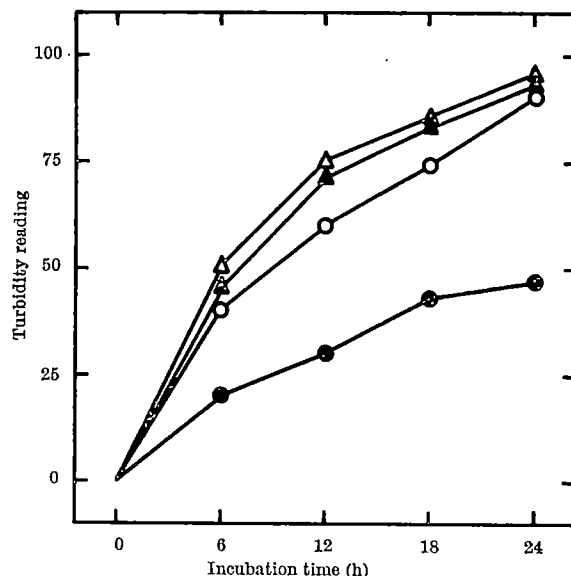


Fig. 1. Growth curves of *E. coli* N.C.T.C. 4144 in broth (Δ) and broth plus sodium chloride (▲) compared with growth curves of *A. aerogenes* N.C.T.C. 8172 in broth (○) and broth plus sodium chloride (●)

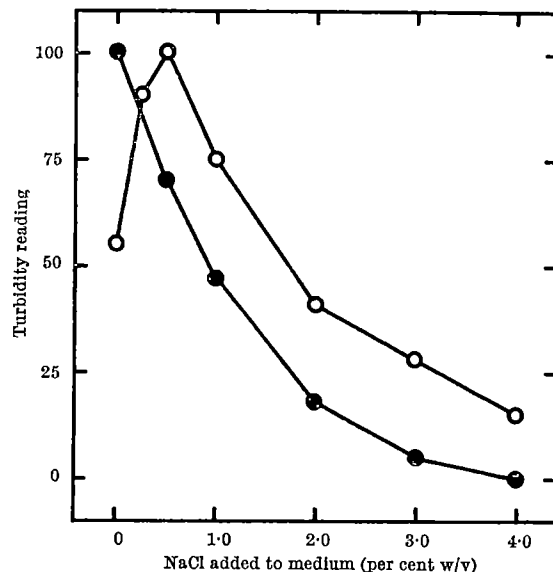


Fig. 2. Growth of *A. aerogenes* N.C.T.C. 8172 (●) and *Proteus vulgaris* 7149 (○) at 37° C, over a range of sodium chloride concentrations

8172 over the entire incubation period. In contrast to this the growth of *E. coli* N.C.T.C. 4144 is unaffected by the presence of sodium chloride. The effect of variations in sodium chloride concentrations on *A. aerogenes* N.C.T.C. 8172 and *Proteus vulgaris* 7149 is shown in Fig. 2. At no concentration of sodium chloride is the growth of *A. aerogenes* greater than growth obtained in the absence of sodium chloride whereas *Proteus* gives a peak of growth at a concentration of 0.5 per cent sodium chloride.

The apparent growth inhibition of *A. aerogenes* by sodium chloride may be due to membrane instability associated with cation dependency<sup>1</sup>. Wyatt<sup>4,5</sup> has postulated that the inhibition of growth of staphylococci by cations could be a function of the transport of certain cations which antagonize certain metabolic pathways, for example, pyruvate phosphokinase is antagonized by sodium or calcium ions. Mechanisms of this sort could play a part in the phenomenon described here. Whatever the underlying cause of this effect it would appear that sodium chloride has an inhibitory effect on *A. aerogenes*. This effect should be borne in mind when carrying out

cultural procedures with this organism. Furthermore, such an effect makes one question the validity for the use of sodium chloride at its 'classical' concentration.

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## VIROLOGY

### Role of Temperature and Light in Lesion Development of Tobacco Mosaic Virus

THE optimum post-inoculation temperature for the development of macroscopic lesions of the common strain of tobacco mosaic virus (TMV) on *Phaseolus vulgaris* L. var. 'Pinto' is about 31° C, whereas for the sweet potato strain it is 16° C or lower<sup>1</sup>. This report describes the effects of post-inoculation temperatures of 20°, 25° and 30° C, and light intensities of 640 ft.-candles and 4,000 ft.-candles on the development of macroscopic lesions and the total number of detectable lesions (starch lesions)<sup>2</sup> by three further strains of TMV: 'U<sub>1</sub>', which is an example of the common strain of TMV and is well known to form lesions on Pinto bean leaves, 'U<sub>2</sub>' (ref. 3), which is reported

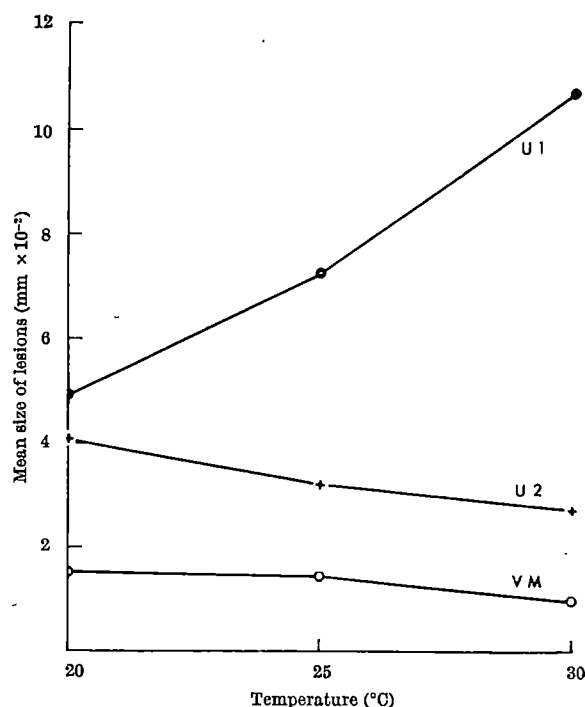


Fig. 2. Effect of temperature and high light intensity (4,000 ft.-candles) on size of lesions (starch lesions) formed by strains of TMV on Pinto bean leaves. Mean data for one experiment. Numbers of lesions measured at 20°, 25° and 30° C were: 168, 283 and 218 for U<sub>1</sub>; 354, 379 and 168 for U<sub>2</sub>; 265, 256 and 115 for VM.

to form lesions on this host by some authors<sup>2,4,5</sup>, but not by others<sup>3-9</sup>, and 'VM' (ref. 10), which previously was not known to form lesions on this host<sup>8,9</sup>.

The Pinto bean seed was obtained from the Haney Seed Co., Twin Falls, Idaho. It was grown in Ceres, the Canberra phytotron, in vermiculite watered once daily with Hoagland's nutrient solution and once with demineralized water. In the first set of experiments, the seeds were germinated (during the winter) in naturally lit bays maintained at a day temperature of 27° and a night temperature of 22° C. Six days after sowing the seedlings were put in artificially lit cabinets at 25° C with an 8-h photoperiod of 4,000 ft.-candles (fluorescent and incandescent light). Three days later they received a pre-inoculation dark period of 24 h followed by 1.5-2 h light at 4,000 ft.-candles before inoculation. There were 10 plants for each treatment in each experiment. U<sub>1</sub> was applied at 0.4 µg/ml., and U<sub>2</sub> and VM at 2.5 or 10 µg/ml. The primary leaves were inoculated with a brush using 0.1 M phosphate buffer at pH 7.0 and 5 per cent 'Celite'. After inoculation, the plants were placed for 72 h in cabinets run at 20°, 25° or 30° C with continuous light of 4,000 ft.-candles. This was followed by 24 h darkness at the same temperatures. The inoculated leaves were then gathered and stored in the dark at 25° C for an additional 24 h to remove excess starch before lesions were counted and measured on leaf disks sampled from leaves<sup>3</sup>. Data for the numbers and size of lesions in each treatment are shown in Figs. 1 and 2, respectively. With U<sub>1</sub>, the number of both classes of lesions increased with increase in temperature; with U<sub>2</sub> and VM the number of macroscopic lesions decreased with increase in temperature, whereas the number of starch lesions reached a maximum at 25° C. Lesions of U<sub>1</sub> were larger than those of U<sub>2</sub>, which in turn were larger than those of VM. For each strain, the patterns for size of lesions in relation to temperature showed similar trends to those for numbers of macroscopic lesions.

In a further set of experiments, inoculated plants were incubated at the same temperatures but with a light intensity of 640 ft.-candles. The pre-inoculation environ-

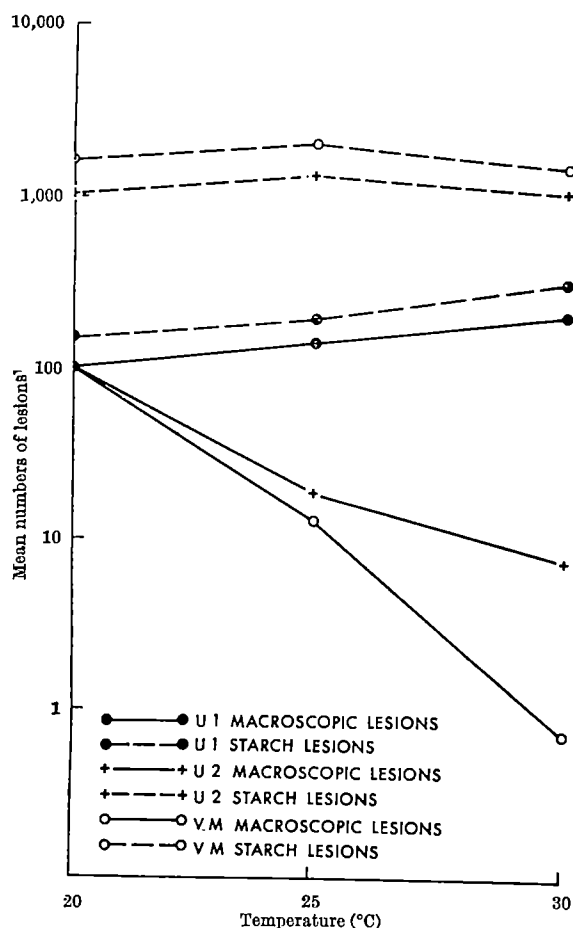


Fig. 1. Effect of temperature and high light intensity (4,000 ft.-candles) on numbers of lesions formed by strains of TMV on Pinto bean leaves. Mean data for 3 experiments. For each treatment of each experiment lesions were counted on two 18 mm leaf disks sampled from each of 20 leaves. For each strain, numbers of macroscopic and starch lesions at 25° C and 30° C were adjusted relative to the number of macroscopic lesions at 20° C, 100. Numbers of lesions were then adjusted for variation of leaf area with temperature.



## GENETICS

## Antimutagenic Effect against Colchicine of Kinetin in Black Currant Seedlings

It is generally known that the juvenility of plant organs can be prolonged by treatment with kinetin; moreover, senescent tissues can be rejuvenated<sup>1</sup>, and several authors have shown increased mutability of older tissues<sup>2</sup>. Treatment of plant leaves by kinetin appreciably inhibits the multiplication of viruses<sup>3</sup>, although the virus contains RNA. After incorporation into the host cells, the virus may cause information disturbances by inducing the synthesis of foreign RNA. In this interpretation, the changes brought about by the virus are in the nature of mutations.

On this basis we have supposed that kinetin has an antimutagenic activity, or, in other words, that it takes part in the protection of the information-system controlling heredity. In order to test this idea, we attempted to neutralize the genome mutation-inducing effect of colchicine by pre-treatment of the plant with kinetin.

We chose black currant ('Altayskaya Desertnaya' × 'Boskoop Giant' *F*<sub>1</sub> seedlings) as a test plant. The treatment of the shoots of the seedlings by kinetin (50 p.p.m. or by kinetin (50 p.p.m.) + colchicine (0.8 per cent) was started at the 3-4 leaf age in the greenhouse, and was continued for 24 days, repeating the treatment daily. Following this, a solution of colchicine (0.8 per cent) and gibberellic acid (GA; 50 p.p.m.) was applied to the shoot of the plants for a period of 21 days, twice a day using the 'cotton cup' method. Colchicine was added to gibberellic acid because in earlier experiments we obtained a much higher percentage of genome mutation by applying this technique than by the use of the classical colchicine treatment<sup>4</sup>. Evaluation was made by the 'squash' technique, using fixed shoots 5 days after finishing the treatment.

Table 1. ANTAGONIZING EFFECT OF PRETREATMENT BY KINETIN ON THE GENOME MUTATION OF BLACK CURRANT SEEDLINGS INDUCED BY COLCHICINE

Pretreatment	Mutagenic treatment	No. plants	Mixoploids No.	%
—	Colchicine + GA	44	12	27.3
Kinetin	Colchicine + GA	55	0	0.0
Kinetin + colchicine	Colchicine + GA	48	13	27.0

The pre-treatment with kinetin provided a complete protection against the mutagenic treatment, without influencing the growth of the seedlings. It seems, however, that a longer time is needed for the realization of the stabilizing effect of kinetin, since colchicine applied together with kinetin—as a pre-treatment—has inhibited the stabilization.

In the past, a number of publications have reported the natural occurrence of kinetin-like substances<sup>5</sup>. Thus, it is possible that these substances play a part in stabilizing the transfer of genetic information. This view is supported by the results of Matthews<sup>6</sup>, who has shown the presence of RNA-attached purine derivatives and attributed a stabilizing role to these compounds.

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mental conditions were unchanged except that the plants were grown (during the summer) under net shades with natural lighting, until 24 h before inoculation when they were placed in the dark at 25° C; the plants then received a light intensity of less than 10 ft.-candles for 1.5 h before they were inoculated. Lesions were counted immediately after leaves were gathered. For U<sub>1</sub> and U<sub>2</sub>, the patterns for the numbers of macroscopic and starch lesions which developed at 20°, 25° and 30° C and 640 ft.-candles (Table 1) resembled those at 4,000 ft.-candles (Fig. 1); for VM, no macroscopic lesions were formed and starch lesions were not identified consistently. In these experiments lesions of all strains were smaller and contained less starch than those in the previous experiments. This made lesions of U<sub>2</sub> and VM more difficult to identify than in the previous experiments, especially when the inoculated plants were incubated at 30° C. In a previous paper, it was shown that the ratio of the numbers of macroscopic lesions to the numbers of starch lesions could be used as a measure of lesion size<sup>2</sup>. In the present experiments, in plants inoculated with U<sub>1</sub> and U<sub>2</sub> and incubated at 25° C and 640 ft.-candles, this ratio was 36.0 per cent and 0.2 per cent, respectively, whereas in plants incubated at 25° C and 4,000 ft.-candles it was 68.4 per cent and 4.7 per cent respectively. This difference between the size of lesions in the two sets of experiments is considered to be due primarily to the light intensity used for the incubation of inoculated plants. It may be due in part to the light intensity the plants received immediately before inoculation.

Table 1. EFFECT OF TEMPERATURE AND LOW LIGHT INTENSITY (640 FT.-CANDLES) ON NUMBERS OF LESIONS FORMED BY STRAINS OF TMV ON PINTO BEAN LEAVES\*

Virus strain	Class of lesions	Temperature of incubation (°C)		
		20	25	30
U <sub>1</sub> †	Macroscopic	100	239	327
	Starch	263	699	747
U <sub>2</sub> ‡	Macroscopic	100	2	0
	Starch	693	1,160	995
VM‡	Macroscopic	0	0	0
	Starch	§	§	§

\* Numbers of lesions were adjusted in the same way as those in Fig. 1.

† Mean data for 2 experiments.

‡ Mean data for 3 experiments.

§ Occasional lesions recognized.

The experiments show that the patterns of number and size of lesions, in relation to post-inoculation temperature, vary with different virus strains and that the size of lesions is affected by light intensity. In several investigations made to examine the phenomena of interference and heat activation, U<sub>2</sub> and VM were not observed to form lesions on Pinto bean plants given environmental conditions, suitable for the development of lesions of U<sub>1</sub> (refs. 6-9). The temperature used for the incubation of inoculated plants was 30° C and the light intensity was 400 or 500 ft.-candles. The present results indicate that although these conditions are favourable for the development and identification of lesions of U<sub>1</sub>, they are relatively unfavourable for the development and identification of lesions of U<sub>2</sub> and VM.

I thank Mrs. E. Valtas for her assistance.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, January 25

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E./I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 10.30 a.m.—Colloquium on "Logic Circuits".

UNIVERSITY COLLEGE, LONDON (in the Botany Theatre, Gower Street, London, W.C.1), at 5 p.m.—Dr. P. N. Magee: "The Biochemical Pathology of Intracellular Alkylating Reactions".\*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. Hyden (Göteborg): "Molecular Biology Studies in the Nervous System".\*

UNIVERSITY OF LONDON (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. D. H. Hubel (Harvard Medical School): "The Eye, The Brain and Perception" (further lectures on January 27 and 29).\*

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—discussion on "The Problem of Obtaining Training Places for Six-Month Sandwich Course Students".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. J. Hardwood: "Flame Retardant Processes".

## Tuesday, January 26

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Short Paper Meeting.

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (Joint I.E.E./I.E.R.E. meeting at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 3 p.m.—Colloquium on "The Achievement of High Accuracy in Analogue to Digital Conversion".

UNIVERSITY OF LONDON (in the New Lecture Theatre, Wye College, near Sharnford, Kent), at 4.30 p.m.—Dr. G. A. Pitt: "Recent Advances in the Physiology of Vitamin A".\*

LONDON SCHOOL OF ECONOMICS AND POLITICAL SCIENCE (at Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Prof. P. J. O. Self: "Bureaucracy or Management?".\*

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Mr. L. J. Lawler: "The British Contribution to Educational Television in the Commonwealth" (Sir Thomas Holland Memorial Lecture).

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, W.1), at 5.30 p.m.—Mr. E. R. Bryan and Mr. W. M. El-Dakhkhni: "The behaviour of Sheeted Portal Frame Sheds. Part 1—Theory. Part 2—Experiments".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. N. J. Felici: "Electrostatic Generators".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. King: "Electrons in Solids". (Afternoon lecture for Sixth Form boys and Girls in Schools from London and the Home Counties. To be repeated on January 27, February 2 and 3.)

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 3.30 p.m.—Prof. Victor Gold: "Analysing Chemistry" (Inaugural Lecture).\*

UNIVERSITY OF LONDON (at the Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. Stanley Gill: "Automatic Computing; its Problems and Prizes" (Inaugural Lecture).\*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. Goldberg: "New Outlook in Toxicology". (Fourth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. W. Abrahamson (Case Institute of Technology, Cleveland): "The Macromolecular Aspects of Vision".\*

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—discussion on "Running-In of Diesel Engines".

## Wednesday, January 27

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. A. J. Grimes: "Metabolism of Red Cells".\*

FAUNA PRESERVATION SOCIETY (at the Offices of the Zoological Society of London, Regent's Park, London, N.W.1), at 5.30 p.m.—Dr. E. J. H. Corner, F.R.S.: "The Mt. Kinabalu National Park in North Borneo".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Protection Problems associated with Marine Electrical Systems" opened by Dr. J. R. Mortlock and Mr. W. C. Robertson.

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Mr. R. Gambell: "Current Research upon Whales at Durban".

ASSOCIATION OF THE WILLIAM PENGELLY CAVE RESEARCH CENTRE, in conjunction with Imperial College Caving Club (in the Physics Department, Imperial College, London, S.W.7), at 6 p.m.—Dr. Trevor Ford: "The Fossil Earst in Derbyshire".\*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at Bedford Square, London, W.C.1), at 6 p.m.—Symposium on "Enhancement and Absorption of Radar Radiation".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Vice-Admiral R. S. Hawkins: "Progress in Naval Machinery During the Last Thirty Years" (Thomas Lowe Gray Lecture).

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. A. S. Basford and Mr. S. S. Brown: "Automatic Vending".

ROYAL AERONAUTICAL SOCIETY, GRADUATES' AND STUDENTS' SECTION (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Mr. S. R. Hughes: "Hovercraft".

## Thursday, January 28—Friday, January 29

CHALLENGER SOCIETY, AND REPRESENTATIVES FROM THE MARINE LABORATORIES (Development Commissioners' Scheme) (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N.W.1), at 10.15 a.m. daily—Symposium on "The Movement of Sediments on the Sea Floor and Their Significance to Benthic Animal Communities".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m. daily—Discussion Meeting on "Recent Advances in the Technique of Seismic Recording and Analysis" organized by Sir Edward Bullard, F.R.S., and Sir William Penney, F.R.S.

## Thursday, January 28

INSTITUTION OF AGRICULTURAL ENGINEERS (at the Institution of Mechanical Engineers, 1 Birdcage Walk, London, S.W.1), at 10.15 a.m.—Mr. J. B. Holt: "The Handling of Unit Loads in Agriculture and Horticulture"; Mr. R. D. Hall: "Work Study in the Mechanization of Farming"; Mr. H. C. Green: "The Susceptibility of Fruit and Potatoes to Damage during Handling".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Standard Methods of Measurement of Civil Engineering Work" introduced by Mr. A. Goldstein.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. Noble: "Biophysics of Cardiac Muscle". (Fifth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

UNIVERSITY OF LONDON (at the School of Pharmacy, 29-39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. F. Sandberg (Stockholm): "The Effect of Various Drugs on the Human Uterus".\*

## Friday, January 29

ROYAL AERONAUTICAL SOCIETY, ROTOCRAFT SECTION (at 4 Hamilton Place, London, W.1), at 2.15 p.m.—Dr. I. C. Cheeseman: "Powered Lift Applications in the Commonwealth"; Mr. W. A. Hibbert: "Helicopter Trials on Sand and Sea".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. G. F. Tagg: "Measurement of Earth Electrode Resistance with particular reference to Earth Electrode Systems covering a Large Area".

UNIVERSITY OF LONDON (at the School of Pharmacy, 29-39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. F. Sandberg (Stockholm): "Arrow Poisons—Their Origin, Chemistry and Action".\*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. R. L. Gregory: "Seeing in Depth".

## Saturday, January 30

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Norman Cook: "Unearthing London's History".\*

## Monday, February 1

SOCIETY OF ENGINEERS (in the Apartments of the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Inaugural Meeting for the year 1965. Mr. C. L. N. Laing: Presidential Address.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Members' Discussion of "The Society's Publications". Discussion Leader: Prof. W. G. Overend.

INSTITUT FRANCAIS DU ROYAUME-UNI (at Queensberry Place, South Kensington, London, S.W.7), at 8.15 p.m.—French Scientific Films.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SCIENTIFIC LIAISON OFFICER (with a university degree in science) at Long Ashton Research Station, to be responsible for organizing public functions, making arrangements for receiving visiting scientists and for editing the Station's Annual Report—The Registrar, University of Bristol, Senate House, Bristol, 2 (January 30).

SENIOR LECTURER, a LECTURER and an ASSISTANT LECTURER in PURE MATHEMATICS—The Secretary, The University, Aberdeen (January 30).

LECTURER/ASSISTANT LECTURER IN PHILOSOPHY—The Principal, St. David's College, Lampeter, Cardiganshire (January 31).

ASSISTANT LECTURER IN HISTORICAL GEOGRAPHY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (February 1).

LECTURER or ASSISTANT LECTURER (with an honours degree in engineering or physics and experience in industrial acoustics) in INDUSTRIAL ACOUSTICS—The Deputy Secretary, The University, Southampton (February 1).

ASSISTANT LECTURER IN PSYCHOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (February 5).

LECTURER or an ASSISTANT LECTURER IN STATISTICS—The Registrar (Room 22, O.R.B.), The University, Reading (February 5).

LECTURER or an ASSISTANT LECTURER (with qualifications in pure mathematics) in MATHEMATICS—The Registrar, The University, Keele, Staffs (February 5).

LECTURER or SENIOR LECTURER IN CLINICAL EPIDEMIOLOGY IN THE DEPARTMENT OF INFECTIOUS DISEASES at Ruchill Hospital—The Secretary of the University Court, The University, Glasgow (February 5).

LECTURERS (2) (mathematicians) IN THE DEPARTMENT OF THEORETICAL MECHANICS (Faculty of Engineering)—The Registrar, University of Bristol, The Senate House, Bristol, 2 (February 5).

ASSISTANT IN AGRICULTURAL BOTANY—The Secretary of the University Court, The University, Glasgow (February 6).

LECTURERS or ASSISTANT LECTURERS (2) (graduates with an honours degree in chemistry, biochemistry or agricultural chemistry) IN THE DEPARTMENT OF AGRICULTURAL SCIENCES—The Registrar, The University, Nottingham (February 6).

PROFESSOR OF MATERIALS SCIENCE—The Registrar, Ref. 72Y/X, Bradford Institute of Technology, Bradford, 7 (February 8).

READER IN STATISTICS—The Secretary, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (February 9).

ASSISTANT LECTURER IN THE DEPARTMENT OF AGRICULTURAL BOTANY—The Registrar, University College of North Wales, Bangor, North Wales (February 10).

ASSISTANT LECTURER (with qualifications in any branch of genetics) IN GENETICS—The Registrar, The University, Sheffield (February 13).

CHAIR OF THEORETICAL MECHANICS AND HEAD OF THE DEPARTMENT—The Registrar, The University, Nottingham (February 15).

LECTURER (preferably with an interest in X-ray techniques or geochemistry or metamorphic petrology) IN GEOLOGY IN GEOLOGY (Room 22, O.R.B.), The University, Reading (February 16).

LECTURER or ASSISTANT LECTURER (with qualifications in plant physiology or in mycology with a physiological background) IN BOTANY; and a LECTURER or ASSISTANT LECTURER (qualified in the field of general zoology) IN ZOOLOGY at the University of Basutoland, Bechuanaland Protectorate and Swaziland—The Secretary Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (February 20).

LECTURER (preferably with experience in the field of fisheries biology, either freshwater or marine, or of experimental zoology) IN THE DEPARTMENT OF ZOOLOGY, University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (February 24).

LECTURER (preferably with qualifications in structural geology, geophysics and/or economic geology) IN THE DEPARTMENT OF GEOLOGY, University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (February 24).

SENIOR LECTURER and a LECTURER IN THE DEPARTMENT OF PHYSICS, University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (February 24).

LECTURER or an ASSISTANT LECTURER IN THE DEPARTMENT OF GEOGRAPHY, University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (February 27).

LECTURER or ASSISTANT LECTURER (preferably with industrial experience and research interests in the field of aircraft propulsion) IN AERONAUTICAL PROPULSION—The Deputy Secretary, The University, Southampton (February 27).

LECTURERS (2) (with special interests in either soil mechanics or municipal engineering) IN CIVIL ENGINEERING—The Deputy Secretary, The University, Southampton (February 27).

CHAIR OF STATISTICS WITHIN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Nottingham (March 1).

JUNIOR RESEARCH ASSOCIATE IN THE DEPARTMENT OF BOTANY to work on the metabolism and enzymology of aromatic ring fusion in moulds and yeasts—The Registrar, The University, Newcastle upon Tyne, 1 (March 1).

LECTURER/SENIOR LECTURER IN BIOLOGY (in the field of Statistical Plant Ecology) IN THE SCHOOL OF BIOLOGICAL SCIENCES, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, March 1).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS (preferably with a Ph.D. or equivalent research experience, and below the age of 30) IN BIOCHEMISTRY, CHEMISTRY, ENGINEERING, GEOLOGY, or PHYSICS—The Registrar, The University, Leicester (March 6).

ENTOMOLOGIST (national of the United Kingdom or the Republic of Ireland, with a degree in biology and at least two years' postgraduate research) with the East African Common Services Organization, to study ways for improving methods of controlling or eradicating tsetse flies by insecticides and chemical sterilants including both laboratory and field studies—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/214/07.

FISHERIES OFFICER (national of the United Kingdom or the Republic of Ireland, with a good science degree and experience of fisheries management, including marketing and processing) in Malawi, to collect statistics and develop and demonstrate to local fishermen improved methods of fishing appropriate to local conditions—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 289/134/01.

HEAD OF THE MATHEMATICS DEPARTMENT—Grade IV—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

JUNIOR RESEARCH ASSOCIATE (graduate with suitable qualifications) for research on heat and water transfer over cold surfaces—The Registrar, The University, Newcastle upon Tyne, 1.

LECTURER (colloid chemist, preferably with experience of rubber latex and/or synthetic dispersions and emulsions); and a LECTURER (polymer technologist with a knowledge of the chemistry and applications of rubbers and/or thermoplastics)—Clerk to the Board of Governors, National College of Rubber Technology, Northern Polytechnic, Holloway, London, N.7.

LECTURER IN THE CRIPS COMPUTING CENTRE—The Registrar, The University, Nottingham.

RESEARCH ASSISTANT (preferably with a knowledge of Spanish) IN THE GEOGRAPHICAL RESEARCH DIVISION, for work in the field of economic geography of Latin America with special reference to energy—Dr. J. E. Martin, London School of Economics and Political Science, Houghton Street, Aldwych, London, W.C.2.

RESEARCH ASSISTANT (with a Ph.D. or equivalent research experience and some knowledge of electronics) for group centred at Silwood Park, near Ascot, working on rocket measurements of magnetic fields in the ionosphere, currently concerned with Centaur and Skylark rockets launched from Woomera and Andoya Is.—The Geophysics Department, Imperial College, London, S.W.7.

STATE FOREST OFFICER (male, not over 45, with an honours degree in forestry plus at least five years' tropical experience) in Brunei, to be in charge of the Forestry Department, be responsible for the enforcement of the Forest Enactment and rules designed to protect the forests, manage and

develop forests, train field staff and give general advice on the proper utilization of timber—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 324/28/01.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

The Nuffield Foundation Science Teaching Project. Progress Report, October 1964. Pp. 31. (London: Longmans, Green and Co., Ltd.; and Penguin Books, 1964. Published for the Nuffield Foundation.) [2511]

British Antarctic Survey. Scientific Reports, No. 45: The Vascular Flora of South Georgia. By Dr. S. W. Greene. Pp. 58+31 maps+6 plates. (London: British Antarctic Survey, 1964.) 32s. net. [2511]

Royal Holloway College. Descriptive Brochure. Pp. 24. (Englefield Green: Royal Holloway College, 1964.) [2511]

The Association of Commonwealth Universities. Annual Report of the Council, together with the Accounts of the Association for the year 1st August 1963 to 31st July 1964. Pp. 46. (London: The Association of Commonwealth Universities, 1964.) [2511]

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1075, Vol. 256 (12 November 1964): The Volcanological Report of the Royal Society Expedition to Tristan da Cunha, 1962. By P. E. Baker, I. G. Cass, P. G. Harris and R. W. Le Maitre. With appendixes by M. W. Holdgate, J. H. Dickson, D. B. Baird, J. A. Miller and K. M. Creer. Pp. 439-578+plates 16-33. 90s.; 13.50 dollars. Series B: Biological Sciences. No. 742, Vol. 247 (19 November 1964): The Cranial Anatomy of Two Coal Measure Anthracosaurs. By A. L. Panchen. Pp. 593-637+plate 10. 20s.; 3 dollars. (London: The Royal Society, 1964.) [2511]

### Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1194-B: The Hovey Group of Northeastern Maine. By Louis Pavlides. Pp. iii+6. Bulletin 1194-C: The Putnam Group of Eastern Connecticut. By H. Roberta Dixon. Pp. iii+12. Water-Supply Paper 1586-F: On Determining Pollutant Distribution in Tidal Estuaries. By E. E. Pyatt. Pp. iv+56+plates 1 and 2. Water-Supply Paper 1613-D: Relation of Salt-Water Encroachment to the Major Aquifer Zones, Savannah Area, Georgia and South Carolina. By M. J. McCollum and H. B. Counts. Pp. iv+26+plates 1-4. Water-Supply Paper 1698: Ground-Water Resources and Geology of Northern and Western Crook County, Wyoming. By Harold A. Whitcomb and Donald A. Morris. Pp. v+92+plates 1 and 2. Water-Supply Paper 1735: Compilation of Records of Surface Waters of the United States, October 1950 to September 1960. Part 2: Pacific Slope Basins in California. Prepared under the direction of E. L. Hendricks. Pp. xvi+715+plate 1. Water-Supply Paper 1748: Apparatus and Techniques for Measuring Bedload. By D. W. Hubbell. Pp. v+74. Water-Supply Paper 1753: Geology and Ground-Water Resources of the Los Alamos Area, New Mexico. By Roy Griggs. With a section on Quality of Water by John D. Hem. Pp. v+107+plate 1. (Washington, D.C.: Government Printing Office, 1964.) [2611]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1779-H: Ground Water in Cedar Rapids Division of Lower Plate River Basin, Nebraska. By James B. Hyland and Charles F. Keech. With a section on Chemical Quality of the Water by Philip G. Rosene. Pp. iii+12+plates 1-3. Water-Supply Paper 1779-O: Hydrology of Aquifer Systems in the Memphis Area, Tennessee. By J. H. Criner, P.-C. P. Sun and D. J. Nyman. Pp. v+54+plates 1-7. Water-Supply Paper 1779-V: Ground Water Resources of Eastern Arkansas in the Vicinity of U.S. Highway 70. By H. N. Halberg and J. E. Reed. Pp. iv+38+plates 1-4. Water-Supply Paper 1783: Hydrologic Conditions in the Wheatland Flats Area, Platte County, Wyoming. By Edwin P. Weeks. Pp. v+79+plates 1-8. (Washington, D.C.: Government Printing Office, 1964.) [2611]

United States Department of the Interior: Geological Survey. Professional Paper 383-B: Geologic Factors That Control the Occurrence and Availability of Ground Water in the Fort Rock Basin, Lake County, Oregon. By E. R. Hampton. Pp. iii+29+plate 1. Professional Paper 411-H: Mathematics of Dispersion With Linear Adsorption Isotherm. By Akio Ogata. Pp. iii+9. Professional Paper 454-M: Bedrock Valleys of the New England Coast as Related to Fluctuations of Sea Level. By Joseph E. Upson and Charles W. Spencer. Pp. iv+44+plates 1-5. Professional Paper 458-A: Regional Geology of the Steamboat Spring Area, Washoe County, Nevada. By G. A. Thompson and D. E. White. Pp. iv+52+plates 1 and 2. (Washington, D.C.: Government Printing Office, 1964.) [2611]

Smithsonian Miscellaneous Collections. Vol. 146, No. 5: Some Behavior Patterns of Platyrrhine Monkeys. 1: The Night Monkey (*Aotus trivirgatus*). By M. Moynihan. Pp. 84. (Publication 4533.) Vol. 146, No. 7: A New Species of Marine Pennate Diatom from Honolulu Harbor. By Paul S. Conger. Pp. 5. (Publication 4593.) Washington, D.C.: Smithsonian Institution, 1964.) [2611]

The Smithsonian Institution. Masters of Space. By Philip S. Hopkins. Pp. 32. (Washington, D.C.: Smithsonian Institution, 1964.) [2611]

National Science Foundation. Programs for Improving the Dissemination of Scientific Information. Pp. 15. (NSF-64-22.) (Washington, D.C.: National Science Foundation, 1964.) [2611]

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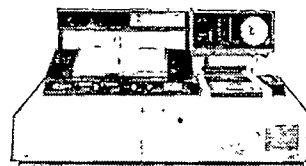
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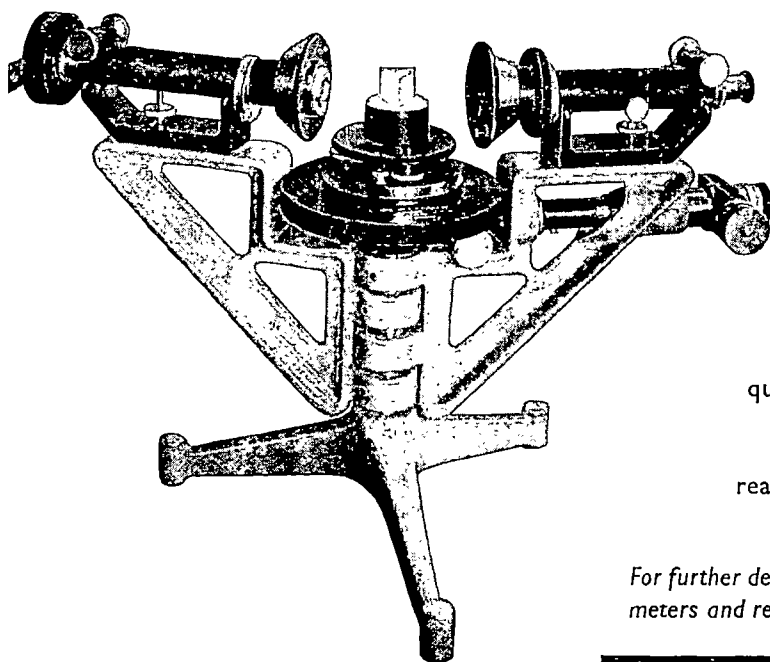
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# Sir Winston Churchill

K.G., O.M., C.H., F.R.S.

SIR WINSTON CHURCHILL, whose death we mourn, was the first British Prime Minister to appreciate the importance of science and technology to our country. His interest and appreciation were of enormous importance in the conduct and outcome of the Second World War. It was fostered by his friendship with Prof. F. A. Lindemann, which started in 1921 on one of Lindemann's social visits, and led to discussions on the scientific interests of 'The Prof.', who was also able to advise on the hydraulic problems of the new water gardens being built at Chartwell—Churchill hoping that "the problems were not beyond Oxford mathematics".

In the 1930's, as a result of this friendship, Churchill began to take a close interest in the problems of air defence and particularly in the applications of radar, and this led, in turn, to the appointment of Lindemann to Sir Henry Tizard's "Scientific Committee on Air Warfare", an appointment which led to the difficulties on the Committee which have already been sufficiently described. In June 1939 he was conducted by Tizard "in a rather disreputable aeroplane" on a tour of the radar establishments on the East Coast. He told the Air Minister that he found his visits to Martlesham and Bawdsey profoundly interesting, and urged the importance of radar to the Navy—"I cannot conceive", he said, "why the Admiralty are not now hot upon this trail".

He became First Lord of the Admiralty in 1939 and was then in a position to press his views, and, in particular, the need for improving radar installations. This led, no doubt, to Admiral Sir James Somerville's urgent demand in September 1939 for the immediate installation around Scapa Flow of radar sets to detect low-flying aircraft. As a result, a team of physicists from the universities, helped by industry and Government establishments, completed the installation of the "C.D.U." radar stations at Sumbrough Head in early December 1939 and on Fair Isle by February 1940. Without the drive from the top, this rapid work would have been impossible.

Sir Winston also founded a Statistical Office with Prof. Lindemann in charge, and a team of six economists was very speedily installed under the leadership of G. D. A. MacDougall. This later became the Prime Minister's statistical section, which provided him with speedy and direct information through charts and an endless stream of minutes from 'The Prof.' on quantitative aspects of the conduct of the War.

As the bombing attack on Britain developed in 1940, the Prime Minister took a still more intense interest in the application of radar. His minute of October 25, 1940, directed that "The very highest priority in personnel and materials should be assigned to what might be called the radio sphere. We must impart a far higher accuracy to the A.A. guns and a far better protection to our warships and harbours".

Eight months earlier the high-power centimetre cavity magnetron had been born in the Physics Laboratories of the University of Birmingham, and by May 1940 centimetre radar was operating in a primitive way on the Downs above Worth Matravers and, with unparalleled rapidity, centimetre radar sets were installed in ships and aeroplanes. It was not unusual for scientists directing the experimental teams to be summoned to the presence of the Prime Minister, dressed in his boiler suit, to be

interviewed about the progress of their work and commanded to bring the latest devices into operation "by the next moon phase".

The Prime Minister was responsible for authorizing the Tizard Mission to proceed to the United States in September 1940 to disclose all our scientific war secrets to the United States authorities. Fortified by his blanket instructions, the Mission got together the famous "black box" collection of samples, reports, drawings, including a working centimetre magnetron. The Mission was successful in establishing complete scientific collaboration and led to the foundation of the successful Radiation Laboratory at the Massachusetts Institute of Technology, which developed centimetric radar for the U.S. forces and provided a great deal of equipment for Britain at critical times.

The Mission also established collaboration with the United States on atomic energy developments, and reports on the work being carried out in Britain under the auspices of the MAUD Committee were rapidly transmitted. This Committee's final report of July 1940 led to a great intensification of work on the atomic bomb by the United States. Personal intervention by Sir Winston led to the Quebec Agreement which enabled British scientists to make an important contribution to the joint war effort, and also enabled them to develop, through the Canadian-U.K. Chalk River project, an almost complete knowledge of nuclear reactor technology.

In the later stages of the War, the Prime Minister took a personal interest in the threat of the flying bomb and the V.2 rocket. There were at times differences of opinion between Lord Cherwell and other scientists advising the Prime Minister, and it was not unusual for him to convene a meeting of the protagonists and responsible Ministers and to insist on the scientists being allowed to give their direct views.

After Sir Winston's retirement from office in 1955, he decided to devote a large part of his remaining energy to awakening Britain to the importance of improving her scientific and technological education. Out of this interest emerged the concept of the foundation of Churchill College with the objective of increasing the supply of highly trained scientists and technologists. It was to have a student body of 540—one-third of them to be postgraduates and 70 per cent working in science and technology. Sir Winston became chairman of the Trustees, who collected the funds for the greater part of the building and endowment of the College. The College reflects Sir Winston's world-wide interest by having more than 60 overseas graduate students and up to 10 Fellows from overseas in an academic year.

In a statement, after the death of Sir Winston, the Right Hon. Harold Wilson said that "Details will be announced shortly by Field Marshal Lord Alexander of plans for an appeal which is being organized on an international basis by close friends and colleagues of Sir Winston Churchill. Sir Winston Churchill himself regarded Churchill College as his national memorial, and the new appeal will enable the public in this country and abroad to show their respect for his memory by contributing further to that foundation and to other similar causes which the organizers will announce in due course."

JOHN COCKCROFT

## SCIENTIFIC POLICY-MAKING IN BRITAIN

THE paper on "Government Scientific Policy and the Growth of the British Economy"\* which Mr. C. F. Carter and Prof. B. R. Williams contributed to the September 1964 issue of *The Manchester School*, makes pertinent reading now that the shape of the Government's organization and plans for civil science and for technological and economic development are beginning to appear. It may well be some time before lines of scientific policy are definitely laid down by the Government, but meanwhile this paper takes the more general discussion which was stimulated by Dr. S. Toulmin in his paper in 1963 on the foundations of scientific policy a stage further, and into a specific British context. (Dr. Toulmin continued his theme in a talk, "Science and the State", broadcast on the B.B.C. Third Network and printed in *The Listener* for June 11, 1964, as well as in an article in *Minerva* last spring, entitled "The Complexity of Scientific Choice".) Pertinent to the discussion also are such papers as Mr. J. Maddox's "Choice and the Scientific Community" (*Minerva*, Winter 1964) and those contributed to *Scientists and National Policy-making*, edited by Prof. R. Gilpin and Dr. C. Wright (see p. 428 of this issue of *Nature*). Nor should it be overlooked that, besides Sir William Slater's discussion, in the Joule Memorial Lecture, of Government as the patron of science, Lord Todd, in the Dalton Lecture of the Royal Institute of Chemistry, also in October, considered the relations between Government and technology, and Prof. R. H. Kantorowich, again in Manchester, called for a national strategy of land use and settlement as an essential counterpart of defence policy or economic policy.

From varied and independent sources there is thus being gathered much authoritative material for the formulation of scientific policy, as well as for its assessment. Mr. Carter and Prof. Williams begin by pointing out that a country's eminence in science is not a good guide to its economic strength and growth, nor indeed something to be measured with precision. Current economic growth depends much more on success in applying existing science and technology than on current rates of expenditure on research and development: the stock of knowledge rather than the flow of new knowledge is the important factor, and the relations between science and growth are too complex to be derived from national expenditure on research and development.

Accordingly, Prof. Williams and Mr. Carter begin by showing how the use of scientists and engineers—the supply of whom depends on education—in the interests of economic growth raises questions about their distribution in different but related activities such as research, development, production, technical sales or management—even in education. However, they do not raise as specifically as did Sir Richard Clarke in his Stamp Memorial Lecture the question of whether or not Britain could afford to employ in teaching some 350,000 out of 750,000 persons in Britain with a full-time higher education.

The appropriate way of using scientists will depend on the relative costs of developing one's own innovations or

buying them on licence from abroad, and on the related cost of using scientists in research instead of in production. Growth can be impeded if, as in Britain, too high a proportion of scientific manpower is engaged in adding to the stock of knowledge and too small a percentage in using it. This point is seldom considered in discussions on industrial research and development or the balance of the research effort. However, its importance can be at least as great as the efficiency with which research or development is conducted, and the soundness of the judgment with which projects are selected or managed. The same factor may also influence the effectiveness of communication between experts in research and development in production, finance and marketing: unbalanced distribution in one sector reacts on the effectiveness of work elsewhere. The paper emphasizes especially the importance not merely of a wise distribution of Britain's limited scientific manpower but also the importance that decisions about its distribution should not be made piecemeal or sectionally but from a balanced view of the whole situation. This is, indeed, the fundamental purpose of Government policy and action, but it is one which since the War no Government seems to have possessed the wisdom or courage to pursue. Yielding to sectional interests has usually destroyed any semblance of a national and balanced policy.

Few would now dispute Prof. Williams and Mr. Carter's argument that the question is no longer whether Government should take action but rather the extent of such action. Directly, they maintain that the Government must provide for the education of a sufficient number of scientists, technologists and supporting technicians, and for certain scientific information services, such as major libraries. It must also accept responsibility for scientific research and for certain applied research for which the Government is the major customer. There are other spheres where Government action is more prudently exercised indirectly, and it is the selection of the points of intervention that tests the soundness of judgment and policy. To apply effort at points where little result can be achieved is a waste of effort, however satisfying to some political dogma. There is no substitute for clear thinking, and the wise maker of policy uses many instruments and none to excess.

Most of the instruments listed by Mr. Carter and Prof. Williams have already been mentioned in Parliamentary debates in the present session. There is direct control of production, of which they observe that unitary control may sometimes lead to a centralization of research policy, so nothing is done which does not fit the ideas of one man or a small group. Of encouraging production by subsidy or large initial orders by the Government, they note that, where the Government is an important buyer, its buying power can be used relatively easily to encourage applications of science. Control of developments by direct Government contracts can promote desirable changes in organization as well as specified developments, but the use of tax incentives may present difficult problems. Again, they point out that although direct Government action in applied research through research stations solves the problem of getting research done for an industry unwilling or unable to conduct such research itself, the

\* "Government Scientific Policy and the Growth of the British Economy". (Reprinted from *The Manchester School of Economic and Social Studies*.) By C. F. Carter and Prof. B. R. Williams. Pp. 20. (Manchester: *The Manchester School*, Department of Economics, The University, 1964.)



problem of applying the results remains. Moreover, even though it is supporting research which industry would undertake to do itself, the Government, by making tax concessions, specific research grants or grants to research associations, etc., increases the probability that the results will be applied. However, the influence on what research is carried out is less, and it is not easy to influence a research association which chooses to ignore the chance of a major change in technology and concentrates on minor refinements of instrumentation. Finally, they refer to the way in which Government action can encourage the employment of scientists and technologists, notably by increasing the supply as well as improving the use made of them by increasing the supply of technicians.

On this last-mentioned point it is surprising, in such a forthright paper, to find no condemnation of the hoarding of manpower. Particularly in the context of Government proposals for dealing with redundancy this is probably one of the most serious dangers threatening the British economy. The problem of scientific policy, it is rightly urged, is not one of devising totally new methods, but of applying more forcefully, and in new areas, methods which have long been used or considered. Along with this more forceful application must go the recognition that there is no general answer to the problem and that to make a measure with the appropriate degree of force in particular circumstances is going to hurt some groups or interests much more than others. Failure to be sufficiently ruthless and mistaken attempts to treat everyone alike are responsible for much that is amiss in industry to-day.

Accordingly, Mr. Carter and Prof. Williams suggest that a Government requires many means of intervention as well as some method of assessing what should be done in different parts of the economy and of repeating such assessments from time to time competently and rapidly. For this purpose they lean towards the idea of a small research team of scientists and men with business or economic training, advised by knowledgeable assessors. Any such team should be attached to a Ministry in day-to-day touch with the relevant sector of the economy and responsible for Government policy towards that sector. It follows that little value is attached, on this view, to independent assessment, but at the same time it is recognized that Government departments generally are nowadays rarely staffed with specialists of the type required. Creation of strong research divisions (composed of small project units) is recommended in each department which has direct responsibilities for relations with industry, commerce and services, even if some changes are in consequence necessary in the structure of the Civil Service. Similarly, relating applied research and development as closely as possible to Ministries responsible for production is advocated in the paper, and Mr. Carter and Prof. Williams do not agree that the applied research of the Research Councils should fall under a separate Ministry for Science, even if this necessitates drawing another line between pure research and higher education. In this context they make a wise comment as to the value of alternative sources of finance to the University Grants Committee as a guarantee of freedom in research—a factor which has received little recognition in comments either on the Government's present proposals or on those of the Trend Report.

This line of thought leads to a recommendation to transfer to a reconstructed National Research Development Corporation, with new terms of reference, that aspect

of the work of the Department of Scientific and Industrial Research concerned with awarding development contracts and grants to applied research; meanwhile the work of its Industrial Grants Committee would go to the Board of Trade, which would be assisted by an outside advisory committee. They would place the Road Research Laboratory in the Ministry of Transport, the Building Research Station in the Ministry of Public Buildings and Works, and other research stations would be treated similarly where a clear functional responsibility could be seen—the Agricultural Research Council going to the Ministry of Agriculture, Fisheries and Food. Such proposals, however, might well be subject to second thoughts in the light of some comments which Dr. S. Toulmin makes about United States experience. Responsibility for postgraduate training awards would be transferred to the Ministry of Higher Education, while research grants in pure science would become the responsibility of a Science Research Council, which would also be responsible for the National Physical Laboratory, and with research stations. This structure would be flanked by the Medical Research Council, the Nature Conservancy, and new Councils for Social Science and the Humanities, all of which would be responsible to the Lord President of the Council.

Clearly this is considerably different in various respects from the structure recommended by the Trend Committee or that which the Government is now establishing. It possesses, moreover, some significant defects, even where superficially most attractive. The proposal to locate research stations within the Department ignores the value which is sometimes attached, as with the Building Research Station, to independence in advice, nor has account been taken of considerable practical difficulties which would arise in determining alignment. Again, although there is a welcome recognition of the importance of information services and a survey of such services to determine whether the most effective work is based on provision by industry or locality, the proposal to distribute among the production departments the information functions of the Department of Scientific and Industrial Research is highly dubious and more likely to hinder than to promote the development of the national library and information services which Britain needs.

So far as the instruments for the formulation or execution of scientific policy are concerned, the proposals by Mr. Carter and Prof. Williams are debatable; however, they are to be welcomed for the emphasis which they put on needs which must be served, some of which the Government's present proposals tend to ignore. As regards actual policy, they give a high priority to improvements in educational selection and measures to counteract social influences which lead to waste of ability, as well as to the more sensible use of scarce manpower. There is a timely reminder that Britain's resources are limited. We cannot possibly lead in every area of pure science and technology, and it is urged that we might benefit by transferring resources from science to industrial technology, and that we might participate more fully in international trade in technical knowledge. Wisely, too, it is urged that large new commitments involving scarce manpower should be viewed with great suspicion, and once again a high place is given to the extension of information and advisory services and the general increase of scientific and technical literacy in the nation.

One blemish unworthy of a stimulating and suggestive paper is sour and unjustified comment on the Advisory

Council of Scientific Policy, which has consistently sought to prevent dissipation of scarce resources in such fields as space research and whose policy of filling in gaps (which has been pursued realistically) is far from being nonsensical as is here suggested. That apart, it is reasonable to suggest that first priority in the use of scarce resources should go to strengthening those few industries in which Britain already has a technical lead which could be maintained or increased. Next, the Government should direct attention to those activities in which at small extra effort, perhaps by purchase of technique, Britain could do at least as well as her commercial rivals. Massive concentration of effort on the revival of seriously backward sections is dubious and perhaps to be avoided. Here perhaps, above all, may come the call for resolution and a certain ruthlessness which, at least as much as good advice and appropriate organization, are what Britain needs for the health of her economy.

Some of these points were emphasized by Prof. B. R. Williams in his presidential address to Section F (Economics) at the meeting of the British Association for the Advancement of Science in August last. Speaking on "Economics in Unwanted Places", he pleaded that cost-benefit analysis should be used to guide Government expenditure on research and development, referring particularly to the second nuclear power programme and the aircraft industry, and to the desirability of judging investment in scientific research in the light of the probable social benefits. Even before the election it seemed fairly clear that whatever Party was returned to power the new Government would have to devise some new and more satisfactory system for the formulation of scientific policy; it is worth noting that in a further paper at the Southampton meeting of the British Association, presented before Section N (Sociology), Mr. D. S. Pugh and Mr. C. R. Hinings, of the Industrial Administration Research Unit at the Birmingham College of Advanced Technology, described some results in an empirical examination of bureaucracy in the management of some fifty organizations in the Birmingham area. The relevancy of such an examination is well brought out by Prof. C. Frankel's article, "Bureaucracy and Democracy in the New Europe", in the Winter 1964 issue of *Daedalus*.

Such investigations are, in fact, vital when the forms of Government organization are being transformed. Prof. Frankel points out that the problems of bureaucracy and of the relation of specialists are all too frequently ignored, and of the major social philosophies of the nineteenth century only Comte and Tocqueville gave the problem systematic attention. Reconciliation of a conception of popular Government based on models drawn from small communities, with the imperatives of Government in large nation States, is not easy, and even in present discussions of the reform of Parliament the issues are sometimes overlooked. Prof. Frankel's realistic discussion displays the real issues: the working touchstone of a democratic system of authority is simply the degree to which it gives individuals legitimate instruments for reaching those who made the decisions that affect them, and for bringing influence to bear on them. That applies where science and technology are concerned just as in other fields, and here too it is imperative that besides the electoral system itself there must be independent resources such as newspapers and periodicals, professional or learned associations, which allow professional men to make their views known and exert effective pressure on their leaders.

Prof. Frankel refutes the idea that bureaucracy is incompatible with this conception of democracy, which accepts the necessity for central leadership; at the same time he shows that political leaders to-day cannot discharge their tasks competently and intelligently without the advice and assistance of a large corps of specialists whose office is not dependent on election and whose loyalties are given equally to any Government. There are important considerations in judging the appropriateness of new institutions, and Prof. Frankel points out that an independent judiciary, free institutions of enquiry and education and private associations all create a vested interest in maintaining constitutional guarantees of liberty, nor has the loss of initiative by Parliament diminished its function of airing grievances. If the importance of personal rights as opposed to administrative authority has yet to be fully recognized the problem is already receiving some imaginative attention.

Beyond this, Prof. Frankel makes some suggestive points about the desirability of new political associations in the light of the new forces of science and technology, productivity and efficiency. It is a major function of the democratic method to prevent decisions being taken that are based on narrow definitions which leave out vital aspects of the interests and issues at stake. Bureaucratic decision-making has to be brought into the open where it can be surveyed and controlled, and those who exercise bureaucratic power placed under pressure from visible and significant competitors. Changes in a democratic system should be such as to encourage such democratic participation and foster the use of all the interest and experience such a society can provide. Conceived on such lines, planning, while providing a framework in which individuals can make their choice, does not necessarily increase the specific interventions by the State, while it can make the citizen aware of larger social purposes in everyday life and supply a sense of meaning and direction.

These criteria for assessing the appropriateness of the Government arrangements for civil science indicate that professional organizations have an important, indeed an essential, part to play, and the lead recently given by the Royal Society should be widely followed. On the content of policy itself, the complexity of the problem implies flexibility and renders almost inevitable a wide divergence of opinion. The pragmatic approach of Prof. Williams and Mr. Carter is almost diametrically opposed by Prof. M. Polanyi's, as Dr. Toulmin points out in the attempt to find common ground which he contributed to the Spring 1964 issue of *Minerva* under the title "The Complexity of Scientific Choice: a Stocktaking". Nor is this any condemnation of the scientist or such a body as the Advisory Council on Scientific Policy. Perhaps the most important passage in Dr. Toulmin's article is that in which he emphasizes that the cardinal choices in national policy are necessarily political choices, to be made at Cabinet level; by the time they reach that status the points of decision will have had to be restated, so ceasing to be technical questions about scientific importance, and becoming political questions about the rival claims of health, defence, higher education and the rest. Unless this central principle is firmly grasped, neither the content of scientific policy nor the arrangements for its formulation and execution can be fairly assessed—not that Dr. Toulmin is entirely satisfied with the arrangements which existed when he wrote. Some of his criticisms have been met by subsequent changes, but his point about the real cause of any weakness in the Advisory

Council on Scientific Policy still stands. In his view that Council should be responsible not to the Lord President of the Council in his role as Minister for Science but to the Cabinet. That the point had been taken might follow from certain tentative arrangements which the Prime Minister has already made for strengthening the Cabinet Office. Meanwhile, it is to be hoped that both inside and outside the Government new arrangements will be impartially assessed in the light of the momentous value of informed criticism.

## ORGANIZATION OF SCIENTIFIC ADVICE

Scientists and National Policy-making

Edited by Robert Gilpin and Christopher Wright. Pp. viii + 307. (New York and London: Columbia University Press, 1964.) 7.50 dollars.

A PART from a review by Sir Solly Zuckerman and comment by Dr. S. Toulmin in *Minerva* on taking stock of the complexity of scientific choice, this collection of essays has attracted little attention in Britain, in spite of the stimulus to thinking about scientific policy given by the international ministerial meeting on science at the Paris headquarters of the Organization for Economic Co-operation and Development in October 1963. Nevertheless, although primarily concerned with the making of policy in the United States, most of the essays are of even greater interest in the light of the new measures which the present Government has taken for the organization of civil science in Britain and to associate scientists with the formulation of policy. Whatever basis there may be for the suggestion that the new arrangements follow the Presidential structure, these essays are, as Dr. Toulmin points out, a most useful contribution to the discussion of the general problem and to assessing the soundness of particular proposals.

A few of the essays have already been published. These include D. K. Price's discussion of the nature and role of the scientific establishment in the United States; Prof. W. S. Sayres's "Scientists and American Science Policy"; and Prof. W. R. Schilling's "Scientists, Foreign Policy and Politics". Apart, however, from R. N. Kreidler, who writes on "The President's Science Advisers and National Science Policy", and H. Brooks, who deals with the Scientific Adviser, it is not made clear how far the authors are themselves writing from inside knowledge and association with the task of formulating policy and not simply from academic studies. Of the remaining essays, Prof. R. C. Wood contributes one on "Scientists and Politics: the Rise of an Apolitical Elite"; Prof. A. Wohlstetter writes on "Strategy and the Natural Scientists" and B. Brodie discusses "The Scientific Strategists". Dr. C. Wright's "Scientists and the Establishment of Science Affairs", which concludes the book, makes an appropriate counterpart to the admirable introductory essay by his co-editor, Prof. R. Gilpin, "Natural Scientists in Policy-making".

The essays thus range widely, though the common theme is clear and strong. Likewise, much of the stimulus is derived from Sir Charles Snow's Godkin Lectures in 1960, and it differs from some other American books in that the British contribution to the debate is reasonably reflected. Mr. Gilpin's introduction brings out very clearly the way in which the participation of scientists in the formulation of policy in the United States has developed in the past quarter of a century and the character of their contribution in relation to national support for science and to national security. These questions are explored more fully in the subsequent essays, but it is chiefly in those by Brodie and Wright,

which discuss the possibility that new types of expertise must be developed to cope satisfactorily with the problems now created by the interactions of science, technology and society, that any essentially fresh contribution is made. By and large, the value of the symposium lies in presenting in reasonable compass the position of present-day developments in this field in the United States and the trend of an important section of informed opinion.

Nevertheless, certain important points clearly emerge. If the crucial nature of the scientific contribution to national strength is manifest, too much is never claimed. Scientists in Government do not suggest that their advice should be overriding, but they do insist on the value and importance of scientific advice in reaching a balanced decision where scientific issues are involved. This is not simply a matter of the arrangements for tendering scientific advice or the calibre of the adviser: it is a matter also of public understanding, and herein lies the value of Christopher Wright's analysis of the bridges which in recent years have been built between science and society. The implications of this essay deserve pondering, not simply by scientists or technologists as individuals but also by their professional associations. There is wide scope for creative thinking on this point to which the social scientist, as well as the physical scientist and the politician, has his own contribution to make. Moreover, as Harvey Brooks points out, the magnitude of scientific and technological enterprise to-day and the influence which the scientific adviser already exerts on public policy expose the scientist to criticism which could be dangerous to science itself in the absence of public understanding. Scientists can no more afford to neglect to make the organization for advice as effective and efficient as possible than they can to promote public understanding of the nature and limits of science. Nor are scientists likely to tender usable advice without some understanding of the nature and limitations of politics.

It is thus mainly for its constructive approach to the problems of the organization of scientific advice that the book is important. It does not ignore the question of the content of scientific policy: the form of organization most appropriate can scarcely be determined without reference to that content, to the purposes to be served. Although such reference, even in Christopher Wright's essay, is slender, it has wide implications for the scientist and is perhaps one of the most constructive features of a timely and stimulating book.

## SCOTLAND'S GREEN MANTLE

The Vegetation of Scotland

Edited by Dr. John H. Burnett. Pp. xiii + 613 + 96 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1964.) 126s.

THIS is an account of Scottish plant life in its environment, written by the members of a group of ecologists and edited by Prof. J. H. Burnett, formerly of the University of St. Andrews and now of the University of Newcastle upon Tyne. Prof. Burnett contributes the introductory chapter, in which the history of plant ecology in Scotland is reviewed. The Perthshire brothers Robert and W. G. Smith, in the years immediately following 1898, laid the foundations of the study in Britain; the premature death of Robert, and his brother's move to Leeds, directed the focus of activity south of the border, where its first major fruit was A. G. Tansley's *Types of British Vegetation* (1911). In Scotland, Robert Smith's immediate successors soon disappeared from the scene and the early momentum was lost; the next thirty-five years produced only isolated papers of consequence on Scottish vegetation. Just after the Second World War the work of Gimingham, McVean, Poore and Ratcliffe stimulated a Scottish revival.



Poore adapted the technique of 'successive approximations', favoured by prominent continental ecologists, to the investigation of Scottish hill plant communities, and the method proved so successful that it has been applied to most of the vegetation zones examined in the present work. As a result, the basic vegetational units are smaller, more natural and more precise than those used by Tansley in his later work, *The British Islands and their Vegetation* (1949).

The remainder of the text is in three sections: two chapters on physical background (climate and soil), followed by twelve on vegetation and a final two on history and pattern. The plant nomenclature adopted is the most recent available: Clapham, Tutin and Warburg's *Flora of the British Isles* (1962) for flowering plants and ferns, and for the other cryptogams the present census catalogues.

The account of climate, given by F. H. W. Green of the Nature Conservancy's Speyside Research Station, is an analysis of Scottish weather as it affects plant life. The evolution of Scottish soils from pre-glacial times and the characteristics of more than thirty types of modern soil are dealt with in the chapter on soil—the work of Dr. E. A. Fitzpatrick. These two chapters on the physical background cover between them some 50 pages; the middle section, on vegetation, takes almost 500. The order of treatment here is altitudinal, from the sea's edge to mountain summits. The chapter on the maritime zone is the work of Dr. C. H. Gimingham, of the Botany Department in the University of Aberdeen. The great length of coastline bounding the Scottish mainland and islands, and the diversity of its scenery, result in a wide variety of habitats; the plant communities of cliff faces, rock ledges, shingle, saltmarsh, dune country and machair all come under review.

Three chapters are devoted to the forest zone. One, on woodland and scrub, written by Dr. D. N. McVean, formerly of the Nature Conservancy's Speyside Research Station, recounts the chequered history of Scottish woodlands; a history complicated by introductions as well as by felling, burning and grazing, so that distinguishing the native from the planted is not always easy. Two members of the staff of the Hill Farming Research Organization, Dr. J. M. King and Mr. I. A. Nicholson, contribute a chapter on low-level grasslands. The fluctuating fortunes of the woodlands are reflected in the history of grassland communities; as the authors point out, Scotland's economic history over the past 200 years has been closely linked with the evolution of her grasslands, in which the main factor has been biotic. Dr. Gimingham's chapter on dwarf shrub heaths concludes the account of the forest zone; this type of country has long been the author's special study, and here he is very much on his native heath. He concludes with an account of economic heathland management, involving controlled burning, and traces the stages in recovery.

Another three chapters are taken up with the lowland aquatic zone. The first, on phytoplankton in Scottish lochs, is written by Dr. A. J. Brook, of the Botany Department in the University of Edinburgh. Relatively little work has been done on this subject since the original investigations by the brothers West fifty years ago; indeed, the author of the chapter is personally involved in about half the small number of papers quoted in reference. On the other hand, the chapter on the macrophytic vegetation of these habitats by Dr. D. H. N. Spence, lately of the Botany Department in the University of St. Andrews, is the longest in the book, and the length of the reference list indicates that this is a topic which many workers have found attractive. Dr. D. A. Ratcliffe, now at the Conservancy's Experimental Station in Monk's Wood, concludes the account of this zone in a chapter on "Wetlands" (mires and bogs); the term 'mire' is equated to 'soligenous bog'. The author estimates that, while about one-tenth of Scotland's total land-surface is at present bogland, the

rapidly growing demands of agriculture and afforestation will drastically reduce this fraction.

The final section in this part of the volume consists of five chapters devoted to the montane zone; all but the last of these are by Dr. McVean. No altitudinal limits are specified for this zone, but it is apparent from the text that it extends upwards from about 1,000 ft., and that all the communities here described can be found in the 2,000–3,500 ft. range. Some of the associations already described in the lowland zones have their parallels here: thus Chapters 11 ("Dwarf Shrub Heaths"), 12 ("Grass Heaths") and 15 ("Montane Mires")—the last-mentioned by Dr. D. A. Ratcliffe—are all projections to higher altitudes of the comparable habitats in the lowland zone. Here changed conditions of altitude, slope and aspect have so modified the climatic and biotic factors as to alter the composition of the plant communities. Two new types of association are recognized in this zone; these are herb and fern meadows (Chapter 13) and moss heaths (Chapter 14). The latter, which do not occur below 2,000 ft., and are beginning to be significant at 2,500 ft., are the associations occupying the highest levels.

Dr. McVean is the author of both chapters in the short third and final section of the volume; they deal with history and pattern. In the first of them the theory of a post-glacial origin for the flora is accepted, and the changes wrought by 15,000 years of climatic fluctuation, as evidenced by plant remains and pollen deposits in peat, are outlined. The final chapter is on regional pattern; in it the author postulates that vegetation regions do exist in Scotland, and makes an arbitrary division of the mainland into four of these. Cultivated land is excluded, and the islands are treated separately—not from natural causes, but for reasons of convenience; in fact their vegetational affinities lie with the adjacent mainland regions.

The text is followed by four indexes, occupying some 35 pages; one is general and the others list the names of authors, places and plants. In the list of authors the name of the late W. H. Pearsall has a prominent place; he would have delighted in this work, of which his own *Mountains and Moorlands* was a natural precursor. References to literature occur at the end of each chapter; the book would be valuable to students for these classified lists alone. There are close on a hundred photographic illustrations; three of these, depicting the horizons identifiable in characteristic Scottish soils, are in colour. Many of the others are full-page; most of these are very good and some are excellent. Dr. Berry's photograph of Rassal ashwood is one of the finest in the book, but others in the same and later chapters also convey the atmosphere of the habitat very successfully. Presumably considerations of space dictated the reproduction of two or even three photographs to the page in other cases; these are mainly of good quality, but for some a larger scale would have been an advantage. Of particular interest is a series of illustrations of fresh-water habitats, for comparison with others taken at the same sites by George West, more than half a century earlier. There are more than 60 maps and line diagrams of various kinds, and 65 tables analysing the vegetation in the various communities.

There seem to be few misprints; some occur in footnotes (pp. 169, 222, 292) and are not misleading. There is an obvious misnomer in the caption to Plate 90, where *sanguineum* should be *sylvaticum*, and two page-headings (pp. 496, 497) are misplaced. The accuracy of the tabulated data must be assumed.

In a volume which contains so much of absorbing interest, one must guard against being over-critical on minor omissions. I felt that there might have been room for some discussion of the effect on pasturage of the Highland clearances during the first half of the nineteenth century, with the consequent replacement of cattle by sheep; nor does there seem to be any reference to records made in the Hebrides, from 1934 onwards, by exploring

parties from the Botany Department in the University of Durham. On points of detail, the application of the name *Juncus fluitans* to the fluitant state of *J. bulbosus* may be unfamiliar to some readers; the term is at present out of favour with systematists, and is not quoted in modern Floras. The list of species given for the tall herb association of flushed ledges in the montane zone seems unduly restricted; in this habitat one is accustomed to meet *Anemone nemorosa*, *Silene dioica*, *Filipendula ulmaria*, *Rumex acetosa*, *Oxyria digyna*, *Carex binervis* and some species of *Dryopteris* as frequently as other plants included in Table 61; again, it is surprising to note the absence of *Carex lepidocarpa* from low-level basic flushes (Table 64).

This is not a book for the pocket; it weighs almost 4 lb., and is built in proportion. Nor, despite its size, would its authors claim any finality for it; the editor himself points out the poverty of published work on the vegetation of montane lochs and of the islands. Scotland's lochs remain "relatively unstudied", and the analytical study of its "vast acreage of bogs and mires" has scarcely begun. Indeed, the author of the longest (and one of the best documented) chapters admits that "the total number of localities (fresh-water lochs) studied throughout Scotland barely exceeds the number of such habitats in Galloway alone; the Outer Hebrides... were omitted, and the larger Highland lochs are under-represented". For all five chapters on the montane zone, as for that on phytoplankton, the reference lists are pathetically short; indeed, the pointers to the future are implicit in most chapters. Yet the publication of this work is a major event in British botany, and one which must bring great satisfaction to its authors; the laborious years of preparation are over, and they have produced a volume which, for years to come, will be invaluable both as a reservoir of existing knowledge and as a stimulus to future workers. Nothing like it exists for any other part of Britain, but it is surely appropriate that the country in which the Smith brothers began, almost seventy years ago, the investigation of British plant ecology should now be the first in these Islands to produce so detailed an account of its vegetation. Its appearance will be hailed with satisfaction overseas as well as at home—a satisfaction tinged, perhaps, with regret that its production could not have been timed for a year earlier; there would have been no better briefing for visitors to the Edinburgh Congress in August 1964 than a few months with this fascinating book.

R. MACKECHNIE

## ORAL BIOLOGY

### Advances in Oral Biology

Vol. 1. Edited by Peter H. Staple. Pp. xiii + 353. (New York and London: Academic Press, 1964.) 100s.

THIS is the first of a projected series of volumes designed to present to research workers in oral biology and related fields critical review articles of recent work. It is hoped in this way, as the editor informs us in his preface, to facilitate the dissemination of information among the small, and geographically widely separated, groups who are interested in oral health and disease.

The present volume contains articles by workers whose names will be familiar to those who are interested in research in the oral field. These articles range over a wide area and give exhaustive and authoritative surveys of the subjects under discussion, with illustrations and bibliography. Some of the topics are likely to appeal to a relatively restricted readership, though others will attract a wider audience. In the former category is Mühlemann and Schroeder's paper on the dynamics of supragingival calculus formation. This is a review of recent research on the structure and variations in rate of formation of dental

calculus (tartar), including techniques for qualitative and quantitative estimation. In this class also are Walsh's review of the relationships of surface active agents, the enamel surface and dental caries, Carlström's account of the polarization microscopy of dental enamel and the incipient lesion of dental caries, and Armstrong's paper on the modifications of the properties and composition of the dentine matrix caused by dental caries. But even in these accounts, highly specialized though they may be, there are aspects that will be of interest to workers in fields apparently quite unconnected with oral pathology and dental disease. This is certainly the case in most of the remaining reviews; they will undoubtedly be of interest and value to biochemists and others concerned with bone growth. Among these reviews may be mentioned Hartles's paper on citrate in mineralized tissues, which gives a valuable résumé of the work done on this subject in the past twenty years—since, in fact, the discovery that citrate is present in bone in appreciable amounts. Bevelander reviews the effect of tetracycline on mineralization and growth—again a useful summary for those who use this technique for bone growth investigations. Morphologists will also be interested in Ness's account of movement and forces in tooth eruption, a very fully documented review of recent observational and experimental work on rodents and man.

The remaining articles, on the secretion of saliva by Schneyer and Schneyer, the physiology of mastication by Kawamura, cytochemical aspects of oxidative enzyme metabolism in the gingivae by Eichel and Shahrik and a review of recent investigations of mucins and blood group substances by Pigman and Hashimoto, will also be of considerable interest to research workers in the oral field and to those with related interests.

The editor of this volume hopes that the series will help many workers in the basic sciences to see that the investigation of oral biology in relation to oral health and disease offers great opportunities for fundamental investigations in their own disciplines. If succeeding volumes maintain the standards set by this one, the editor's hopes deserve to be fulfilled.

R. B. LUCAS

## ANALYTICAL METHODS FOR INSECTICIDES

### Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives

Vol. 2: Insecticides. Edited by Gunter Zweig. Pp. xvii + 691. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 164s.

WHILE a putative purchaser might have felt some doubts as to whether the first volume of this treatise, entitled *Principles, Methods and General Applications*, was worth buying, he need have no hesitation about Volume 2. Anyone concerned with the analysis of modern insecticides will need it as an essential tool.

The book is a model of its kind. Each of its forty-seven chapters deals with a separate insecticide. Chapter 1 about aldrin is followed in alphabetical order by the next about allethrin and so on to aramite, baytex and chlordane. Then, after dylox, guthion and heptachlor; rhotane and ronnel; sevin, systox and tedion; thiodan, toxaphene and trithion, there come, in Chapters 46 and 47, vapona and zectran. The system followed in each chapter is the same. The chemical formula of the active substance and its systematic name are followed by the empirical formula, the alternative trivial or trade names—for example, DDT, it seems, may also be called 'Dicophane', 'Gesapol', 'Gesapon', or 'Neocid'—the source from which a standard sample of the material can be obtained, a sentence or two about its biological properties and reference to the first publications in which it was described. Next come

details of its physical properties, the method by which it is synthesized where this is available, its main chemical reactions, and the types of formulations in which it is most commonly marketed.

The main part of each chapter and the essential core of the book, however, are the clear and explicit descriptions of analytical methods, first for formulations of the insecticides and then for crops or animal tissues likely to contain residual quantities of them. The principles of the available analytical methods are first outlined and references given to alternative procedures. Recommended methods are then described in full detail.

The advance of scientific understanding to-day redounds to the glory of the human intellect. Scientific theory, however, is based on accurate observation and observation depends on technique. This is a book on technique. For example, to determine traces of tedion (2,4,5,4'-tetrachlorodiphenyl sulphone) in fruit, it can be nitrated and treated with alkali in the presence of pyridine to give a red colour. It is, however, useful to be told that uniform nitration cannot be achieved without the addition of a 'keeper' and that the best 'keeper' for tedion is lanolin. The book is full of such useful detail; in some cases drawings of apparatus are included as well as precise working instructions. Sometimes, the very virtue of the full and painstaking precision with which methods are described may present problems for the analyst who lives on the right bank of the Atlantic. It may not be convenient for a British chemist to obtain premixed reagent for aldrin analysis from Austin, Texas, or adsorbent "attaclay" from Menlo Park, California.

The new synthetic insecticides with which this book deals are not only a striking example of the great advances that have taken place during the past few years in general toxicology, insect biochemistry, plant physiology and organic chemistry. As we know all too well, their advent has demonstrated how subtly progress in one facet of biology can disturb the delicate balance of Nature. This admirable compilation of analytical methods is an example of the painstaking laboratory work on which all our efforts to achieve a rational solution of the different problems raised by these new discoveries ultimately depend.

MAGNUS PYKE

## EXPLOSIVES, CHEMISTRY AND TECHNOLOGY

### Chemistry and Technology of Explosives

Vol. 1. By Prof. T. Urbanski. Translated by Irena Jeczalikowa and Sylvia Laverton. Pp. xv + 635. (London and New York: Pergamon Press, 1964.) 140s.

THIS volume is the first of a series of three volumes covering the chemistry and technology of explosives. The original Polish edition of this book was published in 1953-54 and was followed by Czech and German editions. The present fourth edition, translated from the Polish, is a considerably revised and expanded version of the earlier ones.

Since the classic four-volume treatise on explosives by Marshall (1917) a number of books have been published on the subject, to cover the expanding knowledge in the field. Some of these deal more with the physics of explosion/detonation processes, while others emphasize the technology of manufacture and applications. There has, however, been no publication covering the chemistry of explosive compounds, their physical and physico-chemical properties and details of manufacturing processes, in a wide perspective, suitable for explosive chemists and research workers. The present book adequately fills this gap.

The scope of the present volume is indicated by the following chapter headings: 'Nitration and Nitrating

Agents'; 'Nitration Theories'; 'Nitration Agents and Methods More Rarely Used'; 'Nitration with Nitrating Mixtures'; 'General Information on Nitrocompounds'; 'Aromatic Nitrocompounds'; 'Nitro Derivatives of Benzene'; 'Nitro Derivatives of Toluene'; 'TNT Manufacture'; 'Nitro Derivatives of Higher Benzene Homologues'; 'Nitro Derivatives of Naphthalene'; 'Nitro Derivatives of Halogeno Benzenes'; 'Nitro Derivatives of Phenol'; 'Manufacture of Picric Acid'; 'Other Nitro Derivatives of Phenols'; 'Picric Acid Ethers'; 'Nitroderivatives of Aniline'; 'Nitro Derivatives of Azo and Hydraso Benzenes'; 'Aliphatic Nitro Compounds'; and 'Nitronitroso and Nitroso Compounds'.

All the foregoing chapters are not merely descriptive but provide copious experimental data in the form of figures and tables, illustrating the theoretical concepts, chemical reactions and technical processes. The treatment of the subject is thorough in all the chapters, for example, the chapter on 'Nitro Derivatives of Toluene' details general information on toluene nitration with mixed acid, such as extent of nitration with different compositions of mixed acid, change of nitration rate with temperature; physical, chemical and thermochemical properties, toxicity, laboratory and industrial methods of preparation and separation of isomers and mono-, di- and tri-nitrotoluenes; effect of heat and light on  $\alpha$  TNT, reactions of  $\alpha$  TNT with various chemical agents, addition products, elucidation of constitution, kinetics of nitration of DNT to  $\alpha$  TNT both in homogeneous and heterogeneous systems; explosive properties of TNT; unsymmetrical isomers of TNT, their physical and thermochemical properties and chemical reactions with various agents; other by-products of toluene nitration, and tetranitrotoluene.

All chapters are provided with literature references at the end, covering publications up to 1960. One must not expect the same up-to-dateness in a comprehensive treatise like this as in a newspaper, and the author is to be commended for the literature coverage presented. An author index and subject index are also provided at the end.

The book is a mine of information for the explosive chemist. Not only is it a collected source of published information on what has been done but it is also a fertile source of new ideas to a discerning reader. To many the enormous amount of work reported from East European countries will become available for the first time. Details of a number of German and Japanese processes of explosives manufacture, disclosed at the end of the Second World War, in the CIOS, BIOS and FIAT publications, have also been included.

There are some very minor omissions; for example, there is no mention of the "white compound" formed in the continuous TNT manufacture. Its constitution and that of its degradation products have consequently not found a place in the nitro derivatives of azoxy compounds. Similarly, work on action of light on TNT, formation of 2-nitroso-4:6-dinitro-benzoic acid, the precursor of "white compound" and the possible relation of the "white compound" to the early products of photodecomposition of TNT (*Chem. Abstr.*, 48, 13656 a; 1954), has also escaped notice. It has also been overlooked that the formation of a eutectic of tetryl with  $\alpha$  TNT (p. 295) has been questioned (*Chem. Abstr.*, 40, 209; 1946).

The book is well produced and the translators have done a fine job. There are a few misprints (for example, reaction 11, p. 57; reaction C" on p. 63; ref. to p. 274 for p. 271 on p. 65), but these are not likely to cause confusion.

This is an excellent volume and can be wholeheartedly recommended for students and research workers in both explosives and organic chemistry.

W. D. PATWARDHAN



# FISSION PRODUCT FALL-OUT FROM THE NUCLEAR EXPLOSIONS OF 1961 AND 1962

By D. H. PEIRSON and R. S. CAMBRAY

Atomic Energy Research Establishment, Harwell, Berks

THE massive programme of nuclear weapon testing during 1961–62 started three years after the previous substantial series of explosions in 1958. In the 16-month period of testing 1961–62 the amount of fission reported<sup>1</sup> was roughly equivalent to that during 1952–58, but obviously at an increased rate. In addition to an increased rate of fission production in 1961–62 there were fewer high-yield explosions at or near ground-level, thereby reducing the proportion of fission products injected directly into the troposphere, and some individual explosions were very large, implying insertion to higher altitudes. It is to be expected that, compared with the earlier period, debris from the more recent series of nuclear tests would reside in greater proportion in the upper atmosphere and, on return to Earth, would thus contain a smaller proportion of short-lived fission products.

This article, based on fission product measurements already reported<sup>2–4</sup>, is concerned with the physical behaviour and fate of the fission debris produced during the nuclear explosions of 1961 and 1962. This debris is significant, primarily because of the potential radiological hazard to human populations but also because of its usefulness as a meteorological tracer. It is convenient to divide the debris into tropospheric and stratospheric components. The tropospheric debris remains in the troposphere after the cloud has stabilized, is dominated by short-lived radioactivity and could be observed during periods in 1961, 1962 and 1963 without interference by tropospheric debris from the earlier tests that had disappeared by decay. Stratospheric debris originates from the portion of the cloud that penetrates into the stratosphere and remains for a period, dependent on the latitude and altitude of injection, long compared with the residence time of tropospheric debris. It follows that when the new stratospheric debris has returned to the troposphere it is accompanied by a background of long-lived fission products from the residue of stratospheric injections of past years.

## Tropospheric Fission Products

In the troposphere, which in the middle and high latitudes extends to 10–14 km, the wind field varies with level so that debris injected at different levels will be carried on different tracks. Although vertical motion is very slow in comparison with horizontal winds, the cloud of debris will be spread over a very large area of complex shape after several days. Twice-daily charts of the wind distribution over the northern hemisphere are drawn in the Meteorological Office from routine observations for a series of standard upper levels. Vertical motion, too small to be observed directly, may be inferred at least qualitatively. Thus, in the lower half of the troposphere surface anticyclones are associated with slow descent of air, which if continued over several days may bring down debris from great heights to near the surface. Slowly ascending air is associated with the extensive cloud and rain areas of depressions, but in this case wash-out by precipitation counteracts upward transport.

In middle and high latitudes the average circulation is circumpolar from west to east, although frequently subject to large and changing disturbances. In the lowest levels these disturbances are often closed circulations—anticyclones and depressions—which, over limited sectors,

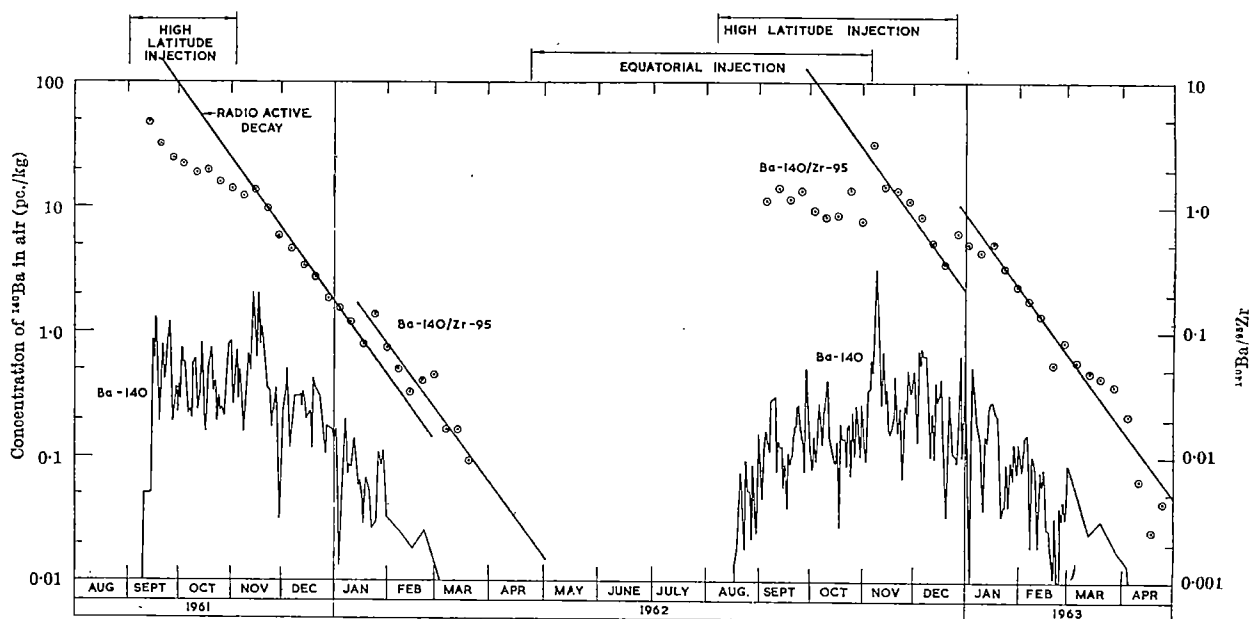
reverse the general west-to-east flow or give rise to broad meridional currents. At higher levels the circulations are replaced by wave-like features in the westerly current, having a wave number for the complete hemisphere of three to six. The westerly component of wind usually increases with height, reaching a maximum near the tropopause; easterlies present at the lowest levels tend to decrease with height and reverse at some upper level.

*Trajectories.* Debris from nuclear explosions will therefore usually reach the British Isles from the west and, in the case of explosions at the Russian testing sites in the Arctic (75° N., 55° E.) and in Central Asia (52° N., 78° E.), will have made nearly a complete circuit of the hemisphere in a time between 12 and 25 days dependent on season, level and the strength of the large-scale circulation. Occasionally debris from the Russian sites will reach the British Isles on more direct tracks from between east and north with a shorter transit time, particularly material injected into the lower levels of the troposphere.

The activity of barium-140 (half-life 12.8 days) may be considered as representative of short-lived fission products. The daily fluctuation in surface air at Chilton (52° N., 1° W.) is illustrated in Fig. 1 together with the ratio of barium-140 to zirconium-95 (65 days)<sup>2,3</sup>. It is clear that the source of barium-140 activity and hence of tropospheric debris detected at Chilton was the explosions at the Russian sites. No significant short-lived activity from the American explosions during 1962 (at Johnston Island, 17° N., 169° W., and Christmas Island, 2° N., 157° W.) was measured in the United Kingdom during March–August.

The general correlation of these fission product parameters with average wind speeds in 1961 has already been investigated<sup>1</sup>. For the tropospheric debris from the Russian series in the autumn of 1962 it has been possible in a few cases to track the path of a cloud from the United Kingdom to the testing site. A trajectory at about 5.5 km (500 mb) drawn in Fig. 2 is related to the peak of barium-140 about October 6–9, 1962. The trajectory was obtained by back-tracking from southern England from October 6 using the twice-daily routine charts of winds and contours. Over the Pacific and east Asia the tracking was less uncertain than usual—most trajectories cannot be tracked realistically much beyond the west coast of North America—and it is likely that the trajectory passed near central north China on about September 26. At that time the wind over northern Siberia was north-westerly, so that the corresponding trajectory was probably like the broken line in Fig. 2. After allowing an uncertainty of one or two days in timing it seems reasonable to associate the large explosions at Novaya Zemlya on September 25 and 27 (ref. 8) with the enhanced air concentrations in the United Kingdom about 12 days later.

The two trajectories shown in Fig. 3 are shorter ones. The first was constructed by forward-tracking from a known explosion of intermediate yield in central Asia on November 4, 1962 (ref. 8), to which are attributed the markedly enhanced concentrations of barium-140 at Chilton on November 10 and at other stations in the United Kingdom during the week November 5–11. The geographical distribution<sup>3</sup>, with very high concentration in the extreme north (Lerwick), high values in the north (Eskdalemuir) and east (Orfordness) and smaller but still

Fig. 1.  $^{140}\text{Ba}$  and  $^{95}\text{Zr}$  in air at Chilton

enhanced values in the south (Chilton) and west (Milford Haven), may be qualitatively explained by a lateral falling-off of activity from the centre line of this trajectory. The high levels of activity and the lack of spatial uniformity are consistent with a low cloud of fresh debris: in fact, over central Asia the easterly winds near the surface changed to westerly at about 2.5 km. The second trajectory in Fig. 3 was obtained by back-tracking at about 5.5 km (500 mb) from Chilton on December 29, 1962, when there was a peak in barium-140 concentration. The trajectory started from explosions in Novaya Zemlya on December 24 or 25 (ref. 8), passed round the pole in the Siberian-Canadian sector and approached the British Isles by northern Greenland and western Norway.

A trajectory for the first Russian test explosion in 1961 has been published by Machta *et al.*<sup>9</sup>: Hvinden *et al.*<sup>33</sup> have described the passage of a radioactive cloud over Norway in November 1962.

**Fission product ratios.** The transport may be timed in a more general way by examining in Fig. 1 the ratio barium-140 to zirconium-95, which has a value of 5 (ref. 10) at the time of formation during fission. Apart from an anomalously high initial value in September the ratio in 1961 tended during the first few weeks to 2.5, which would correspond to a transit time of 16 days. This represented debris passing Chilton during a first circuit; subsequent dilution of first circuit debris by debris on second or later circuits caused a gradual decrease in the ratio until mid-November 1961, the time of arrival of debris from the last explosion in the 1961 series. After this time the ratio of barium-140 to zirconium-95 decreased with the characteristic radioactive decay implying uniform mixing with debris from previous explosions: meanwhile the barium-140 activity was removed from the troposphere at a slower rate (Fig. 1) than required

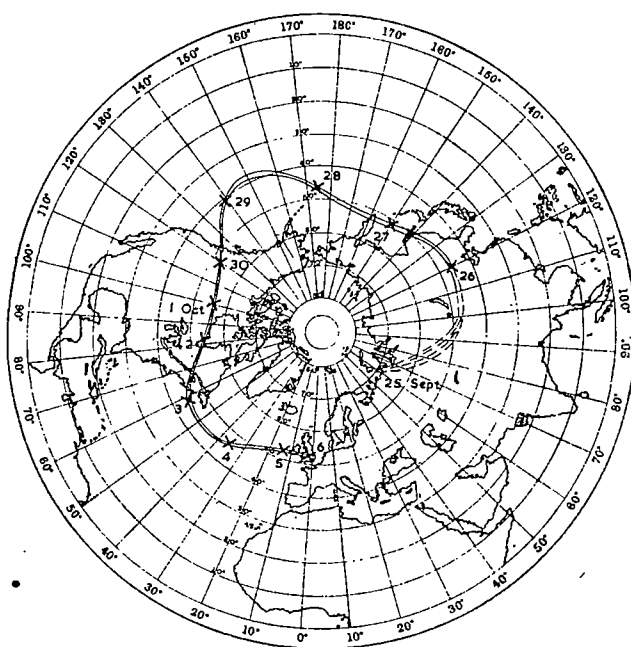


Fig. 2. Trajectory at 500 mb (5.5 km), September–October 1962

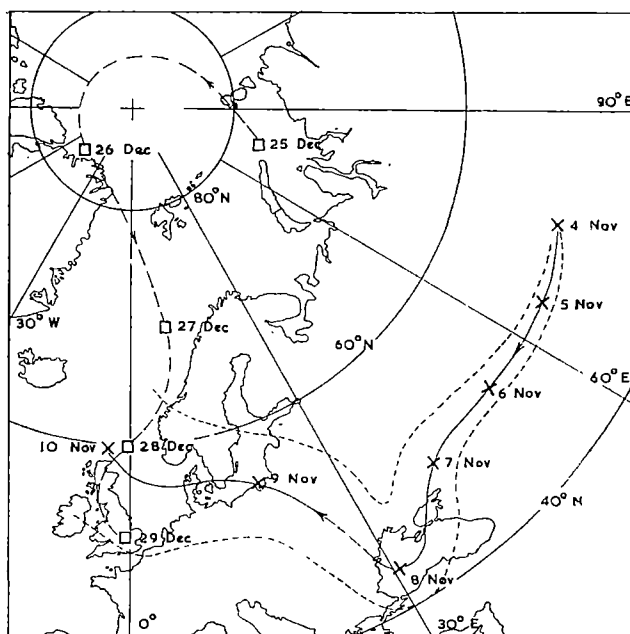


Fig. 3. Trajectories: November–December 1962. □, 500 mb (5.5 km), December 25–29, 1962; x, &lt;2.5 km, November 4–10, 1962; ---, rough indication of spread

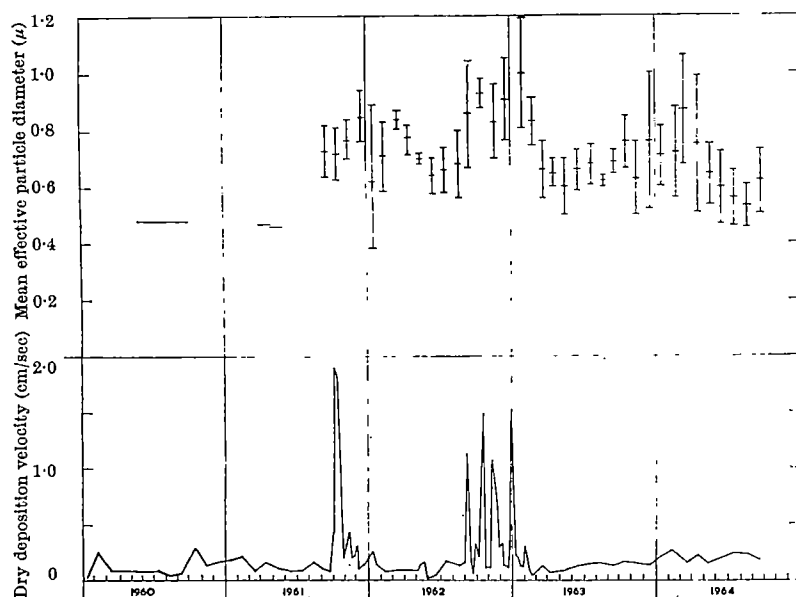


Fig. 4. Dry deposition velocity and mean effective particle diameter (Chilton)

by the combined effects of radioactive decay (half-life 12.8 days) and wash-out (half-residence time 20 days<sup>11</sup>). This evidence strongly suggests the onset by mid-November 1961 of significant reinforcement of the troposphere by stratospheric debris. It is remarkable that the stratospheric source, composed of debris from so many explosions, should have become so well mixed so soon. The behaviour of the barium-140/zirconium-95 ratio in the autumn of 1962 followed a similar pattern. The purely tropospheric régime lasted from August to early November, although the ratio fell gradually from an initial value that was lower than in 1961 due to dilution by old zirconium-95, residue of the 1961 test series<sup>8</sup>. The stratospheric régime started in early November 1962 and continued through the substantial addition from the explosions in late December 1962 and hence overlapped the tropospheric period by about three months.

**Dry deposition.** An important characteristic of a period of tropospheric fall-out is the increase in the rate of dry deposition; this characteristic has become well established during the test series of 1961 and 1962. The effect of dry deposition may be observed by the deposition velocity<sup>12</sup> (cm-sec<sup>-1</sup>) given by:

$$\frac{\text{rate of dry deposition (pc.-cm}^{-2}\text{-sec}^{-1})}{\text{concentration in air (pc.-cm}^{-3})}$$

and has been measured at Chilton by exposing artificial surfaces beneath a rain shelter<sup>7</sup>. The variation of dry deposition velocity on to a horizontal sheet is shown in Fig. 4; increases sometimes ten-fold occur during the tropospheric periods. At the same time the 'mean effective' diameter of the airborne particles was determined by measurement of the retention efficiency of the filter media at various air speeds<sup>8</sup>.

It is clear from Fig. 4 that the increase in particle diameter is accompanied by an acceleration of dry deposition. It is not possible to account for the dry deposition velocity at any time by gravitational settling, since the terminal velocities, calculated by Stokes's Law, are much smaller than the observed deposition velocities. Presumably alternative processes such as diffusion and impaction<sup>12</sup> are mainly responsible; these processes must react sharply to the changes in particle diameter that are shown in Fig. 4. Conditions most favourable to enhanced dry deposition are produced in low-level clouds of fresh debris on more direct trajectories, which could account for the peaks in October 1961 and in October and November and December 1962 (Fig. 3) but not for September

1962. The increase in dry deposition has practical significance for the short-lived fission products, for example iodine-131 (8.1 days), that dominate the tropospheric debris, in that the dry deposition rate at Chilton (annual rainfall, 60 cm) approaches the rate of wash-out by rain<sup>7</sup>.

### Stratospheric Fission Products

In the long term the stratospheric component of nuclear debris is obviously more important both for meteorological tracer investigations and for the assessment of radiological dose. However, for reasons of expense and practicability it has been possible to measure this debris adequately and continuously only as it appears in the troposphere; its stratospheric history must be deduced mainly from these measurements in the troposphere.

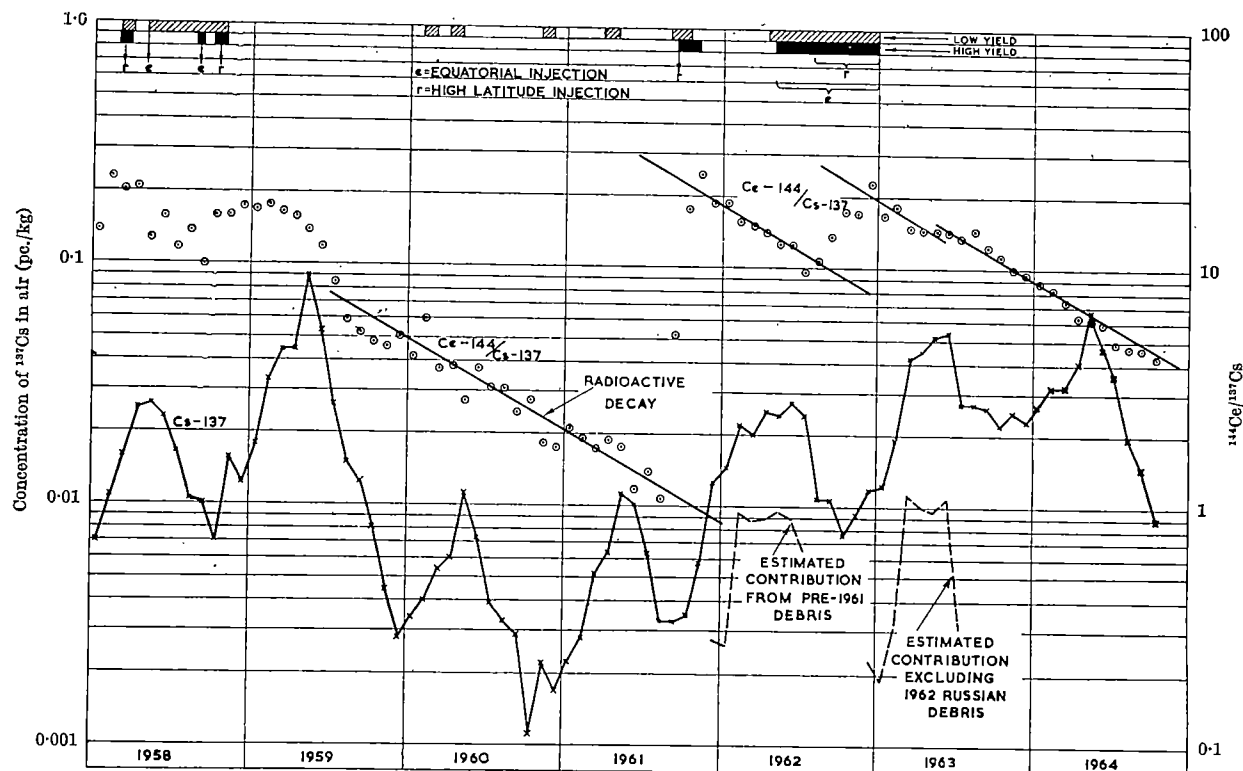
#### *Caesium-137 in surface air at Chilton.*

The concentration in surface air at Chilton of the long-lived fission product caesium-137 (30 years) recorded in Fig. 5 continues to demonstrate the annual oscillations observed by Stewart *et al.*<sup>13</sup>, although with peaks in 1962 and 1963 that are broader than in previous years. The end of the first phase of nuclear weapon testing was marked by an intensive series of explosions at the Russian sites in the autumn of 1958. In the following year the concentration of caesium-137 in air rose to an unprecedented maximum value (Fig. 5), fell rapidly to low but sustained levels during 1960-61, a quiescent period apart from minor explosions at French sites in Africa, and then late in 1961 rose in response to the latest phase of weapon testing.

Additional information may be extracted from these measurements by considering the ratio to caesium-137 of the shorter-lived fission product cerium-144 (285 days). The earlier behaviour of this ratio has been discussed in a previous paper<sup>14</sup> in which it was shown that the discontinuity and downward displacement in the curve for cerium-144/caesium-137 (Fig. 5) in mid-1959 corresponded to a substantial reduction in the proportion of Russian 1958 autumn debris and to a virtual exhaustion of the source of new debris in the lower stratosphere. It was then postulated that the uniform mixture (effective date of origin about January 1958) during the following two years contained only a minor contribution from the most recent explosions but consisted mainly of older debris from *Hardtack* (1958), *Redwing* (1956) and *Castle* (1954) presumably lodged in the upper stratosphere.

The mixing ratio of debris during this period was unaffected by the annual reinforcement of the lower stratosphere during the winters of 1959-60 and 1960-61. The mixture was, of course, violently disturbed by the later phase of nuclear weapon testing that started in September 1961. The relative amount of fresh long-lived radioactivity may be estimated from the ratio cerium-144/caesium-137 in Fig. 5 or from other fission product ratios<sup>4,5</sup>. The proportion of new caesium-137 in 1962 was not less than 60 per cent. The fission product ratios plotted in Fig. 5 also show that, at least until the next Russian test series started in August 1962, no radioactive material from the present American explosions at the equatorial test sites was transferred to the latitude of the United Kingdom. In 1963 the proportion until mid-year from the Russian series of 1962 was 75 per cent; the remainder was due to debris from the American 1962 series together with that from before 1962. These proportions may be used to estimate the continuing contributions of aged debris revealed in 1960-61. It can be shown<sup>4,5</sup> that the level of caesium-137 of pre-1961 vintage continued



Fig. 5.  $^{137}\text{Cs}$  and  $^{144}\text{Ce}$  in air at Chilton

without appreciable diminution into 1962 and, with greater uncertainty, into 1963 (Fig. 5). This is evidence of a long residence in the upper atmospheric reservoir of the earlier debris, evidence that is supported by other work, for example, by Gustafson *et al.*<sup>15</sup> and Lockhart *et al.*<sup>16</sup>. Since 1961 manganese-54 has appeared in samples of fall-out. If this nuclide is, as reported<sup>15,17</sup>, peculiar to the 1961 Russian explosions as a high-altitude tracer, then its behaviour is consistent with a long residence time in the upper atmosphere<sup>6</sup>.

The curves of fission product ratio used as described here to estimate the proportion of new debris show an additional feature that is important in the interpretation of atmospheric behaviour. Thus in the first halves of 1959, 1962 and 1963 the mixing ratios given by the cerium-144/caesium-137 ratios of Fig. 5—or more precisely by the shorter-lived and more sensitive ratios zirconium-95/caesium-137 and strontium-89/strontium-90 (refs. 4, 5 and 18)—reached uniform values during or immediately after the tropospheric periods of the preceding autumns. This rapid approach to a homogeneous mixture applies both to fresh debris injected into the lower stratosphere from equatorial and arctic latitudes (1959 and 1963) or to the mixing of fresh debris with the annual increment of old debris.

Another aspect of atmospheric mixing that has even greater significance in the long term is demonstrated by the behaviour of the fission product ratio at mid-year in 1959 and 1963, when the new debris directly injected into the lower stratosphere has been exhausted and is then succeeded by debris transferred from the long residence store above. In contrast to the reduction in cerium-144/caesium-137 ratio in 1959 noted here, the ratio increased slightly in mid-1963 to a value corresponding to a proportion of caesium-137 from the 1962 Russian series of ~90 per cent; this proportion has been sustained at least until the autumn of 1964. The implication of this increase in mid-1963 is that the upper atmospheric store, assumed to be responsible for the regular seasonal pattern of caesium-137 in 1960–61, has been massively reinforced at least for the 1963–64 season. The surface air concentra-

tion of caesium-137 observed in 1964 is consistent with the current proportion of 1962 Russian debris after allowing for a diminution of the 1960 level to (say) two-thirds by 1964 (corresponding to a mean residence time of 10 years). This comparison is uncertain because of the unknown but probably small contribution from the high-altitude component of the other recent series.

The meteorological significance of the fission product ratio and its effect on estimates of the content of the atmospheric reservoir will be discussed later.

**Measurements at other stations.** The results already discussed here for the Chilton samples may be compared with measurements made in samples from other stations in the United Kingdom and also from the network of rain collectors overseas<sup>6</sup>.

The annual mean value of caesium-137 in surface air at Chilton lies within 20 per cent of the average value for all the stations<sup>4,6</sup> in the United Kingdom (five stations in 1963). Similarly the annual mean value of the concentration of caesium-137 in rain collected at Milford Haven (52° N., 5° W.) lies within 20 per cent of the average value for all the stations<sup>4,6</sup> in the United Kingdom (ten stations in 1963). The annual mean values since 1954 of caesium-137 at Chilton are shown in Fig. 6 together with other parameters; the variation of caesium-137 in surface air at Chilton followed generally that of the peak value each year<sup>6</sup>. The ratio of the concentration in the composite sample of effective altitude 1.3 km over the eastern Atlantic to that at ground level (for equal masses of air) is a measure of the gradient of caesium-137 in the troposphere. This ratio had a steady value about 2.5, but then rose during the latest phase of nuclear testing. The increase in gradient was unlikely to have been caused solely by an additional burden, compared with previous years, of tropospheric debris directly injected, but probably reflected a source of debris in the stratosphere that was substantially sustained beyond the season of injection. The ratio of caesium-137 in rain (at Milford Haven) to that in surface air at Chilton (for equal masses of rain and air), conventionally defined as the 'wash-out factor', varied slightly about 700, but increased to 1,100 when the new

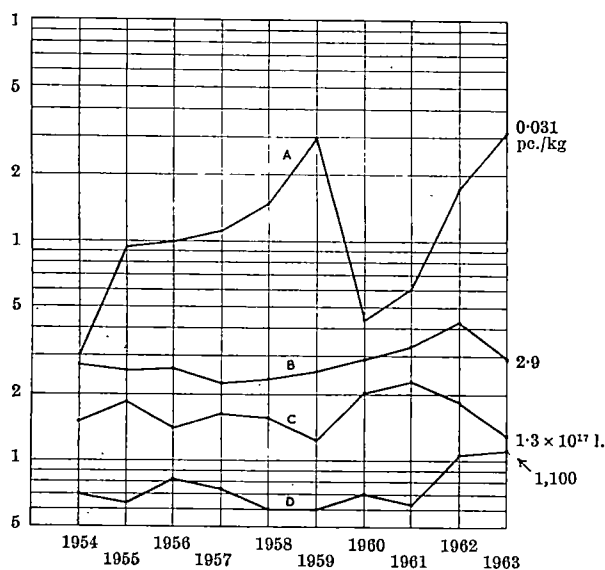


Fig. 6. Annual mean values

- A,  $^{137}\text{Cs}$  in air at Chilton (pc./kg)  
 B, Atmospheric gradient of  $^{137}\text{Cs}$ ,  $\sim 1.3$  km E. Atlantic (pc./kg)  
 C, Global deposition of  $^{90}\text{Sr}$  (Mc.)  
 D, Wash-out factor,  $\frac{^{137}\text{Cs} \text{ in rain at Milford Haven (pc./l.)}}{^{137}\text{Cs} \text{ in air at Chilton (pc./kg)}}$

debris appeared during 1962–63. The increase of the conventional wash-out factor with increase of gradient in the lower troposphere suggests that the true wash-out factor related to the air concentration at some indefinable rain-forming altitude has remained unchanged. An alternative explanation based on anisokinetic sampling of the larger particles of debris since 1961 would account for both the apparent increases in gradient and wash-out. However, the increase in particle size shown in Fig. 4 is probably insufficient to explain the magnitude of these effects.

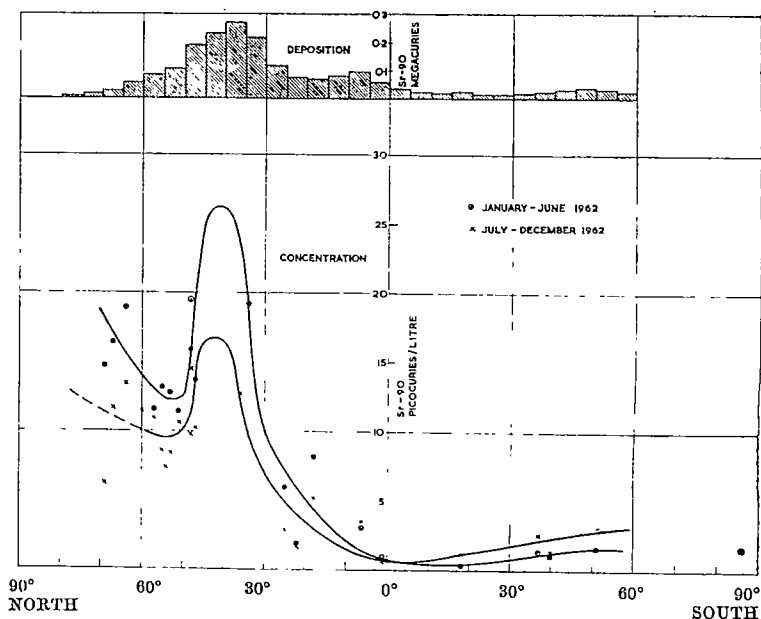
The relation between fission product concentrations measured in the United Kingdom and overseas may be derived from the strontium analyses of rain-water collected at about 25 stations<sup>5</sup>. The variation with latitude of the concentration of strontium-90 (28 years) in rain is shown in Fig. 7 for the two halves of 1962. The curves show the well-marked dependence on latitude observed by Stewart *et al.*<sup>13</sup>, namely, a peak in middle latitudes of the northern hemisphere and low concentrations in the southern hemisphere falling to virtually zero in the equatorial region. In addition there is some evidence of a rise northwards of 60° N. The seasonal variation is demonstrated over most of the northern hemisphere; the reversal of this relationship in the southern hemisphere, which would correspond to an out-of-phase seasonal variation, may be due to tropospheric debris from the American test series during 1962. Similarly the peak in the northern hemisphere in mid-latitudes in the second half of the year, contrary to 1958–59 (ref. 14), may have been enhanced by tropospheric debris from the 1962 Russian test series. To reduce the effect of local variations the annual comparison made in Fig. 6 is between the integrated global deposition of strontium-90 and the concentration in rain at Milford Haven. The global deposition<sup>6</sup> is obtained by integrating the latitudinal distribution of strontium-90 concentration in rain weighted by the distribution of rainfall with latitude<sup>19</sup> over the Earth's surface. In Fig. 7 the latitude distribution

of strontium-90 deposition for 1962 shows the same peak in mid-latitudes as does the concentration, with less-marked variation due to the modifying influence of the distribution of rainfall and surface area with latitude. The global integration is uncertain because of incomplete coverage by the measuring stations, including none over the sea which covers two-thirds of the Earth. Nevertheless, within this recognized limitation the strontium-90 ratio, nominal global deposition/concentration in rain at Milford Haven has varied by less than 50 per cent about  $1.7 \times 10^{17}$  l. during the period 1954–63. An integration by Collins<sup>20</sup> using American data from a greater number of sampling stations gives values of the annual global deposition of strontium-90 that are generally 20 per cent lower than those used here.

Having related the United Kingdom and overseas stations quantitatively, it is now of interest to make a qualitative comparison of effective age or origin of debris by investigating fission product ratios on the global scale. As a preliminary it is necessary to note that the behaviour of the fission product ratio strontium-89 (50.4 days) to strontium-90 in rain at Milford Haven behaved generally as the ratios zirconium-95/caesium-137 (ref. 6) or cerium-144/caesium-137, shown in Fig. 5, in surface air at Chilton. The variation with latitude of the ratio strontium-89/strontium-90 in rain<sup>4–6</sup> is illustrated in Fig. 8 from September 1961. Except during periods of weapon testing the ratio continued to be independent of latitude in the northern hemisphere, again demonstrating uniform mixing of debris<sup>14</sup> from the equator to 70° N. despite the marked dependence on latitude of strontium-90 shown in Fig. 7. There was no substantial transfer of Russian debris across the equator, although the rapid minor excursion during September 1961 (ref. 4) has considerable meteorological interest. The American explosions during April–November 1962 increased the ratios in the equatorial region, with greater proportionate effect in the southern hemisphere.

### Atmospheric Mechanisms

The introduction of artificial radioactivity from nuclear weapon tests has added to the methods available for tracing atmospheric movements. Thus fission products formed in the troposphere and stratosphere may be investigated in conjunction with the radioactivity produced by cosmic rays (stratospheric), radioactivity from radon emanation (terrestrial), water vapour (terrestrial)

Fig. 7.  $^{90}\text{Sr}$  in rain versus latitude, 1962

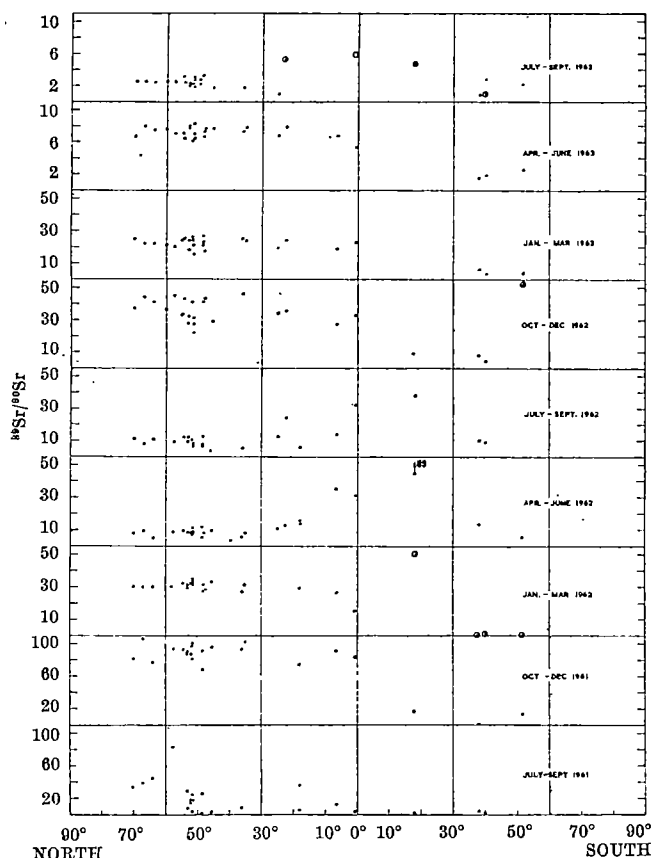


Fig. 8.  $^{88}\text{Sr}/^{90}\text{Sr}$  in rain versus latitude. ○, uncertain ratios due to low activity

and ozone (stratospheric) in order to follow the movement of air masses on a global scale.

**Transfer from stratosphere to troposphere.** The distinction between the behaviour of tropospheric and stratospheric debris was established by Stewart *et al.*<sup>11</sup>, who demonstrated that the fission products from low-yield tests in Nevada in 1951 had a mean residence time in the troposphere of about one month assuming exponential removal, whereas debris from the high-yield tests near the equator in 1954 returned to ground much more slowly. The mean residence time of one month in the troposphere has been implicitly applied also to debris transferred from the stratosphere. The principal agent of removal is rain; the mean residence time of water vapour is 10 days<sup>21</sup>.

Debris injected directly into the troposphere will therefore be removed virtually completely after several months; the presence in the troposphere of continuing fission product radioactivity with a regular seasonal variation must therefore be explained by transfer of debris from the stratosphere through the tropopause, conventionally regarded as a semi-permeable boundary. The mechanism of this transfer is not clear owing to the lack of direct experimental measurements in the vicinity of the tropopause, but the overall characteristics of the process may be calculated using an extension of the method of Staley<sup>22</sup>.

Thus the rate of change of fission product concentration  $C$  in surface air is given by the rate of transfer of stratospheric concentration  $S$  less the rate of removal from the troposphere:

$$\frac{dC}{dt} = KS - kC$$

where  $K$  is the fractional rate of net transfer across the tropopause and  $k$  the fractional rate of removal from the troposphere to the ground. Let the transfer activity  $K$

and the stratospheric concentration  $S$  be represented by sinusoidal factors such that:

$$\frac{dC}{dt} = \text{const.} \times [1 + a \sin(\omega t + \alpha_1)] [1 + b \sin(\omega t + \alpha_2)] - kC$$

where  $\omega = 2\pi/12$  (or  $30^\circ$ ) when  $t$  is measured in months.

Each sinusoidal factor describes a variation about a mean value having a period of one year and a phase angle measured from January 1. For the purpose of this calculation the removal factor  $k$  is considered independent of time and no attempt is made to account for the variation of each term with latitude. After multiplication:

$$\frac{dC}{dt} + kC = \text{const.} \times [1 + A \sin(\omega t + \phi_1) + B \sin(2\omega t + \phi_2)]$$

where the amplitudes  $a$ ,  $b$  and phase angles  $\alpha_1$ ,  $\alpha_2$  may be derived from  $A$ ,  $B$ ,  $\phi_1$  and  $\phi_2$ . Integrating, the steady state solution for  $C$  is given by:

$$C = \text{const.} \times \left[ \frac{1}{k} + \frac{A}{(k^2 + \omega^2)^{1/2}} \sin(\omega t + \phi_1 - \psi_1) + \frac{B}{(k^2 + 4\omega^2)^{1/2}} \sin(2\omega t + \phi_2 - \psi_2) \right]$$

where  $\tan \psi_1 = \omega/k$ ,  $\tan \psi_2 = 2\omega/k$ . The 12 measured monthly mean values of the concentration of caesium-137 in surface air at Chilton in 1963 have been fitted by the method of non-linear least squares to this expression. In principle it should be possible to extract values for the five unknowns—two amplitudes, two phase angles and  $k$  the tropospheric removal factor. However, the minimization process proved insensitive to variation in  $k$ , so it was necessary to assign an arbitrary value to  $k$  and then determine the other four parameters.

The result of the analysis, after putting  $k=1$ , that is, by assuming a mean residence in the troposphere of one month, is shown in Fig. 9. The lower pair of curves represent the stratospheric concentration and the transfer activity: the upper pair represent the measured values and a curve fitted to the computed values. Mathematically the two lower curves are interchangeable, but it seems reasonable to have the concentration in the lower stratosphere peaking in January–February in accordance with measurement<sup>23</sup> and the meteorological evidence of a winter subsidence.

This choice requires the curve of 'transfer activity' to peak in June–July. Staley has suggested<sup>22</sup> that the mechanism of transfer from stratosphere to troposphere was by baroclinic disturbances having maximum intensity in winter, in association with vertical movement of the tropopause, having maximum altitude in summer<sup>24,25</sup>—except in the equatorial region—thereby exposing air formerly in the stratosphere to tropospheric weathering and wash-out. The transfer activity curve of Fig. 9 is in phase with the variation of tropopause height but out of phase with the disturbance intensity, implying that the former effect is more important in transferring debris from stratosphere to troposphere. Similar curves, derived from analysis of the Chilton data for other years, show phase angles and relative amplitudes similar to 1963. The sinusoidal representation is an over-simplification since, for example, the lower stratospheric concentration probably has a sharper pulse-like shape<sup>23</sup>; this may explain the conflict with the meteorological evidence. Nevertheless the curves derived in Fig. 9 provide an explanation of the 3–4 month lag (in United Kingdom latitudes)<sup>23</sup> between maxima in the long-lived fission product concentrations in the lower stratosphere and troposphere. Furthermore, in the curve of lower stratospheric concentration the minimum in July–August (in the case shown in Fig. 9 for 1963) marks the end of the stratospheric 'season'. This is the time of year when the current store of debris has been exhausted and replenishment from the upper atmosphere is about to begin, with debris possibly



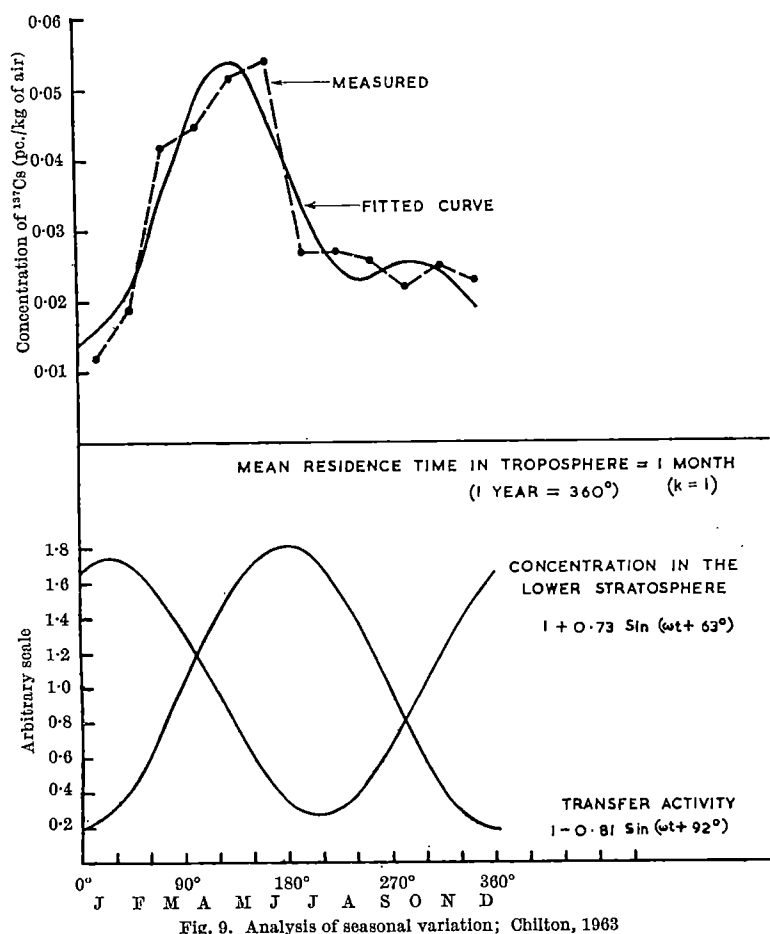


Fig. 9. Analysis of seasonal variation; Chilton, 1963

of a different mixing ratio or effective age as in 1963 (younger) or 1959 (older) but of the same age in 1960 and 1961 (Fig. 5).

This treatment of the mechanism of transfer between stratosphere and troposphere does not specify any variation with latitude. The existence of a latitude effect is indicated by Fig. 7; thus the peak of strontium-90 in middle latitudes is probably associated with the major gap in the tropopause and also with the tropospheric circulation system. Since the mixing ratio is independent of latitude, as shown by the strontium-89/strontium-90 ratios in Fig. 8, then the debris must be well mixed before distribution over the hemisphere.

**The stratosphere.** The model most suited to explain the behaviour of nuclear debris in the stratosphere seems to be that of meridional circulation limited in altitude. The circulation was originally postulated by Brewer<sup>26</sup> to explain measurements of humidity, and extended by Dobson<sup>27</sup> to account for seasonal variations in ozone. In the Dobson-Brewer circulation air rises through the equatorial tropopause, moves polewards and subsides into the lower stratosphere of temperate latitudes in late winter or spring. Goldsmith and Brown<sup>28</sup> proposed that the circulation did not extend upwards above roughly 25 km in order to accommodate, *inter alia*, the stationary behaviour of tungsten-185 tracer at the higher altitudes as observed by Feeley and Spar<sup>29</sup>, who considered that this behaviour was best explained by turbulent mixing rather than by an organized meridional circulation. In this compromise the circulation becomes slower with increasing altitude until above 25 km mixing is by small-scale turbulent diffusion or gravitational settling. The stratosphere, which is characterized by a zero or positive temperature gradient, is therefore seen as two regions. The lower region is affected by tropospheric circulations but is not mixed so rapidly as the troposphere, which has a

negative temperature gradient; in the upper stagnant region mixing is inhibited by the temperature structure. Exchange will occur between regions by limited vertical mixing and on a larger scale by subsidence in late winter at polar latitudes. Murgatroyd and Singleton<sup>30</sup> suggest that the subsidence extends above the stratosphere (above 50 km) into the mesosphere.

The evidence presented previously (see preceding section on "Stratospheric Fission Products") gives qualitative support to this model. The seasonal peaks observed (Fig. 5) in the troposphere—only wholly meteorological during 1960–61 and 1964 in that the amplitudes were not enhanced by recent injections—continue to support the concept of a phased polar subsidence. The lower stratosphere mixes debris injected at equatorial and arctic latitudes over a period of six months but only within the hemisphere (Fig. 8). The uniformity of mixing demonstrated by the fission product ratios after mid-1959 until disturbed by the new explosion series, and after mid-1963 relates to debris stored in the upper atmosphere. It is difficult to reconcile this homogeneity of debris of such different ages with the characteristics of the upper stagnant region of the stratosphere. It is possible that the high-altitude debris penetrated into the mesosphere where comparatively rapid mixing is permitted by a reversal of the temperature gradient to negative values. In such an extension to the stratospheric model, as invoked by Kalkstein<sup>31</sup> to explain the distribution of rhodium-102, the reservoir of nuclear debris would lie above the stratosphere, the upper stagnant region

of which would serve as a buffer rather than as a store. If the mean residence time in the reservoir is taken to be 10 years (nominal), then the annual winter subsidence would remove about 10 per cent downwards to the lower stratosphere for subsequent distribution to the troposphere.

Having established that the debris sampled in the 1963–64 season originated at high altitudes, it is now possible to estimate the content of the atmospheric reservoir, again assuming exponential depletion, by comparison of caesium-137 concentrations in air (Fig. 5) and the estimates of the global deposition of strontium-90 (ref. 6). The mean concentration of caesium-137 in air at Chilton during 1964 will probably lie between that for 1962 and 1963. The corresponding global deposition derived from the average for these two years<sup>6</sup> would be about 2.5\* megacuries (Mc.) of strontium-90; one-tenth of this (= 0.25 Mc.) would represent the contribution from the aged debris of the period 1960–61 (see preceding section on "Caesium-137 in Surface Air at Chilton") and is consistent with a mean residence time for that debris of 10 years. The reservoir of aged debris therefore contains  $10 \times 0.25 = 2.5$  Mc. If the newer debris from the 1962 Russian explosions is effectively exhausted during 1964, which is unlikely, then the total reservoir at January 1964 is  $2.5 + 2.25 = 4.75$  Mc. However, the trend of the fission product ratios up to the autumn of 1964 suggests that the 1962 Russian debris has reinforced the reservoir more permanently. In this case the content, again assuming a nominal 10-year mean residence time, would be  $10 \times 2.5 = 25$  Mc. of strontium-90. In this manner it is possible to extract two estimates for the reservoir of strontium-90 at January 1964, namely, a lower limit\* of

\* Volchok—*Science*, 145, 1451 (1964)—extrapolates from the New York deposition and predicts that the global deposition of strontium-90 in 1964 will be 2.48 mc.

4.75 Mc. and an upper limit of 25 Mc. For comparison the cumulative global deposition at this time was nearly 10 Mc. (ref. 6). According to Machta *et al.*<sup>32</sup> the atmospheric inventory in January 1963 was 6.6 Mc.: this reduces to about 4 Mc. in January 1964 after subtracting the 1963 deposition<sup>6</sup>. This inventory is based on sampling by aircraft and balloon up to 30 km and would ignore any debris stored, as postulated here, in the upper stratosphere or mesosphere at ~50 km.

Because of the well-marked seasonal behaviour observed in the troposphere it is clear that the bulk of each annual increment from upper to lower stratosphere occurs by the winter/spring subsidence. It is possible that more continuous delivery also occurs by turbulent diffusion and gravitational settling that might account for the shallowness of the autumn trough observed in 1963 (Fig. 5). Comparison of the effectiveness of diffusion and gravity is prevented by the lack of experimental estimates of the size of the particles while in the stratosphere. By calculation of the time of fall from, say, the top of the stratosphere at 50 km to the bottom of the stagnant compartment at 25 km, it can be shown that, at present, gravity would have had significant effect for particle diameters (unit density) in the stratosphere of ~1 $\mu$ . For the aged debris observed in the troposphere in 1961 the corresponding lower limit to particle size while in the stratosphere tends towards 0.1 $\mu$ . The 'mean effective' particle diameter<sup>6</sup> observed since 1958 near ground-level (ignoring tropospheric debris) lies in the region 0.4–0.8 $\mu$  (Fig. 4); this includes the effect of coagulation after the particles have entered the troposphere. It follows that the existence of a significant gravitational component in the upper stratosphere is possible in 1961 but improbable during 1963–64.

We thank Dr. A. G. Forsdyke and Dr. R. J. Murgatroyd of the Meteorological Office at Bracknell for their advice. Dr. Forsdyke provided the meteorological material for the discussion leading up to and including the trajectories of Figs. 2 and 3.

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## FOREST GROWTH AND GLACIER CHRONOLOGY IN NORTH-WEST NORTH AMERICA IN RELATION TO SOLAR ACTIVITY

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FOR the period A.D. 1750–1960, a correlation of +0.47 ( $P < 0.05$ ) was found between bole growth per decade of forest-grown Engelmann spruce (*Picea engelmannii* Parry) in the Yoho Valley, B.C., Canada, and mean yearly sunspot number per decade<sup>1</sup>. Information on sunspot number from 1700 until 1749 not previously available<sup>2</sup>, together with a sample of *Picea* growth from a nearby area, prompted a reconsideration of this correlation. The additional sample was made in the Little Yoho Valley (51° 31' N., 116° 34' W.) 6 km south-south-west of the Yoho Valley. Increment borings were taken from *Picea engelmannii* which grow in a forest adjacent to the end moraine of the President Glacier<sup>3</sup>. Immediately after each increment core was collected, a drawing of the core was made in order to avoid the distortion in ring width which may occur with differential shrinkage during storage. From these drawings, measurements were made of ring width per decade and the yearly increase in bole basal area per decade calculated by the method previously described<sup>1</sup>.

The result of bole growth measurements in the Little Yoho Valley showed higher growth in late 1700, mid-late

1800 and early mid-1900 and lower bole growth from mid-1600 to early 1700, early 1800 and late 1800. This temporal pattern was similar to that found in the Yoho Valley<sup>1</sup>. For this reason, data on bole growth from the two adjacent valleys were combined (Table 1) for all trees 300 or more years of age (total 49 trees). The correlation coefficient for these data between tree bole growth per sunspot cycle and mean yearly sunspot number per cycle is +0.63 ( $P < 0.001$ ), demonstrating a more significant correlation than in the original investigation.

Trees growing in the Yoho Valley were suppressed in growth up to 150 m from the end moraine during the decade of maximum advance (1840–50) of the Yoho Glacier<sup>1</sup>. Following this decade, forest growth suppression was present only within 5 m of the end moraine. The President Glacier, which advanced in the Little Yoho Valley during 1830–40, had a similar depressant effect on the growth of some nearby forest trees<sup>3</sup>. If tree growth data in Table 1 for the two sunspot cycles during 1834–56 are eliminated because of the growth-depressant effect of nearby glacier ice, then a recalculation of the correlation coefficient between tree growth and mean yearly

Table 1. BOLE GROWTH OF FOREST GROWN *Picea engelmannii* IN EASTERN B.C., CANADA, IN RELATION TO SUNSPOT ACTIVITY, 1699-1964

	Mean yearly sunspot No. per cycle	Length sunspot cycle (yr)	Length sunspot cycle, previous to given maximum (yr)	Mean basal area increase (cm <sup>2</sup> /yr)
1695-1964*	96	10.0	10.4	9.6
1945-1954	75	10.1	10.1	9.8
1776-1784	69	9.2	8.7	10.0
1834-1843	65	9.6	7.3	8.4†
1785-1798	60	13.6	9.7	9.5
1707-1775	59	9.0	8.2	10.2
1868-1878	56	11.7	10.5	9.3
1934-1944	55	10.4	9.0	9.4
1724-1733	54	10.5	9.3	8.9
1844-1856	53	12.5	10.9	9.0†
1734-1744	52	11.0	11.2	9.0
1857-1867	50	11.2	12.0	9.7
1914-1923	45	10.0	10.6	9.2
1756-1766	43	11.3	11.2	9.5
1924-1933	41	10.2	10.8	8.5
1745-1755	40	10.6	11.6	9.1
1824-1833	40	10.6	13.5	8.4
1890-1901	38	12.1	10.2	8.6
1879-1889	35	10.7	13.3	8.8
1902-1913	31	11.9	12.9	9.4
1713-1723	30	11.5	12.7	9.0
1799-1810	23	12.3	17.1	9.0
1811-1823	18	12.7	11.2	8.6
1699-1712	16	14.0	12.5	8.6

\* Sunspot number of 18 estimated for 1964, the probable last cycle year<sup>20</sup>.

† Growth diminished from influence of nearby ice mass.

sunspot number per cycle results in a value of  $+0.76$  ( $P < 0.001$ ).

Since standardized measurements began in 1700, mean sunspot number per cycle has tended to alternate between periods of three or more low-activity cycles and periods of three or more high-activity cycles. Six such periods can be distinguished between 1700 and 1964. The first began in 1724, extended to 1798, and can be separated into an interval of 3 cycles from 1724 until 1755 which had a mean yearly sunspot number of 48 and an interval from 1756 until 1798 which had a mean yearly sunspot number of 57. These intervals were followed by a period of three low-activity cycles during 1799-1833 which had a mean yearly sunspot number of 26. A high-activity period of 4 cycles occurred during 1834-78 which had a mean yearly sunspot number of 56 and which was followed by three low-activity cycles with a mean yearly sunspot number of 35 during 1879-1913. A high-activity period began in 1914, is still occurring, and may extend to near the end of the twentieth century<sup>1</sup>. Mean yearly sunspot number from 1914 until 1964 was 62.

In addition to the foregoing six sunspot periods, two previous periods can be extrapolated from data on sunspot cycle length. From 1699 until 1964, there was a highly significant negative correlation ( $r = -0.53$ ;  $P < 0.01$ ) between mean yearly sunspot number per cycle and cycle-length (Table 1). A closer correlation ( $r = -0.64$ ;  $P < 0.001$ ) existed over the same period between mean yearly sunspot number per cycle and the interval from the maximum of a given cycle to the maximum of the preceding cycle. The lower significance of the correlation between cycle length and sunspot number is the result of the tendency for a high-activity cycle to be longer than is usual when it is followed by a low-activity cycle in which the new sunspots are of low vigour and tardy appearance. An example of this is the high-activity cycle from 1785 until 1798 which had a mean yearly sunspot number of 60, but had an unusually long cycle length of 13.6 years which included an interval of 10.7 years between the cycle maximum and subsequent minimum. The cause of this 10.7 year interval was presumably the tardy appearance of sunspots of the subsequent cycle (1799-1810) which was of very low vigour with a mean yearly sunspot number of 23. The period from the maximum of the 1785-98 cycle to the maximum of the preceding 1776-84 cycle was 9.7 years which more accurately characterizes the vigour of the 1785-98 cycle.

The first of the two extrapolated periods was judged to occur from around 1656 until 1723 and contained 6 cycles of low sunspot activity as determined by: (1) a mean cycle length of 11.3 years and a mean length from previous

to given maxima of 11.5 years; (2) a sunspot dearth reported during 1645-1715 (ref. 4), 1660-1720 (ref. 5) and 1639-1720 (ref. 1); (3) a mean yearly sunspot number for the last two cycles of the 1656-1723 period of 22 (assuming 1699,  $R = 5$ ). The other extrapolated sunspot period was from 1611 until 1655 and was probably of medium activity, since the mean cycle length was 11.0 years and the mean length from previous to present maxima was 11.1 years.

Table 2. BOLE GROWTH OF *Picea engelmannii* AND GLACIAL ACTIVITY IN NORTH-WEST NORTH AMERICA IN RELATION TO SUNSPOT ACTIVITY, 1611-1964

Sunspot activity period	Mean yearly sunspot No. (R) per period	Mean sunspot cycle length previous to given maximum (yr)	Mean bole basal area increase cm <sup>2</sup> /yr	No. glacier advances (40% time lag) All data	Best data only
1914-1964	62	10.2	9.3	2	1
1756-1798	57	9.4	9.8	5	1
1834-1878	56	10.2	9.1 (9.5)†	4	1
1724-1755	48	10.7	9.0	3	1
1611-1655	—	11.2	9.0†	3	0
1879-1913	35	12.1	8.9	7	3
1856-1723	22*	11.5	8.7	10	4
1799-1833	26	13.9	8.7	13	6

\* Data for 1699-1723 only.

† Neglecting 1834-1850 because of tree growth depression from nearby glacial ice.

‡ Based on 18 trees only, growth relative to 1856-1723.

Mean forest tree growth, in relation to the foregoing eight sunspot periods, decreased with decreasing solar activity per period (Table 2). The sunspot cycle periods in Table 2 were aligned in order of decreasing mean yearly sunspot number which was supplemented by sunspot cycle length for the two periods for which sunspot numbers were not available. Forest tree growth decreased from 9.3 cm<sup>2</sup>/yr for 1914-64 and 9.8 cm<sup>2</sup>/yr for 1756-98 to 8.9 cm<sup>2</sup>/yr for 1879-1913 and 8.7 cm<sup>2</sup>/yr for 1656-1723 and 1799-1833. This relationship was considered to reflect growing season temperature<sup>1</sup>, with higher tree growth likely to occur during a longer and warmer growing season and lower growth to occur during a shorter and colder season. There is, therefore, indirect evidence that summer temperatures in the Yoho area may be related to solar activity, with higher temperatures occurring during periods of greater sunspot number. Yearly world temperature patterns since 1750 have also been tentatively related to sunspot activity<sup>8</sup>. The recent demonstration<sup>7</sup> of atmospheric temperature above 300 km increasing with higher solar activity may provide a mechanism for a solar activity-climate correlation if a connexion can be found between upper air and surface temperatures.

**Glacier chronology.** A shortened season for tree growth is probably coincident with an increased period in spring and autumn when glacier budgets at high elevations are positive. Such an increased ice accumulation period may be sufficient to result in a positive yearly ice budget and, eventually, in ice advance at the terminus. An increased likelihood of ice advance during the close of and following periods of lower solar activity was demonstrated for all maximum advances and major readvances of glaciers in Alberta and British Columbia, Canada, and Washington and Oregon, U.S.A.<sup>1</sup>. To examine further this relationship, dates from subsequent investigations and for minor ice advances were added to the original compilation as follows: subsequent work, major advance, Boulder Glacier about 1750 (ref. 8), President Glacier 1714 (ref. 3); readvance, Boulder Glacier 1850, 1880, 1954 (ref. 8), President Glacier 1832 (ref. 3); minor readvance, Yoho Glacier 1890's, 1914 (ref. 1), Emmons Glacier 1896 (ref. 9), Nisqually Glacier 1893-1907 (ref. 10); Freshfield Glacier 1881 (ref. 11); Helm Glacier 1700-12 (ref. 12); Commander Glacier 1890's (ref. 13), 1954 (ref. 14). A total of 47 dates was available from this compilation.

Sixty-eight per cent of glacial advance since 1640 in the area noted here occurred during 1700-32, 1811-53 and 1890-1914 towards the close of, and immediately following, the three periods of lowest sunspot activity, 1656-



1723, 1799–1833 and 1879–1913. There may be a lag of up to 18 years between a period of heavy snow accumulation in an icefield and the conversion of this snow to ice and its maximum distance flow from the icefield<sup>1</sup>. The existence of this lag period may explain the tendency for glacial advance to occur immediately after, as well as towards, the close of periods of low sunspot activity. In summarizing the glacier chronological dates noted here, therefore, a period of about 15 years (40 per cent of the length of the subsequent period) was added to the close of each sunspot period and subtracted from the following period (Table 2). The result of this manipulation clearly shows a tendency for glacial advance to occur during and immediately after low sunspot periods. There is a  $\chi^2$  of 7.7 ( $P < 0.006$ ) for the hypothesis that an equal number of glacial advances should occur in the 4 high sunspot periods and in the 4 low periods which demonstrates the improbability of the greater number of ice advances in the low sunspot periods occurring by chance.

The majority of data on periods of glacial advance noted here were determined by adding the age of the oldest tree on a given mineral soil surface to the average length of time for tree invasion of that surface following its deposition at the time of maximum ice advance. The most useful mineral surface for this examination was the material of the end moraine on the side away from the ice. This period for tree invasion has been measured or estimated as 5 years on Mount Hood, Oregon<sup>16</sup>, 1–16 years on Mount Rainier, Washington<sup>9</sup>, 30–50 years on Mount Rainier, Washington<sup>10</sup>, 5–23 years for the same area as the preceding investigation<sup>9</sup>, 12–17 years on unspecified sites in the Canadian Rockies<sup>11</sup>, 20–43 years on surfaces of known age in the Yoho Valley, Canadian Rockies<sup>1</sup>, and 25–32 years on the basis of a tree which started growth in 1862 and which grew adjacent to a mature forest in which there was a marked growth decline indicating glacial advance from 1830–37 in the Little Yoho Valley<sup>3</sup>. These results suggest that the period for tree invasion increased from the south to the north and from lower to higher elevations. Other reasons for these differences in invasion period have been discussed<sup>1</sup>. A more rigorous selection of glacier chronologies was made by choosing only dates from direct observation and from information on mature trees which grew immediately outside the glaciated area and which gave evidence for a period of ice advance through: (1) drastically reduced growth caused by cold-air drainage or the nearby presence of ice; (2) drastically increased growth through removal of tree competition; (3) frost cracks; (4) changes in ring pattern through tilting of the bole; (5) scarring from boulders or ice blocks. These dates (last column, Table 2) support the glacier activity–sunspot cycle relationship noted here. More than three-fourths of the glacial advances occurred during 1707–15, 1832–47 and 1893–1914, during the close of and immediately following the three periods of lowest sunspot activity.

**Climatic pattern.** In an analysis of climatic pattern during A.D. 800–1950<sup>1</sup>, evidence was summarized for a period of generally lower solar activity from around 1400–1900. This period was concurrent with a period of increased glaciation in West Greenland<sup>18</sup>, north-west North America<sup>1</sup>, western Europe<sup>15</sup>, the Caucasus and Soviet Arctic<sup>17</sup>, East Africa<sup>18</sup> and presumably elsewhere. Further evidence for a low solar activity during the 1400–1900 period is the length of the sunspot cycle during this period compared with cycle length in preceding and subsequent periods. Data in Kanda<sup>16</sup> on the number of Oriental solar observations from 27 B.C. to A.D. 1930 were analysed for cycle length. Sufficient observations are available to establish cycle length for the fourth, ninth, twelfth and thirteenth centuries (Table 3). If solar observations were separated by more than one-half an average cycle length (about 5 years), they were regarded as representing separate sunspot maxima. The result of this analysis, compared with sunspot cycle lengths during

Table 3. PERIODICITY OF SUNSPOT MAXIMA AND LENGTH OF SUNSPOT CYCLE IN FOURTH, NINTH, ELEVENTH AND TWELFTH CENTURIES A.D.

(Data from ref. 16)			
300–399	800–899	1100–1199	1200–1299
302.0 } ††	807.9	*1100.4	1202.8 }
*306.0 }	Missed	1108.7	*1204.3 }
311.3 }	*827.3 }	*1117.1 }	Missed
*313.9 }	830.0 }	1120.5 }	*1227.0 }
322.1	840.0	*1129.2 }	1239.0
Missed	851.9	1130.2 }	*1247.6 }
342.2	865.1	1137.7 }	1258.7 }
354.2	875.6	*1140.0 }	*1261.1 }
359.9	*882.6	1148.4	Missed
372.6	888.0	*1150.7 }	1277.4
388.9	8 cycles 80.1 yr	*1158.7 }	*1288.9
398.4	10.0 yr/cycle	1160.5 }	8 cycles 86.1 yr
9 cycles 96.4 yr		1171.9	10.8 yr/cycle
10.7 yr/cycle		*1181.2 }	
		1185.8 }	
		*1193.0 }	
		1193.9 }	
		1202.8 }	
		*1204.3 }	
		10 cycles 102.4 yr	
		10.2 yr/cycle	

† Brackets couple observations made during the same sunspot maximum period.

†† Dates with asterisk from aurorae observation, other dates from sunspot observation.

1600–1964 (Table 4), shows that cycle lengths tended to be lower in the fourth, ninth, twelfth and thirteenth centuries than for the 1600–1900 period, and to be more comparable with the period 1900–64. This suggests that in view of the negative correlation between sunspot number per cycle and cycle length demonstrated earlier, sunspot activity in the fourth, ninth, twelfth and thirteenth centuries was higher than for the 1600–1900 period. These results support information on the total number of Oriental solar observations noted earlier<sup>1</sup> and reproduced in Table 4.

Evidence is accumulating that for Asia, Europe, North America and, probably, the southern hemisphere, an era of widespread glacial advance occurred at high elevations from the late fifteenth and early sixteenth centuries to about the end of the nineteenth century. This minor glacial epoch was preceded by a period of 1,000 or more years during which many mountain glaciers were smaller or had completely melted<sup>1,3,15,17,18</sup>. As suggested from the evidence summarized in Table 4, this minor glacial period was apparently synchronous with a period of lower than average sunspot activity. Since 1900 and for many, if not all, of the 12 centuries before about A.D. 1500, there has apparently been a higher average sunspot activity and a period of mainly glacial retreat or stagnation.

I believe this presentation strengthens the evidence previously given in support of the solar radiation climatic hypothesis<sup>1</sup>. During the period 1610–1964 for which sunspot observations are available, the great majority of advances of ice in north-west North America occurred at the close of and immediately following periods of low sunspot activity. Glacial retreat or stagnation occurred during periods of higher sunspot activity. Tree growth in the Yoho National Park area of British Columbia was positively correlated with sunspot activity during this period, which may reflect a relationship between growing season, climate and solar activity. There is also evidence, although fewer data are at present available, that glacial advance during the past several millenia has predominated in centuries of lower average sunspot activity, and glacial

Table 4. SOLAR ACTIVITY, ICE OFF ICELAND AND GLACIAL ACTIVITY

Period A.D.	Mean sunspot cycle length (yr)	No. observations sunspots plus aurorae*	Ice off Iceland weeks/yr†	Glacial activity in mountain regions, northern hemisphere
801–900	10.0	9	0.1	Stagnation
1101–1200	10.2	16	0.0	Stagnation
1301–1400	10.5	—	8.6	Retreat
1501–1600	10.7	11	—	Stagnation
1701–1800	10.8	8	7.8	Stagnation
1801–1900	11.0	4	22.0	Advance
1901–1964	11.3	5	25.3	Advance
1965–1990	11.6	0	40.8	Advance

\* From ref. 16.

† From ref. 19.

retreat and stagnation have predominated in centuries of higher average sunspot activity.

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<sup>3</sup> Bray, J. R., *Science*, **144**, 287 (1964).

<sup>4</sup> Maunder, E. M., *J. Brit. Astron. Assoc.*, **32**, 140 (1921-1922).

<sup>5</sup> Douglass, A. E., *Climatic Cycles and Tree-growth* (Carnegie Inst. Wash. Publ. No. 289, 1928).

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<sup>19</sup> Schell, I. L., *Geograf. Ann.*, **43**, 354 (1961).

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## COMMUNAL FOOD DISTRIBUTION AND DIVISION OF LABOUR IN AFRICAN HUNTING DOGS

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IN the Serengeti Plains of East Africa a pack of hunting dogs (*Lycaon pictus lupinus* Thomas 1902) was observed from a specially equipped Land Rover for a total of 523 h between February and May 1964. The pack contained 6 males and 2 females, of which one had 4 and the other 11 pups. These were about 1 and 3 weeks old, respectively, as observations began, and lived in one of the numerous burrows which aardvarks, warthogs, or hyaenas dig in this area. All members of the pack behaved very tolerantly to one another. Only the females had minor disagreements, because they competed in caring for the pups. Each of them nursed all the young and tried to steal them from the others. When the older young at about seven weeks could already run well, lions repeatedly investigated the burrow, causing the pack to move to another burrow about 1,000 yards away from the first. The 4 younger pups were thereupon carried singly in their mother's mouth to the new site. Some days later the pack again moved, and thereafter increasingly readily, until the pack with 3-month-old cubs no longer sought out a burrow to rest, but lay on the grass in the open. Although initially the adults chased off (but did not kill) any hyaena or jackal approaching within a distance of 500 yards from the home burrow, they now allowed these animals to approach within 50 yards of the half-grown pups.

The hunting range of the pack in February, as the great antelope herds trekked across the plains, covered about 20 square miles. In May but little game remained, so the dogs had to extend their forays to an area of 60-80 square miles. Four other packs of hunting dogs adjoined this area. Apparently hunting dogs remain in the same area as long as they can find food there, and there is then no mixing of packs.

The pack had fixed times for hunting—in the morning about sunrise (6.50) from 6.30 until 7.30 and in the evening from 18.00 until 19.00 (sunset at 18.50). Observed hunts were always successful. The numerous Thomson's and Grant's gazelles took flight only when the racing dog pack had approached within 600-800 yards. From this distance, however, a hunting dog can overhaul either of these antelope in direct pursuit. When the pursuer is hard on its heels, the quarry tries to escape by zigzagging and often actually does succeed in gaining some ground. If the hunter has overtaken his quarry two or three times without being able to seize it in the flank, he is so exhausted that he has to give up the chase, panting heavily. Success is more certain when several dogs pursue the same antelope. In its avoiding manoeuvres it is likely to come closer to one of the other dogs, which can then catch it. I never saw a true relay hunt.

Besides gazelles, the hunting dogs killed gnu calves, which they tried to separate from the herd. As soon as

the dog pack approached a herd of gnus, the bulls standing around the edges tried to drive them off by charging. The dogs avoided the attacks and tried each on his own to penetrate to the core of the herd, where the calves and their mothers were congregated. So long as these all packed tightly together the hunters were powerless, but sooner or later a calf broke out of the herd, followed by its mother, and in an instant all the dogs were after it. The mother defended it for a time, but soon followed the stampeding herd. Outside the daily hunting periods, Thomson's gazelles and straying gnu calves several times approached within 300-500 yards of the pack as it lay by the pups' burrow, but were not attacked. At night these visually oriented dogs did not go out hunting.

The quarry was felled by bites in the flanks and hind quarters; as soon as one prey was down, all dogs congre-

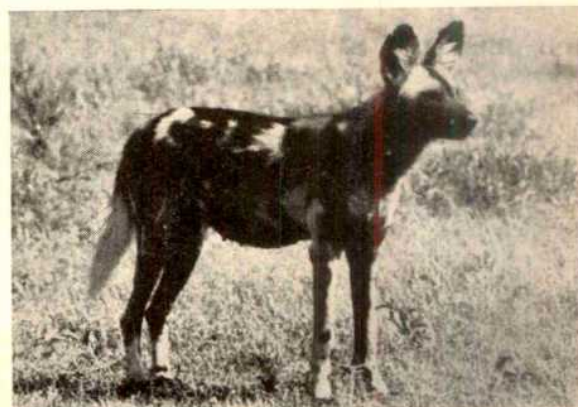
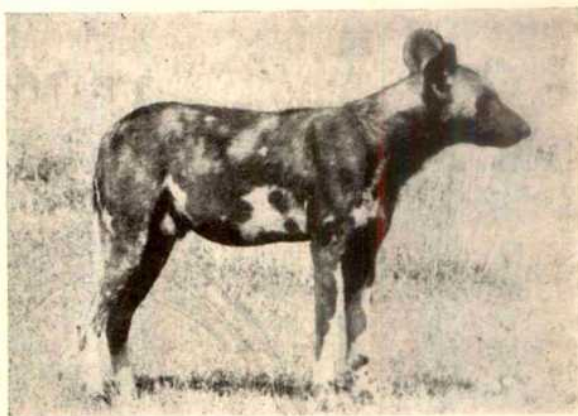


Fig. 1. African hunting dogs. Top, male; bottom, female (Photos: W. Kühme)



gated at this kill. A second kill was sometimes made afterwards. The dogs tore open the belly of the still living quarry (without a killing bite) and bolted down hand-sized scraps of meat and skin without chewing. After 5 min they returned to the burrow and disgorged the meat in front of the begging pups and the guards who had stayed behind. Pup-guarding during the hunt was shared in turns by the females and by particular males. The guards could also disgorge in their turn, and when this meat was then taken up by another adult, which passed it on further later on, such a piece of meat could wander through several stomachs before finally being digested. So long as the pups were very small and could not easily swallow the big pieces of meat, the females begged for more meat, which they chewed up and then (over a period of hours) repeatedly disgorged for the pups; they were the storage and preparation containers for the pups' food. The stomachs of all members of the pack, however, served to transport the hastily dismembered prey into safe cover or, when pups were present, into the centre of the pack, where the meat was distributed to begging, and thus hungry, members of all ages. Although the young swallowed meat as early as 14 days, they were suckled for 10–12 weeks. Up to that point they lived in burrows, and there was no indication that they left them to visit water-holes. They were seen to go to water for the first time when they accompanied the adults on their long forays and no longer returned to the burrows. In the first months the pups evidently obtained enough moisture from the fresh bloody meat and from their mothers' milk.

The communal treatment of food supply among pack members made a true division of labour between guards and hunters possible. The first groups of pre-humans

may have formed under similar conditions. A few members of the horde took over as guards the protection of the young, while the rest went about gathering food. In many mammalian societies the greatest share of the food goes to the strongest, dominant individual, generally a male. He then generally has especially important functions within the group. In a pack of hunting dogs all the adults are equally qualified to fulfil any of the functions: catching prey, defending the family, or feeding meat to the young. This ought to require absolute absence of rank, and no indications of rank order were discerned in the pack investigated. One particular male was very often seen in company with one of the females, but did not otherwise stand out in the society. In hunting, at the kill, in distributing the food or during the mid-day sleep at the burrow, all combinations in working and living together were possible. This tolerance of competitors, which human beings find so difficult, was achieved by a special ritual.

In the same way as the pups, the adults also begged from one another, by pushing from the side with the nose against the mouth of the food-bearer and even, in undoubtedly aggressive mood, biting him in the jowls. This juvenile behaviour was also shown by the adults in ritualized form as a greeting, on meeting one another. Not only did they push at one another's jowls, but also a male sometimes licked at the udder of a female, or a female crept under a male as a nursing pup under its mother. All thus behaved rather like children of one great family, in which only the mother occupied a special position, in that she greeted less intensively than the rest.

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## REFLEXIONS ON MANAGEMENT SCIENCE

**I**N his inaugural lecture at the Imperial College of Science and Technology, University of London, on November 3, Prof. Samuel Eilon, head of the Department of Industrial and Management Engineering, discussed the difficulties of defining precisely the scope of his Department. While Prof. Eilon has been variously described as a production engineer, an industrial engineer, an operational research worker, and a management scientist, there is a measure of overlap between these various titles. An authoritative definition by the Institution of Production Engineers states that a production engineer is "one who is competent by reason of education, training and experience in technology and management to determine the factors involved in the manufacture of commodities and to direct production processes to achieve the most efficient co-ordination of effort with due consideration to quality, quantity and cost".

While the knowledge and understanding of production processes are important aspects of the production engineer's work, the definition also stresses the need for managerial competence. Determination of factors which affect production, the characteristics of the product and its cost, the need to plan utilization of resources and to co-ordinate activities—all these call for the same faculties of analysis and synthesis that are expected of a competent manager. Though the definition does not specifically state involvement in human problems or in top management policy decision processes, the understanding of these topics is often implied.

Fig. 1 provides one concept of the interrelation between subjects in which the production engineer is involved, and these can be broadly grouped in two areas.

(a) Production technology, which is the investigation of manufacturing processes and machines, their technical

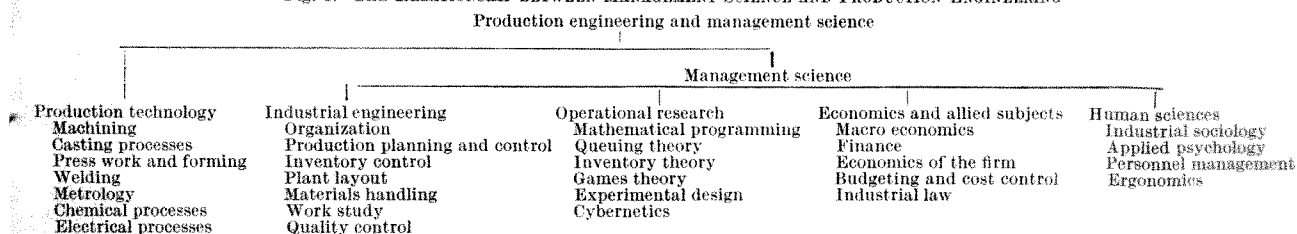
characteristics and limitations, as well as economic considerations relating to selection of processes and machine utilization. Mechanical processes (such as machining, casting, forming and welding) also play a prominent part in production technology, and chemical and electrical processes could also be added.

(b) Management science, which is the investigation of methods by which industrial activities are planned and controlled. This area could be considered under four headings: First, industrial engineering—a generic term which relates to the methods of planning and control of technical industrial operations, involving men, machinery and plant. This includes organization, production planning, stock control, plant lay-out and materials handling, work study and quality control. Secondly, operational research, or the application of scientific research—analytical, experimental or quantitative—to industrial and business problems with the object of providing a more analytical basis for making predictions and decisions. The third field is that of economics (both macro and micro), budgeting, cost control, industrial law and marketing. The fourth is related to the behavioural sciences, and covers the examination of social and psychological problems of human operators in relation to their work.

One aspect of this approach is worth noting, namely, that all subjects listed in the management science area are equally relevant to industrial situations which do not involve manufacturing processes. Scheduling, for example, is an important function in a factory, where resources of materials, machines and men have to be matched to produce a projected plan of integrated activities; but this is just as important a function in designing a time-table for a construction site, for schools,



Fig. 1. THE RELATIONSHIP BETWEEN MANAGEMENT SCIENCE AND PRODUCTION ENGINEERING



or for a transportation system. The latter are not production activities in the conventional sense, unless the definition of production is stretched to include all service activities.

### Industrial Engineering and Operational Research

Turning to industrial engineering, Prof. Eilon indicated that the difficulties in classifying management or business activities are demonstrated in the attempt made by L. F. Urwick, one of the pioneers of industrial management. He suggested three groups which can be clearly demarcated.

(1) Activities "which were regarded as the professional province of the engineer and as requiring a basic engineering training *before* scientific management was ever thought of", including research and development of natural resources, processing installation and maintenance of plant.

(2) Activities "which belong to one of the major functions of business (accounting, personnel, organization, and the like) and require primarily a training in the basic discipline directly applicable to that function".

(3) Activities which are directly concerned with the effective use of mechanical appliances and, "hence, with their adaptation to the human element in the business complex on scientific lines". Here, "a thorough knowledge of engineering principles and practice, requiring a complete technical training as an *engineer* or corresponding practical experience, have so far proved essential to the most effective performance. . . . It is to this third group of activities that the title industrial engineering is properly applicable".

Urwick also asserted that "engineering as practised by the industrial engineer is only a part of the over-all work of managing. . . . Any title which includes the term *engineering* is strictly limited to dealing with things, because engineering knowledge does not include people".

If Urwick's remarks are accepted as a commentary on the historic development of management, rather than as a doctrine of how it should be practised, his observations may well provide one of the reasons why engineers have been kept away from boards of companies in Great Britain for so many years, and perhaps explains why industrial engineering in the past has adopted a somewhat rigid and mechanistic attitude to managerial problems.

The definition of industrial engineering by the American Institute of Industrial Engineers is as follows: "Industrial engineering is concerned with the design, improvement, and installation of integrated systems of men, materials and equipment; drawing upon specialized knowledge and skill in the mathematical, physical, and social sciences together with the principles and methods of engineering analysis and design, to specify, predict and evaluate the results to be obtained from such systems".

The definition does not specify what size of systems is involved: it could be any size, even encompassing the whole organization. The projected reliance on pure and applied science, as well as social science, and the emphasis given to the investigation of integrated systems certainly puts industrial engineering well beyond the confined boundaries suggested by Urwick. Apart from the puzzling references to "principles and methods of engineering

analysis and design", there are no fundamental features which distinguish industrial engineering from operational research. The latter, defined by the Council of the Operational Research Society, is: "Operational research is the attack of modern science on complex problems arising in the direction and management of large systems of men, machines, materials, and money in industry, business, government and defence. The distinctive approach is to develop a scientific model of the system, incorporating measurements of factors such as chance and risk, with which to predict and compare the outcomes of alternative decisions, strategies or controls. The purpose is to help management determine its policy and actions scientifically". This definition is so broad that it covers practically the whole field of management science.

Similarly, in discussing the relation of economics and the behavioural sciences to management, Prof. Eilon shows wide areas of overlap and how futile it is to attempt to draw clear lines of demarcation. There is a lack of unanimity on terminology, and there are many shades of interpretation of the scope and composition of management science.

The task of management was also discussed. A fruitful way of evaluating the place of various subjects and fields of study in the management matrix is by examining them in relation to the tasks that management has to perform in an industrial enterprise. These may generally be described as: (1) determining the goals of the enterprise (short-term and long-term); (2) planning the acquisition and utilization of resources; (3) instituting recording and communication systems; (4) determining control procedures; (5) evaluating the performance of the enterprise and the effectiveness of the control mechanisms.

This management process can be ascribed to every task of the enterprise, and each stage of this process involves analysis and synthesis, design of alternatives, prediction of the outcome of events, and finally a decision (that is, a selection to be made from the given alternatives). Decision-making is involved throughout the management process, and a great deal of research work has been focused on this area in recent years.

What contributions have modern techniques made to the solution of problems in an industrial enterprise? An investigation of educational programmes in industrial engineering in many universities and colleges (particularly in the United States, where the subject has been taught in the universities for many years) has shown that, until recently, the approach of industrial engineering to management problems has been rather narrowly based and mechanistic in outlook. There are signs that this attitude is now changing.

Similarly, operational research has not yet reached the point where it can unequivocally be acclaimed as indispensable to top management. There are several reasons for this. First, many of the papers which are published in professional journals appear to be increasingly detached from any reality. Some are no more than interesting formal exercises in applied mathematics, written by mathematicians for mathematicians, with little pretence that the discussion could be of use to a practising manager. To do justice to the definition of operational research, perhaps some of this activity should properly belong to

departments of applied mathematics and not be called operational research at all. The true task of the operational research man is to explore reality and build a model to resemble it, not to take a model and try to force reality into it.

Operational research started in industry accompanied by a considerable fanfare to herald its great capabilities to cope with complex problems and large systems having many interdependent variables. Few applications at the strategic level in industry have been made. Most of the attention of operational research workers has been directed to tactical problems, in which the system under scrutiny is well confined and its constraints never questioned. A typical transportation problem in linear programming includes transportation costs in the matrix. It may be more profitable to question these costs, the capacities of transportation media, the capacities of stores and production lines, than to find the optimal solution to the linear programming problem. For many years operational research has been too much obsessed with optimization. It is one thing to find an optimal solution—if it can be found—and it is another to know when and how to implement it. It is only in recent years that adequate attention has been given to sensitivity analysis, that is, to determining what happens when we deviate from an optimum solution and how serious it is. Again, operational research has largely been concerned with the physical environment and tended to ignore or circumvent problems relating to organizational behaviour and to human and social environment.

### Research

Prof. Eilon then went on to describe some of the research topics which are being investigated in his Department. One is management structure and control. There are still many people who believe that management control is a summation of functions, such as financial control, personnel control, production control, etc. Precisely how these tools are co-ordinated and integrated is never made clear. This is not helpful in understanding the intricacies of organizational behaviour, and organization charts have often been shown to be too static, too dogmatic and too naïve to reflect reality.

The mechanistic approach to management is particularly evident on the shop-floor level and is seen, for example, in the attitude to fatigue and rest periods. Yet even the effect of different types of work on fatigue is not adequately known, such as the way an operator's performance is affected by various environmental parameters for a given type of work or the way he is affected by the performance of fellow workers. Barnes investigated the daily product curve for heavy manual work and for light work, and it appears that the latter does not exhibit the deterioration and recovery in production rate, which have been the basic assumptions in many models. Here is a clear example of the limitations of the mechanistic approach to the human machine.

In contrast, there are individuals who regard management control as an applied exercise in sociology, and the coining of the term 'organizational behaviour' is symptomatic of this approach. A survey into management control procedures suggests that they fall into two categories: in the first, an attempt is made to examine the individual within the organization, to record what he does and why he does it, and to assess his role accordingly. Investigations in the second category emphasize relationships between individuals and interpret management control as roles of staff functions, demarcation of responsibilities and lines of communication. Some of these investigations are penetrating and have produced useful results; but the picture remaining of the organizational structure is often too static, leaving the term 'control' ambiguous and undefined.

This is where collaboration between industrial sociologists, engineers and operational research workers could

be useful. One team of research assistants is using the 'tracer' method of investigation, that is, a specific task has been isolated and, throughout its progress, its impact on the organization and the decisions of people have been faithfully recorded. It is too early to attempt to summarize this work; but it appears that some insight is being obtained into the way in which independent control systems emerge and how standards of performance are set. Other topics examined were measurement sensitivity analyses, perishables and semi-perishables, inventory depletion and scheduling.

### On Management Education

Finally, Prof. Eilon examined many aspects of management education. It has been estimated in the United States that more than 60 per cent of graduates with engineering degrees become managers of some kind within 10–15 years after graduation. 25–40 per cent of the entrants to some prominent graduate business schools are engineers, and in institutions like the Massachusetts Institute of Technology School of Management or Carnegie's Industrial Administration, engineers form more than 80 per cent of those registered. No comprehensive surveys have been carried out in Great Britain to show what engineers and scientists actually do in industry. Some investigations show that engineers who reach senior positions spend little time on technical matters and most of their work is administrative in nature. This raises a cardinal issue: if engineers and applied scientists expect to spend so much of their time on management activities, should they not be primed in these matters in the same way that they are being educated in their own specialization? And if they should be so educated, at which stage in their career is it best to do so?

The opposition to academic educational programmes falls, broadly speaking, into three camps: the first objects to any formal training or education in management *per se*; the second asserts that management should be learnt 'on the job'; the third firmly believes that management courses should be organized in industry and taught by practising managers.

It has been argued by members of the first group that management education does not produce managers, that we cannot expect leaders of industry to emerge as a result of a few courses. The question whether management education can produce good managers or not is, however, not relevant. The question is whether it can produce better managers. Naturally, individuals must have a flair for management and certain personal attributes, and without appropriate pre-requisites it is doubtful whether any educational programme can guarantee to produce good managers, good engineers, good doctors, or indeed good practitioners in any field of human endeavour.

Those who oppose formal management education and advocate 'learning on the job' resort to a variety of arguments to prove their point. One is a reference to the famous engineering feats of the past, such as the Great Wall of China or the Suez Canal. These projects were constructed and led by engineers with no sophisticated network analysis and with no special training in management. Even for comparatively small plants we argue that we require managerial expertise and the use of modern management techniques.

Those who argue in this way are not comparing like with like. Competition, the abolition of sweated labour, and the need to treat employees as individual human beings, the necessity to subject most industrial projects to a scrupulous economic examination are such that none of the projects mentioned could be economically and socially feasible in our times with methods of execution used in the past. Industrial operations are getting more complex than ever before, involving an increasing degree of interdependence and technical specialization, and these must be matched by equally sophisticated managerial methods for utilizing available resources. Management is

becoming too difficult for many present-day managers, and by equipping themselves with the appropriate skills they stand a better chance of being effective at their job.

The assertion is often made that management is best taught by practising managers, that the material taught should be based on the lecturer's own experience and that management training is best carried out in industrial firms, using the experience of the firms and their employees. But any manager's experience is necessarily limited by the type of activities he has been engaged on and the kind of situations he has had to face. It is doubtful whether such experience will always be valuable, or even relevant, when this manager and his trainees have to face different circumstances from those recorded in the past. The manager's claim to success does not preclude the possibility that the same level of success, or even better results, could not have been attained by other methods than those he advocates. Moreover, the fact that firms call in industrial consultants, who are not necessarily more mature in years and industrial experience than the firm's own managers, is an admission that other approaches

could be helpful in controlling a changing environment. Resorting to management-training programmes within firms can lead to the worst kind of inbreeding, coupled with stifling of initiative and imagination; and even if his past experience is relevant, the manager is rarely equipped with the skills required to impart his knowledge to others in a systematic fashion or to be an educator.

Industrial firms should re-examine the assumptions on which their management training schemes are based. It would be far better, according to Prof. Eilon, if they were to send their management trainees to a course at an academic institution and then subject them to a short course in the firms, the purpose of which would be to tell these young people about the work and structure of the companies, rather than to teach them their own brand of management.

The form of management education at the university was also examined. Prof. Eilon is doubtful whether the undergraduate student is mature enough to appreciate the implications and ramifications of the subject, and recommends that it should be left to the postgraduate stage.

## OBITUARIES

### Prof. D. D. Woods, F.R.S.

PROF. D. D. WOODS, who died on November 6, 1964, at the age of fifty-two, was the first holder of a chair of chemical microbiology in Britain.

Donald Devereux Woods was a student at Trinity Hall, Cambridge, and obtained first-class honours in both parts of the Natural Sciences Tripos. After graduation in 1933 he began research in the Sir William Dunn School of Biochemistry, Cambridge, where he came under the influence of Sir Frederick Gowland Hopkins and also of Dr. Marjory Stephenson, and it was his association with the latter that led to the development of his interest in the biochemistry of micro-organisms. He obtained the degree of Ph.D. in 1937 and was awarded a Beit Memorial fellowship. He left Cambridge in 1939 to join the Medical Research Council Unit for Bacterial Chemistry at the Middlesex Hospital under the direction of Dr. (later Sir) Paul Fildes. During the Second World War he, together with other members of the Unit, was seconded to the Biology Section of the Chemical Defence Research Establishment at Porton. After the War he was invited by Prof. (later Sir) Rudolph Peters to join the Biochemistry Department at Oxford, where he was appointed reader in microbiology in 1946 and Iveagh professor of chemical microbiology on the endowment of this chair by Arthur Guinness, Son and Co., Ltd., in 1955. He remained at Oxford until the time of his death. He leaves a wife and a daughter.

From the time he first began research, Woods worked on the biochemistry of micro-organisms. During his period at Cambridge, he was mainly concerned with amino-acid and energy metabolism of *Clostridia* and with mechanisms of hydrogen production in bacteria. When he moved to the Medical Research Council Unit, he turned his attention to the nature of the substance present in yeast extract which antagonizes the antibacterial action of sulphanilamide. His demonstration that this substance is *p*-aminobenzoic acid, a structural analogue of sulphanilamide, first brought him international recognition. Together with Fildes, he then put forward the hypothesis that substances which are structural analogues of essential metabolites could inhibit the metabolism of the latter and so prevent growth. This hypothesis proved a great stimulus to research in the design of drugs as "metabolite analogues" and, indeed, the hypothesis has to-day become one of the few principles available for the rational develop-

ment of chemotherapeutic agents. Woods was not able to participate in the early exploitation of the idea, as he was seconded to work at Porton at that time. During the Second World War, workers in the United States showed that *p*-aminobenzoic acid forms part of the molecule of the growth factor folic acid.

When Woods was able to resume academic research in Oxford, he showed that the sulphonamides inhibit the conversion of *p*-aminobenzoic acid to folic acid. Together with research students and colleagues, he then began a series of investigations, using combinations of nutritional mutant and enzymological techniques, to elucidate the biosynthetic functions of folic acid and vitamin B<sub>12</sub>. Investigations of the nutritional requirements of bacteria mutants requiring folic acid or vitamin B<sub>12</sub> showed that both growth factors are involved in the synthesis of methionine from homocysteine. The inter-relationship of folic acid and B<sub>12</sub> proved to be highly complex and was unravelled in a series of papers culminating in three publications in the September 1964 issue of the *Biochemical Journal*. Folic acid is converted to a methyl-derivative as an intermediate, and a B<sub>12</sub>-containing enzyme is necessary for transfer of the methyl group from folic acid to homocysteine. An alternative B<sub>12</sub>-independent pathway was discovered and proved to be uniquely dependent on the triglutamate form of folic acid. The demonstration that some organisms can synthesize their methionine requirement without the mediation of B<sub>12</sub> while others have undergone a mutation to B<sub>12</sub> dependency is of considerable evolutionary interest. In the course of these investigations on methionine synthesis, Woods also made one of the first observations of enzyme repression when he found that growth of organisms in the presence of methionine results in loss of the formation of the homocysteine methylating system. The disentangling of this complex situation bears witness to Woods's skill, intellectual ability and unflinching patience.

Prof. Woods was a Fellow of Trinity College, Oxford, and in 1951 was elected a Fellow of the Royal Society. He gave the first Marjory Stephenson Memorial Lecture of the Society for General Microbiology in 1953 and frequently took a major part in symposia and discussions of that Society. He had a flair for the exposition of difficult biochemical topics, and his lectures were leavened with delightful touches of humour. It is a matter of great regret that he did not live to deliver the Leeuwenhoek



Lecture which he had been invited to give to the Royal Society in December 1964. Apart from his academic activities, Woods sat on many committees and advisory bodies dealing with microbiology and its application; he also carried out considerable editorial work for scientific journals. His many friends and colleagues will always remember his friendliness and enthusiasm for his subject; a long line of successful students will remember his kindly encouragement, advice and guiding hand. He led a full life in pursuit of his subject, and his death leaves a big gap in the ranks of both microbiologists and biochemists.

E. F. GALE

### Prof. L. W. Pollak

PROF. L. W. POLLAK, most widely known for his work in developing the photoelectric nucleus-counter, died in Dublin on November 24, 1964. He was born in Prague on September 23, 1888, and after a distinguished school and college career there was appointed to the staff of the German University in 1911. During the First World War he directed the meteorological service of the German Eleventh Army in the South Tyrol, and afterwards returned to the University, becoming professor of meteorology in 1927 and then director of the Observatories of Prague and Donnersberg.

Pollak's outlook was too liberal to please the Nazi régime, but his students made sure he learned of it when his name went on the list of disapproved persons. He was thus able to escape, and, after some months in London, found congenial employment in the newly formed Irish Meteorological Service, taking up duty at Foyines in December 1939. There he worked alongside Dr. M. Doporto, refugee from Spain, who died in September 1964. Pollak assisted in the teaching of the cadet forecasters, infecting them with his real enthusiasm for meteorology. He next

proceeded to establish the Service's Climatological Division and to initiate its regular publications. In 1947, however, he welcomed the opportunity to regain his standing of professor, as head of the School of Cosmic Physics in the Dublin Institute of Advanced Studies. He quickly built the premises in Merrion Square into an important observatory. The number of students was small, but research continued in many sections of geophysics, while colleagues extended the scope of the School with sections for astronomy and cosmic rays. In 1955 it was the venue for the first International Symposium on Atmospheric Condensation Nuclei.

Pollak retired when seventy-five, but not to laze. He was much in demand as consultant and spent most of his last year assisting projects in the United States. It was, however, at his home that death came suddenly.

Pollak's lectures were prepared with meticulous care, and yet achieved the appearance of spontaneity, and included homely and topical references. Nearly two hundred papers bear his name, many dealing with the photoelectric nucleus counter, developed during his first years in Ireland. It had been the pioneer work on punched-card techniques in climatology which had established Pollak's name earlier, and he was also largely responsible for the dissemination of Fuhrich's auto-correlation method of investigating periodicities. The only text-book bearing his name is *Methods of Climatology*, published in 1950, written in collaboration with V. Conrad, of Harvard University (formerly at Vienna). Many modern instruments incorporate Pollak's improvements: actinometers, balloon theodolites, kata-thermometers, dosimeters, tonometers: all received his attention, and several were the subjects of patents.

His first wife, Johanna, was a gifted linguist. She died in 1958. In 1962 he married Nessa Falconer, who survives him.

F. E. DIXON

## NEWS and VIEWS

### Council for Scientific Policy

THE following have been appointed members of the Council for Scientific Policy: Sir Harrie Massey, Quain professor of physics, University College, London (chairman); Dr. J. B. Adams, director of the Culham Research Laboratory, Atomic Energy Authority; Prof. D. H. R. Barton, professor of organic chemistry, Imperial College of Science and Technology; Prof. P. M. S. Blackett, professor of physics, Imperial College of Science and Technology (also member of the Advisory Council on Technology); Prof. F. S. Dainton, professor of physical chemistry, University of Leeds; Sir Charles Dodds, Courtauld professor of biochemistry, University of London; Prof. K. C. Dunham, professor of geology, University of Durham; Prof. B. H. Flowers, Langworthy professor of physics, University of Manchester; Sir Willis Jackson, professor of electrical engineering, Imperial College of Science and Technology; Dr. F. E. Jones, managing director, Mullards, Ltd.; Dr. J. C. Kendrew, deputy-chairman, Medical Research Council, Laboratory for Molecular Biology, Cambridge; Lord Rothschild, chairman, Shell Research; Sir Gordon Sutherland, Master of Emmanuel College, Cambridge; Prof. M. M. Swann, professor of zoology and dean of the Faculty of Science in the University of Edinburgh. The Council for Scientific Policy will replace the Advisory Council on Scientific Policy and will advise the Secretary of State in the exercise of his responsibility for the formulation and execution of Government scientific policy. Among the issues which are expected to come before the Council will be the balance of scientific effort in the various fields for which the Secretary of State is responsible.

### Operational Research in the University of Birmingham : Dr. S. Vajda

DR. STEVEN VAJDA has been appointed to the newly established chair of operational research in the Department of Engineering Production, University of Birmingham, as from January 22. Dr. Vajda, who was born in Budapest, and who graduated as a Doctor of Philosophy in the University of Vienna, has been head of the Mathematics Group in the Admiralty Research Laboratory at Teddington since 1952. He had previously been a lecturer in the Technical University of Berlin and held various actuarial appointments on the Continent and in Britain before joining the Civil Establishment of the Admiralty in 1944; he was later assistant director of physical research and assistant director of operational research in the Royal Naval Scientific Service. He has been a member of council of the Royal Statistical Society, chairman of the Department of Scientific and Industrial Research Sub-committee on Automatic Data Processing and chairman of the Programme Committee of the International Symposium on Mathematical Programming. His books on mathematical programming and the theory of games have been translated into French, German, Japanese and Russian.

### The Rockefeller Institute : Prof. C. Pfaffmann

DR. C. PFAFFMANN has been appointed vice-president and professor of the Rockefeller Institute, where he will be responsible for the development of a broad programme of graduate teaching and research in the behavioural sciences, with emphasis on the relations of the natural and social sciences. Dr. Pfaffmann graduated from Brown

University in 1933 and was awarded a Rhodes scholarship. He is a member of the American Philosophical Society, the Society of Experimental Psychologists, the American Psychological Association and the National Academy of Sciences. Dr. Pfaffmann is an authority on the physiology and psychology of the senses of taste and smell and the behaviour they control.

#### Prof. M. McCarty

Dr. M. McCarty has been appointed vice-president and professor of the Rockefeller Institute, where he will have primary responsibility for the development of clinical teaching and research and will supervise all its hospital facilities. Dr. McCarty graduated from Stanford University in 1933 and from the Johns Hopkins Medical School in 1937; he joined the faculty of the Rockefeller Institute in 1941. Dr. McCarty is a member of the Association of American Physicians, the Society for American Bacteriologists, the American Association of Immunologists, and the National Academy of Sciences. Together with Avery and MacLeod, he was a pioneer in demonstrating the biological activity and genetic significance of deoxyribonucleic acid.

#### Agricultural Research Council

THE annual report for 1962-63 of the Agricultural Research Council illustrates the continuing wide scope of both basic and applied scientific work carried out in the Council's Institutes and special Units (Pp. iv+91. (Cmnd. 2519.) London: H.M. Stationery Office, 1964. 6s. 6d. net). The appointment of Prof. S. R. Elsdon as the first director of the new Food Research Institute at Norwich and the establishment of the Unit of Nitrogen Fixation under the direction of Prof. J. Chatt at the University of Sussex are recorded. Developments of general interest are the monitoring of radioactivity in foodstuffs; the transmission of a plant virus by the zoospores of a soil fungus; improvements in electron-micrograph techniques for observing virus particles; and the radiometric assay of acetylcholinesterase, the enzyme which provides a chemical link between muscles and nerves in all living animals and which is inactivated by many insecticides. Important developments have been made in the measurement of body temperature by the use of small radio transmitters fitted inside the body and in the screening of drugs, which on administration to the mother may be embryotoxic or teratogenic, by examining changes in the pre-implantation embryo. General accounts are given of work at four institutes, the Animal Breeding Research Organization, the National Institute for Research in Dairying, the Hannah Dairy Research Institute and the Welsh Plant Breeding Station. Summaries of the work of the two units administered by the Council are also included; these are the Unit of Embryology, under Prof. F. W. Rogers Brambell, and the Unit of Plant Physiology, directed by Prof. C. P. Whittingham. The report concludes with appendixes of the committees of the Council expenditures, and special grants in support of research in universities.

#### The Society of Applied Pharmacological Sciences, Milan

A Society of Applied Pharmacological Sciences (Società di Scienze Farmacologiche Applicate) has been established in Milan. The aim of the Society is to bring together, independently of the organizations, institutes or laboratories where they are employed, pharmacologists, microbiologists, biochemists, pathologists, and clinical research workers concerned with research on new therapeutic agents. The Society intends to promote investigations and discussions of all those problems which concern the development of new drugs, and which, in their complexity, are treated only partially by other scientific societies dedicated mainly to basic research. Analysis and discussion of these problems by investigators

who are engaged in evaluating and realizing new drugs are of particular importance at a time when scientific and procedural criteria concerning the introduction of new compounds in therapy are being critically re-examined. The Society hopes to contribute through its activities, in co-operation with the public health organizations, to the greater safety and efficiency of developing new drugs.

#### International Filariasis Association

IN order to promote the investigation and control of filariasis, an association was established, to be known as the International Filariasis Association (Société Internationale des Filarioses). This grew out of earlier meetings held in 1955 and 1961, which were aimed at examining onchocerciasis under the auspices of the World Health Organization. The first general meeting of the Association was held at Rio de Janeiro on September 8, 1963, during the International Congresses of Tropical Medicine and Malaria, and the second at Rome on September 23, 1964, during the International Congress of Parasitology. Further general meetings will be held when international congresses facilitate them. Steps will be taken, as and when possible, to promote the investigation of all types of filariasis through appropriate local meetings, and to provide an official channel for bringing the problems of filariasis to the notice of Governments and international organizations. It is hoped to enlist the interest of all who are concerned with this subject. The officers of the International Filariasis Association are as follows: *President*, Prof. J. F. Kessel (U.S.A.); *Vice-Presidents*, Prof. P. C. C. Garnham (U.K.) and Medical General P. Richet (France). There is an executive council of fifteen members representing the different regions of the world involved, and the secretaries are: Dr. M. Giaquinto (Italy) and Dr. F. Hawking, National Institute for Medical Research, London, N.W.7, from whom further information can be obtained.

#### The Association of Commonwealth Universities

THE annual report of the Council of the Association of Commonwealth Universities for the year ended July 31, 1964, records a membership of 139 institutions, of which 126 were universities, 9 university colleges and 4 approved institutions of higher education (Pp. 46. London: The Association of Commonwealth Universities, 1964). The work of the appointments department continued to increase, and the Association assisted in filling some 1,294 vacancies compared with 1,146 in 1962-63. Ninety-one advisory committees were set up at the request of universities to interview candidates in the United Kingdom; of appointments made by overseas universities reported to the Association during the year, about 140 were of candidates who had applied from Britain in response to the Association's announcements, 95 being in Australia. Through its work for the Commonwealth Scholarship and Fellowship plan in the United Kingdom, the Association was concerned with the administration of the Marshall scholarships, the Frank Knox Memorial fellowships, the Commonwealth University Interchange. It also continued to assist the Drapers' Company in its scholarship scheme to enable British schoolboys to take first degrees in some universities in Australia, Canada and New Zealand. The Association continued to act as the authority in the United Kingdom to attest the eligibility for admission to British universities of candidates seeking admission to French universities by virtue of examinations passed in Britain: 207 such attestations were issued during the year. A brief account of the work of the committee of Vice-chancellors and Principals of Universities of the United Kingdom is appended.

#### National Museum of Canada

THE National Museum of Canada Bulletin No. 202 (*Anthropological Series* No. 69) consists of a paper by

Asen Balikei on the development of basic socio-economic units in two Eskimo communities (Pp. x+114. Ottawa: Queen's Printer, 1964. 1.50 dollars). The author deals with the Arviligjaarmiut hunters of Pelly Bay and the Puvirnituirmiut trappers of East Hudson Bay, and considers that the cultural changes among the Eskimos described are essentially changes in socio-economic organization.

### Uganda Museum, Kampala

THE Report of the Trustees of the Uganda Museum for 1962 and 1963 records a period in which there have been numerous changes of personnel both among trustees and staff (Pp. 29. Kampala: Uganda Museum, 1964). It has also been a time of great advance in every sphere of the Museum's activities. The primary task has been to collect traditional material before it is too late, and to this end a body of informed collectors, asked to fill specific gaps in the collections, has been organized throughout Uganda. The impact of independence in 1962 gave a great impetus to pride in Uganda's past, and preservation is now seen to be a matter of urgency. The Kebu Association in Arua, for example, sent a large number of specimens to fill an almost complete gap in the collections. A large historical exhibition, *The Nile Quest*, was shown in 1962 as a contribution to the Nile Centenary Festival.

### Museum of Applied Arts and Sciences, Sydney

IN a modern and attractive format, the annual report of the Museum of Applied Arts and Sciences, Sydney, for the year ended December 31, 1963, records a total of more than 206,000 visitors, which is the highest figure reached within the eighty-three years of the Museum's existence (Pp. 28. Sydney: Museum of Applied Arts and Sciences, 1964). This result is due in no small measure to the high standard of recent exhibits, the intensified advertising programme and the increasing attention directed to the comfort and convenience of visitors. A major alteration during the period was the modernizing of the Museum's main entrance, thus relieving congestion at the front door and also creating a pleasing initial impact on visitors. The Planetarium and the 'Transparent Plastic Woman' continue to be very popular, and a major acquisition was the complete notebooks and papers of Lawrence Hargrave, Australia's aeronautical pioneer.

### New Reference Tools for Librarians

*New Reference Tools for Librarians*, compiled and edited by Hans Zell, contains some 2,000 entries of reference books, bibliographies and other publications on librarianship published in 1962-63 (Pp. v+232. Oxford: Robert Maxwell and Co., Ltd.; Long Island City, N.Y.: Maxwell International Inc., 1964. *Gratis*). It is intended to supplement standard reference works on the subject and does not include year books and annuals, annual reviews of progress or membership lists of learned societies and professional bodies. The emphasis is on British and American material and the scope is interpreted liberally to include material likely to be of service to librarians, and is not limited to material concerned with librarianship in the strict sense of the word. Entries are arranged under the Universal Decimal Classification.

The first supplement to *New Reference Tools for Librarians*, issued in March 1964, lists a further 600 new and forthcoming reference books, bibliographies and publications on librarianship, published in 1963-64, now including publishers' names. The second supplement includes a further 550 titles published in 1964, as well as some titles which appeared too late in 1964 for inclusion in previous catalogues. The emphasis is again on British and American publications (No. 1. Pp. 62. No. 2. Pp. 56. Oxford: Robert Maxwell and Co., Ltd.; Long Island City, N.Y.: Maxwell International Inc., 1964).

### Science Citation Index

A BOOKLET issued by the Institute for Scientific Information is intended to assist the scientist or librarian to use effectively the *Science Citation Index* for searching the literature (*Effective Use of the Science Citation Index: a Programmed Text*. Pp. 52. Philadelphia: Institute for Scientific Information, 1964). A general description of the *Index* is followed by explanations of its construction; the abbreviations of titles, authors' names and references which are used, and the characteristic features of the system, are demonstrated.

### Papermaking Research

THE British Paper and Board Industry Research Association at Kenley, Surrey, issues a quarterly journal called *What We Are Doing*. One section is confidential to members, but most of the remainder deals, usually in a general way, with the activities of the Association and contains book reviews and abstracts of scientific papers. Occasionally, for good measure, more detailed accounts of certain research activities of the Association are also released, the guiding principle being the extent to which they are of academic interest as distinct from technical importance. This is a wise policy, since it enables the outside world to assess the quality of some of the work being carried out by the Association. The June 1964 issue contains a typical example of this nature, dealing with the obviously important subject of the mechanism of rupture of paper under tension. An attempt is being made, and with some success, to correlate thin places in the paper with the path taken by the fracture propagated when a strip of paper breaks under tension. In the first example, the light transmitted by the paper through very small areas (approximately 0.23 mm<sup>2</sup>) was used to track the potential weak spots (that is, thin spots) in the paper. Afterwards  $\beta$ -radiography was used to determine the corresponding weights per unit area, an obviously more exact method of measuring a weak spot than from the thickness. A computer was then used to formulate the possible rupture paths, the input being obtained directly from the automatic scanning of the  $\beta$ -radiographs. The latest results are a striking justification of this method of forecasting the rupture propagation, and they have also demonstrated the overwhelming influence of abnormalities in the structure of the edge of the paper in initiating rupture. This work should eventually lead to a more exact understanding of the mechanism of fracture of non-homogeneous materials.

### A New Source of Petroleum

AFTER a quarter of a century's search for oil in the sheikdom of Abu Dhabi, on the Trucial coast of Arabia, aptly described as "a place of sand and sky and wide rolling horizons", pioneer efforts have been rewarded in the discovery of a new source of crude oil and development of a commercial field at Abu Dhabi, inland at a place called Murban, some miles from the sea. There is an offshore oilfield, about fifty miles out to sea, off the Abu Dhabi-Jebel Dhanna coast, which has been in production for two years or more, but the proving of the Murban oil area has been quite a different story. It is most uninviting territory, at least on surface, both from human and commercial points of view. "26,000 square miles of rolling sand dunes which imperceptibly merge into the wastes of Arabia's 'Empty Quarter' . . . noon temperatures of about 120 degrees Fahrenheit coupled with high humidity . . . sandstorms, shortage of water . . . difficulty of keeping food fresh and free from dust . . ."—these are the conditions under which the geologists, geophysicists and engineers had to work during many unrewarding years. Actually, the first well in the Murban area was drilled ten years ago, to 12,500 ft.; it yielded some oil and gas, but had to be abandoned for technical reasons; by unfortunate chance this well was located only about five miles north of the



now proved Murban field, but at the time this fact was unknown to the prospectors. A second well was completed five years ago which, together with the sub-surface information already provided by Murban No. 1, threw more light on the character of the underground formations in this area and prompted the placing and drilling of Murban No. 3; this well penetrated two oil-bearing formations and produced about 700 tons of oil per day on test. By October 1960, the results of these discovery wells were considered sufficiently encouraging to justify commercial production and export. On December 31, 1963, the 36,000 ton tanker *Esso Dublin* discharged the first cargo of Murban crude oil at the marine terminal of the Esso Petroleum Co.'s refinery at Milford Haven, Pembrokeshire. As an article in the *Esso Magazine* (13, No. 4; Autumn 1964) states: "The discovery of this new source of oil follows hard on the heels of the important discoveries in Libya and provides not only another sizeable addition to world proved reserves of oil, but also a further diversification of supply".

### Laboratory Guide to Ion Exchange

A USEFUL 42-page, illustrated manual entitled *Amberlite Ion Exchange Resins Laboratory Guide* was published in 1964 by the Rohm and Haas Co., Philadelphia, Pa., of which a brief summary appeared in *Rohm and Haas Reporter*, the periodical of the Company (22, 3; May-June 1964). Rohm and Haas pioneered development of ion-exchange technology in the United States and claims to be the first commercial organization in that country to produce synthetic, high-capacity ion-exchange resins on a large scale. The Company manufactures at present a complete range of laboratory and commercial ion exchange resins under the trademark 'Amberlite'. For laboratory use these resins are supplied in two different grades: chemically pure and analytical reagent. More than 30 different ion-exchange resins of varying porosity and particle-size are available in these grades, which are marketed by Mallinckrodt Chemical Works, St. Louis, Missouri. This manual describes "the many ways 'Amberlite' ion exchange resins may be used to eliminate difficult and time-consuming steps in laboratory work and explains how ion exchange operations are carried out. The manual emphasizes actual techniques for the use of ion exchange process in preparative and analytical work. . . . While primarily concerned with laboratory applications, the guide also reviews ways of using ion exchange resins in commercial processes". It is pointed out that these resins are exceptionally convenient to use; often, to make an analysis, the only equipment required is a glass column containing the resin beads. Separations can be performed more rapidly and determinations made more accurately by the analyst by this ion-exchange process than by the slower, often more cumbersome, methods of precipitation or crystallization. Copies of this guide can be obtained (gratis) from the Ion Exchange Department, Rohm and Haas Co., Philadelphia, Pa., 19105 (London subsidiary, Lemnig Chemicals Ltd., 26-28 Bedford Row, London, W.C.1).

### Microbiological Contamination of Food

CONTAMINATION of foods by micro-organisms does not constitute the hazard that it once did; however, new methods in processing and distributing foods are introducing new problems in food microbiology. A publication from the U.S. National Academy of Sciences—National Research Council, entitled *An Evaluation of Public Health Hazards from Microbiological Contamination of Foods*, examines the present position (Publication No. 1195. Food Protection Committee of the Food and Nutrition Board. Pp. vi + 64. Washington, D.C.: National Academy of Sciences—National Research Council, 1964. 2 dollars). It is concluded that it would be premature to adopt legal standards but recommends several steps which should be

taken to prepare the ground for their eventual adoption. It notes the increasing disparity between technical change and the level of efforts made to evaluate and control the health hazards with new products and processes. Comment is made on the poor reporting (in the United States) of the incidence of food-borne disease, the inconsistency of such reporting being shown by the fact that one State repeatedly reports 30-50 per cent of all outbreaks recorded by the Public Health Service. Definitions for microbiological specification, recommended microbiological limit and microbiological standard are proposed. The basic principles on which these criteria should be based are given. It is recommended that, before microbiological criteria for a food are adopted as an administrative device, standard methods of sampling and analysis of the foods in question should be agreed on. For this purpose, an organization similar to the Association of Official Agricultural Chemists should be formed in the field of food microbiology. Limits suggested by various workers for nine classes of foods are tabulated in an appendix together with 90 references to their source.

### Natural Radioactivity in Soils

THE dating of relics by radiocarbon analysis, which, in recent years, has come to be accepted as a most useful technique, is only one application of the measurement of the emissions from several radioactive elements. Obviously the principle might be used to estimate the weathering of soils from parent materials and the geological age of soils; moreover, the radioactivity of soils is affected by fall-out contamination from atomic explosions. A most useful review of present knowledge on this topic has been made by O. Talibudeen in *Soils and Fertilizers* (27, No. 5. Commonwealth Bureau of Soils, Rothamsted, 1964). He describes the properties of radioactive elements found in Nature and their contribution to the radioactivity of soils. About two-thirds of the total activity may be from potassium and thorium, and about 20 and 7 per cent from uranium and rubidium respectively. Carbon-14 contributes less than 0.5 per cent, and inorganic carbonate is more active than organic carbon of the same geological age, but carbonates may have isotopic exchange with atmospheric carbon dioxide, so that there are complications in the calculation of the age of organic residues in soil. The relative amounts of radionuclides in the soil are altered by such pedological processes as leaching, oxidation and reduction, and by the levels of sesquioxides, carbonates and organic matter, and the particle size distribution. The uranium in surface soils, for example, is related to their organic matter content, and the radium/uranium ratio is increased by leaching. The ratio of pairs of elements may help to assess the intensity of weathering in a soil profile, the thorium/uranium ratio being a more sensitive guide to leaching than the radium/uranium ratio. Short descriptions of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -assays of radioactivity are given, with notes on corrections and interfering unstable nuclides. There are 9 tables of data and 2 figures of spectra, and a comprehensive list of references.

### Extraction of Forest Produce

THE British Forestry Commission Booklet No. 11 reviews extraction methods in use in Great Britain and also a few that have been tried but which have proved unsuccessful (*Extraction of Conifer Thinnings*. By R. E. Crowther. Pp. 74 + 15 plates. London: H.M.S.O., 1964. 5s. net). It is a timely publication because the extraction of forest produce is an important economic factor in forestry efficiency. Extraction is a costly business and it may account for 25-75 per cent of the total costs of production, excluding the cost of growing the trees. About two-thirds of the cut from State forests is made up of coniferous thinnings and at present some 40 million hoppus ft. of the latter are extracted in a year and the

amount is expected to rise to 85 million hoppus ft. in 1980. Much of this is destined for pulp and chipboard and thus much attention has been directed in recent years to increasing the efficiency of their extraction. The descriptions of the various extraction methods are based on experience and on the findings of the work study section, and supporting data are given in a series of tables. The general conclusions reached are that, of the seven types of extraction compared, the double drum winch is the best for both uphill and downhill hauls. Horse extraction is good for short distances downhill but not very efficient uphill, and under some conditions the wheeled tractor may be the cheapest method. So much importance is attached to the use of the Isachsen double-drum winch that Forestry Commission Booklet No. 12 is devoted to it (*Double Drum Winch Technique*. By R. E. Crowther and S. Forrester. Pp. 38+8 plates. London: H.M.S.O., 1964. 3s. net). This is a useful manual with clear explanations and good illustrations. It and its companion booklet are examples of concinnity.

### Royal Astronomical Society Awards

THE President and Council of the Royal Astronomical Society have made the following awards: *Gold Medals*, to Dr. G. M. Clemence of Yale University, in recognition of his applications of celestial mechanics to motions in the solar system and his fundamental contributions to the study of time and the system of astronomical constants; to Sir Edward Bullard of the University of Cambridge, for his pioneering work in geophysics and, in particular, his leadership in the field of studies of the geology of the ocean floor; *Eddington Medal*, jointly to Prof. R. V. Pound and Dr. G. A. Rebka of Harvard University, for their decisive experiment of measuring the gravitational red shift on the surface of the Earth through the methods of nuclear physics.

### Institute of Metals Awards

THE Council of the Institute of Metals has announced the following medal awards: *Institute of Metals (Platinum) Medal for 1965*, to Prof. A. H. Cottrell, in recognition of his services to the non-ferrous metal industries; *Rosenhain Medal for 1965*, to Mrs. M. K. McQuillan, in recognition of her contributions in the field of physical metallurgy; *W. H. A. Robertson Medal and Premium for 1964*, to Mr. D. Green, for his paper entitled "An Experimental High-speed Machine for the Practical Exploitation of Hydrostatic Extrusion" published in the *Journal of the Institute* (November 1964).

### University News:

DR. E. H. FROST-SMITH, research director with Stavely Industries, Ltd., has been appointed to the chair of electrical engineering.

#### Belfast

#### Birmingham

THE following appointments have been announced: *Lecturer*, Dr. D. A. Lihou (chemical engineering); Dr. K. C. Hooper (physiology); D. Tarin (pathology). *Research Fellow*, J. P. Barton (physics); C. A. T. Harnden (civil engineering); M. Landon (biochemistry); J. W. Dale (Senior Research Fellow in the Department of Experimental Pathology). The following titles have been conferred: *Reader*, Dr. S. A. Barker (carbohydrate chemistry); Dr. A. B. Foster (organic chemistry); Dr. G. J. Lawson (minerals engineering).

#### Bristol

DR. G. M. HUGHES has been appointed to the chair of zoology as from August 1, in succession to Prof. J. E. Harris. Dr. M. P. Banton has been appointed to the newly established chair of sociology as from August 1.

#### Cambridge

SIR EDWARD BULLARD, reader and head of the Department of Geodesy and Geophysics in the University of

Cambridge, has been appointed to the chair of geophysics at the University.

#### Lancaster

IN response to the recent appeal by the new University of Lancaster for funds to supplement its Government grants, Mullard, Ltd., has agreed to donate the sum of £10,000.

#### London

DR. H. R. V. ARNSTEIN, a member of the scientific staff of the Medical Research Council, has been appointed to the chair of biochemistry tenable at King's College. Dr. G. M. Jenkins, reader in mathematical statistics at the Imperial College of Science and Technology, has been appointed to the chair of statistics tenable at Queen Mary College. The following titles have been conferred: *Professor*, Dr. A. Ashmore (nuclear physics). *Reader*, Dr. D. F. Evans (inorganic chemistry); Dr. Marie B. Hall (history of science and technology).

### Announcements

THE name of the International Nickel Company (Mond), Ltd., has been changed to International Nickel, Ltd. This took effect as from January 1.

DR. VAN ZANDT WILLIAMS, president-elect of the Optical Society of America, has been appointed director of the American Institute of Physics with effect from April 1.

DR. H. K. CAMERON, chairman of the London Section of the Society of Chemical Industry, has been awarded the Lampitt Medal for 1965 by the Council of the Society of Chemical Industry.

DR. W. LANE-PETTER is relinquishing his position as director of the Laboratory Animals Centre, as from January 31, 1965, in order to become director of Carworth Europe, a subsidiary of Carworth Laboratories (U.S.A.).

MR. F. N. SUTHERLAND, deputy chairman and managing director of The Marconi Co., Ltd., has been appointed president of the Council of the Television Society as from January 1. Mr. Sutherland succeeds Sir Robert Fraser, who has held office for the past two years.

MR. F. D. OUTRIDGE was appointed director of the Council of the Scientific Instrument Manufacturers' Association, at their January meeting. He succeeds Capt. R. A. Villiers. Mr. Outridge joined the Association in 1961, and was appointed acting director in July 1964.

THE seventh session of the Welsh Soils Discussion Group on "Physical Aspects of Soil Fertility" will be held in Bangor on February 10. Further information can be obtained from Dr. A. H. Charles, Welsh Plant Breeding Station, Gogerddan, near Aberystwyth.

A SERIES of six evening lectures under the general title "Science, Industry and the State," organized by the Office of Health Economics, will be held at the Royal Society of Health during the first three months of 1965. Further information and forms of application for tickets can be obtained from the Director's Secretary, Office of Health Economics, 62 Brompton Road, London, S.W.3.

THREE lectures on "The Scientific Basis of Dentistry", arranged by the British Postgraduate Medical Federation, will be held at the Royal College of Surgeons on February 10, February 24 and March 10. The lectures, which are designed for research workers and specialists in training, will be as follows: "Science in Dentistry", by Prof. D. J. Anderson; "Electron Microscope Autoradiography", by Dr. S. R. Pele; "Histochemical Techniques in the Study of the Cellular Biology of Dental Tissues", by Dr. A. R. Ten Cate. Further information can be obtained from the British Postgraduate Medical Federation, 18 Guilford Street, London, W.C.1.

## CHEMICAL ENGINEERING STUDENTS

A SURVEY of students admitted to chemical engineering departments at universities and technical colleges in 1963 has been carried out by the Institution of Chemical Engineers in conjunction with Research Services, Ltd. \*. It covers all 19 institutions in the United Kingdom offering a full-time first-year degree course in chemical engineering, including students taking sandwich courses. Valid replies to the questionnaire totalled 931, and the 608 from chemical engineers represented an 89 per cent response. The survey indicated that while the difference between these groups of students was not as great as that between science and engineering students generally, students seeking a degree in chemical and mechanical engineering are of higher ability than engineering students in general. Chemical engineers originating from grammar or public schools showed no difference in ability, and while other schools held their own for the better students they provided a higher proportion of poorer students. The proportion of chemical engineers and chemists from public schools is lower than that of engineers or scientists in general.

Chemical engineers at grammar or public schools decide on their course at a considerably later stage than other students, only 8 per cent deciding on chemical engineering before taking the Ordinary Level examinations, compared with 31 per cent of chemists, while 44 per cent choose within the second or third year of their advanced work (22 per cent for chemists). Chemical engineers from other schools decide on their course even later. Motivation of careers is greater for students in technical colleges than for university students. More chemical engineers prefer to work in the field of design, construction, installation and plant maintenance than in other fields, most chemists electing for research and development work. The distribution of the intended field of work of students is often in marked contrast to that of the chartered membership of the corresponding professional engineering institution. Chemical engineers are better informed about the type of work they will be doing after leaving college and about the salary-levels to be expected than are other students, and 60 per cent would like to go abroad after completion of

study compared with 41 per cent of chemists, 'experience' being the motive most frequently given, with 'better opportunities for financial reward' a close second.

Most chemical engineers learned of the existence of their course from their chemistry teacher (28 per cent), general literature on careers (23 per cent) and college or university prospectuses (20 per cent). Relatives and friends were a source of information for 20 per cent of the chemical engineers from public schools, 14 per cent of those from grammar schools and 30 per cent of those from other schools. Almost half the chemists were given this information by their chemistry teacher. The Institution of Chemical Engineers was the second most frequently used single source for detailed information on the course, after college or university prospectuses, this information reaching 35 per cent of the public school boys, 29 per cent of grammar school boys and only 16 per cent of other school boys. The impact of the professional institution on mechanical engineers was only 6 per cent and on chemists only 2 per cent, but there was no indication that chemistry teachers or careers masters who do not teach chemistry were unable to supply detailed information on chemical engineering. For about half of all the students, sources connected with the school were decisive, chemical engineers quoting the chemistry teacher (16 per cent), literature from the Institution of Chemical Engineers (13 per cent) and (for public school boys only) people in the profession (23 per cent) as the most frequent single sources. Chemistry teachers decisively influenced 27 per cent of the chemists compared with 16 per cent of the chemical engineers, but the difference is unlikely to be due to careers masters who teach chemistry.

Of all the chemical engineers, 64 per cent seriously considered taking another course (1 in 3 considered taking chemistry and 1 in 7 a branch of engineering). Among the chemists 1 in 8 and among the mechanical engineers 1 in 28 considered chemical engineering. Of the chemical engineers who considered taking chemistry, 30 per cent decided against it because it offered fewer prospects for a career than chemical engineering. Less than 5 per cent of all chemical engineers could have been students who were refused admission to chemistry departments.

\* *The Institution of Chemical Engineers. The Choice of Chemical Engineering at University or Technical College: a Survey of Students Admitted in 1963.* Pp. vii + 53. (London: The Institution of Chemical Engineers, 1964.) 15s.

## THE ANIMAL VIRUS RESEARCH INSTITUTE, PIRBRIGHT

THE Animal Virus Research Institute is the new name for the Foot and Mouth Disease Research Institute at Pirbright, Surrey, and the report which has recently been published covers the years 1961-63\*. The Institute is still primarily concerned with the prevention and control of foot-and-mouth disease, and includes the World Reference Laboratory for this disease, but it is now intended that activities be extended to cover other virus diseases of animals as well. For this reason and because much of the research carried out at the Institute is of relevance to viruses in general a change of name was indicated.

The main theatres of operation for control of foot-and-mouth disease during the period reviewed were in South-West Africa, where extensive vaccination was carried out to control a large outbreak, and the Middle East, where the

African strain of virus became widespread in 1962. Such operations have naturally led to great demands on the Institute, particularly for live attenuated vaccine. The consequent problem of production has, however, been eased by a major development described in the report, namely the establishment of a co-operative project with the Wellcome Foundation. Under the new arrangements the Foundation will be responsible for production of live vaccine in a new building on the perimeter of the Institute.

This development has freed Institute staff and facilities for the pursuit of the proper function of research. Much effort has gone into methods for producing attenuated strains of virus suitable for immunization, and into studies on the safety of such strains when used in vaccines. The basic research which is reported may ultimately shed light on these and other problems. For example, genetic recombination has been achieved for the first time and may lead to detailed genetic analysis of foot-and-mouth disease virus. Viral replication is under investigation in

\* *The Animal Virus Research Institute, Pirbright. Report for the years 1961-1963.* Pp. 34. (Pirbright: The Animal Virus Research Institute, 1964.) 2s. 6d.



the biochemistry section. Immunity mechanisms are also being studied at a molecular level in the biophysics section.

One major difficulty in work on foot-and-mouth disease virus has been the lack of a suitable line of cells which can be continuously cultivated, and which is still sensitive to infection by the virus. The report describes how this difficulty has been overcome by the use of a diploid line of hamster fibroblasts. Cells of this line are highly sensitive, and are now used extensively for all types of research on the virus. The hamster cells are also proving to be suitable for mass culture and therefore for large-scale vaccine production.

It is gratifying to see from the report that the problem of disease security is continuing to attract the attention it deserves, and much of the building programme for the period under review has been concerned with improved isolation units and air-filtration equipment.

This will be the last report by Dr. I. A. Galloway, who has now retired after twenty-four years as director and who is succeeded by Dr. J. B. Brooksby. The wide scope and large scale of the activities described in the report are a particularly suitable record of the development which has taken place in the Institute under Dr. Galloway.

M. G. P. STOKER

## THE SHIRLEY INSTITUTE

THE idea of co-operative industrial research is well established in Britain, for there are no fewer than fifty research associations, each financed jointly by the industry concerned and the Department of Scientific and Industrial Research. In few instances has the work been more successful than in the case of the Cotton, Silk and Man-made Fibres Research Association, as is made abundantly clear in the 1964 annual report of the director, Dr. D. W. Hill\*, and by the stimulating exhibits which could be seen recently when the laboratories were open to members.

The Research Association, colloquially known as the "Shirley Institute", has established for itself a world-wide reputation based on its work on the constitution, reactivity and processing of cotton, but its activities now include work for the silk and the man-made fibres industries, particularly after the merger with the Rayon Research Association a few years ago. It is not possible in a short note even to summarize the wide range of topics at present being studied in the Association's laboratories, but two or three perhaps deserve special mention, because they illustrate the vision and foresight which have characterized the planning of the research programme and the close collaboration which exists between the Association's scientists and member firms.

One of these is the design of the automated system for producing yarn from raw cotton. The project has been developed to such an extent that mill trials are now in progress. As Dr. Hill states, "the introduction of automated processing is the one way in which this country

can compete with Eastern countries that have a large surplus of cheap labour". The system could be adapted to work in most modern mills, although considerable capital expense would be involved.

The Institute has for some time been carrying out research on the behaviour of fibres during stretching, and it was found that when fibres such as polyethylene are stretched they tend to split longitudinally. A similar phenomenon occurs when films are stretched, and from this observation has emerged a completely new method of producing fibres simply by stretching films under suitable conditions. Yarns can be spun from these fibres and the technique opens up many important possibilities.

Almost all the basic operations involved in converting fibres into finished cloth are being investigated in an attempt to obtain maximum efficiency in conventional techniques and to develop new ones, but an important aspect of the choice of topics is that the probable cost of each project has been assessed in the light of possible financial return. It is interesting that considerable stress is being laid on the production of stretch fabrics.

The work of the Institute is characterized by the careful balance between 'pure' and 'applied' research so that projects range from fundamental investigations to the solving of members' problems. During the past fifteen months covered by Dr. Hill's report, some 3,000 technical enquiries were dealt with. The scientific quality of the work at the Shirley Institute is high, and the present report shows that the Association is pursuing imaginative and profitable lines of research which will ensure that it continues to be one of the most highly regarded centres of fibre research. C. S. WHEWELL

\* Report of the Council and Statement of Accounts for the year ended March 31, 1964, presented at the forty-fifth annual general meeting of the Members of the Cotton, Silk and Man-made Fibres Research Association, held on Thursday, November 5, 1964.

## SCIENCE OF THE FABRICATION OF CERAMICS

THE meaning of the term 'ceramics' is to-day in a state of flux. Materials scientists tend to apply the term to any inorganic non-metallic material. Most of the workers in the field of ceramics—both the traditional and the new—feel that ceramics is a technological subject and that it is the fabrication process which brings the old and the new ceramics together. Basically the process may be described as the shaping of an article from a powder and its consolidation into a monolithic article by a high-temperature 'sintering' treatment. The scope of the meeting of the Basic Science Section of the British Ceramic Society, held at Brighton during October 12-14, 1964, was thus logically divided into three main sections: (1) preparation, characterization and properties of powders (chairman, Prof. J. White, University of Sheffield); (2) forming processes (chairman, Mr. A. Dinsdale, British Ceramic Research Association); (3) sintering and related processes (chairman, Dr. P. Murray, U.K. Atomic Energy Authority, Harwell). Some new and interesting processes

which did not fit into this scheme were dealt with in a fourth section entitled "Other Fabrication Routes" (chairman, Dr. J. Williams, U.K. Atomic Energy Authority, Harwell).

In order to acquaint the participants with recent developments and present-day thinking, the first three sections were each preceded by an introductory lecture. The first of these was given by J. Williams, who leads the very large ceramic team of the Metallurgy Division at Harwell. He emphasized the importance of the surface, since the driving force in the sintering process is the surface-free energy, and considered both its physics and its chemistry. Particle size and shape, and the deformation characteristics of powders, determined by their defect structure, were some of the points discussed.

The usual methods of characterization of a powder are by its particle size (for example, determined by optical and/or electron microscopy), its crystal size by X-ray line broadening, and surface area by the Brunauer-

Emmett-Teller adsorption method. In addition, B. R. Steele and his colleagues from the U.K. Atomic Energy Authority, Springfield, have used the measurement of gas permeability as a means of investigating the pore morphology of compacts. Although interpretation is difficult, because the pores form a three-dimensional interconnecting network of channels whereas flow is in a single direction, the breakdown of particle aggregates into fragments of the very small dimensions of the crystallites occurring during pressing has been examined by this method. Experiments on  $\text{UO}_2$  and  $\text{BeO}$  powders have shown a considerable difference in the bond strength between crystallites.

Many of the oxide powders used in ceramics are produced by thermal decomposition of hydroxides, followed by calcination. P. J. Anderson and his colleagues from Harwell showed, quoting as examples  $\text{MgO}$ ,  $\text{CaO}$  and  $\text{BeO}$ , how markedly the nature of the product of thermal decomposition could be affected by quite low partial pressures of  $\text{H}_2\text{O}$ . Water vapour affects the rate of nucleation of the oxide and enhances crystal growth during calcination (for example, at  $1,050^\circ\text{C}$ ). They doubted whether true vacuum conditions were obtained in many so-called 'vacuum-sintering' experiments because of the build-up of water vapour desorbed from the surface.

K. R. Poole, also of Harwell, discussed the statistics of mixing two or more powders. He showed that a single number that provided a measure of the difficulty of mixing, that is, the mean particle weight, could be deduced from a particle size distribution. Examples were given of how to calculate from this figure the random homogeneity of a mix, which makes it possible to decide whether it is practicable to improve a given mix by further mixing or whether size reduction of the components is necessary.

Comminution of particles is an important process that is little understood. J. Williams mentioned in the introductory lecture that only 5 per cent of the mechanical energy is used to create fresh surfaces, while the rest is converted into waste heat. Attempts to improve efficiency are thus important, and in view of the lack of a fundamental understanding the problem must at present be approached empirically for each material. M. G. Harwood (Mullard Radio Valve Co.) reported a very thorough investigation of the milling of iron oxide. Milling parameters (time, ball size, powder: water ratio, filling factor, dimensions and nature of lining of mill) were varied and their effect on surface area, reactivity, particle size and shape was examined. The following stages were observed: (1) 'de-agglomeration' with an increase of reactivity but not of surface area; (2) constant particle size but coating of the particles with some very fine ones which may arise from the wear of the ball or mill liner; (3) sudden break-up into fine components; (4) re-agglomeration or 'over-milling', resulting in decreased reactivity but not surface area.

Comminution by milling is only one particular method of obtaining fine powders. R. M. Glaister, of Standard Telecommunication Laboratories, Ltd., reported on a comparison of a variety of methods for preparing ultra-fine powders to be used in the sintering of magnetic ferrites to negligible porosity. Besides the conventional ball milling, he and his colleagues had examined chemical and electrolytic coprecipitation, treatment of metallic powders in steam, and a flame reaction process. The finest particles were obtained by decomposing a milled mixture of organic salts ( $0.25\text{ }\mu\text{m}$ ) and by flame reaction ( $0.7\text{ }\mu\text{m}$ ), although powders produced by electrolytic and chemical coprecipitation had much smaller crystallites than their apparent particle size of  $0.5\text{ }\mu\text{m}$  and  $1\text{ }\mu\text{m}$ , respectively. It will be interesting to see how these powders sinter.

A. L. Stuijts, from the Philips Research Laboratories, Eindhoven, gave the introductory lecture on "Forming Processes". After discussing briefly the peculiar rheo-

logical properties of powder-in-liquid suspensions, he dealt with the three most important traditional processes: slip casting, plastic forming (extrusion), and powder compaction. Much in this field is still an art rather than a science; there is no single unit or indeed any combination of properties by which one can assess the workability of a plastic mix and which might replace the 'potter's thumb'. While a certain amount of macroscopic data on compaction is available, little is known about the movement of powder in a die, about cohesion of powder and the properties required of a lubricant to decrease friction between powder and die and between the powder particles themselves. The three papers given in this section were all steps in the right direction, but much more work of this type is required. These seem to be research topics that could very well be tackled in the new technological universities.

Glass spheres and quartz, fluorite and baryte in various fractions between 20 and  $300\text{ }\mu\text{m}$  as model substances were used by A. Turba (Technische Hochschule, Karlsruhe) in his work on the behaviour of powders compacted in a die. The work required for compaction was determined by integration of an oscillogram of force versus displacement. To obtain distribution of porosity the compact was turned down on a lathe in twenty-five steps. Comminution of the original material was examined by comparing the particle size distribution before and after pressing. The effect of adsorbed gas and water molecules was also examined, but was found to play only a minor part. The paper by Capriz and Laratta on the "Extrusion of Non-Newtonian Bodies" was possibly too mathematical to be followed by most of the audience. However, it was highly relevant, as it dealt with pressure flow in a conical die and the behaviour of plastic materials during ram or auger extrusion.

C. F. Cooper and S. F. A. Miskin, of Morganite Research and Development, Ltd., presented new data on the kinetics of slip casting of  $\text{Al}_2\text{O}_3$ . The build-up of the cast was observed by means of  $\gamma$ -ray absorption. Casting rates were observed for casting slips in the pH range 0.5-14. The cast thickness showed a parabolic dependence with time. The temperature dependence of casting and the effect of variations in viscosity of the suspending fluid showed that the process was controlled by the rate of permeation of water through the cast layer.

Recent ideas on sintering were surveyed by Prof. White, an early exponent of the plastic flow theory, in his introductory lecture to the third section. The early stages of sintering, as demonstrated by the neck growth between two spheres, are now fairly well understood, and matter transport is generally believed to be due to volume diffusion. The later stages of sintering, leading to a pore-free material like 'Lucalox', are much less well understood, but it is well established that firing atmosphere and small additions, for example  $\text{MgO}$  in the case of  $\text{Al}_2\text{O}_3$ , can have a pronounced effect. A full understanding of the mechanism of the 'grain growth inhibitor' seems the most important problem still to be solved.

Work on material transport during the sintering of compacts of  $\text{ThO}_2$  and  $\text{CaO}$  was reported by C. S. Morgan (Oak Ridge National Laboratory). He and his colleagues suggested that the initial sintering was primarily by dislocation movement. D. W. Budworth's (University of Sheffield) experiments on the sintering of sodium chloride pointed towards the danger of the too ready acceptance of model experiments which proposed an evaporation-condensation sintering mechanism which is said not to lead to shrinkage. His work with A. Ammar has demonstrated that as much as 19 per cent shrinkage after 30 h sintering at  $740^\circ\text{C}$  on a powder compact of  $53\text{ }\mu\text{m}$  particles.

The sintering of fine alumina powders ( $0.1\text{--}1\text{ }\mu\text{m}$ ) in air and argon was examined by N. C. Kothari, of the Danish Atomic Energy Research Establishment. He concluded that bulk diffusion was the principal mechanism

of material transport. The effect of atmosphere on the density was much smaller than that observed by E. D. Macklen and P. Johns (Standard Telecommunications Laboratories) on manganese-zinc ferrites.

T. Vasilos presented a review paper covering a wide range of theoretical and practical aspects of hot-pressing. Some of the results obtained by him and his co-author, R. M. Spriggs, at the laboratories of the Avco Corporation, Massachusetts, were very impressive indeed; for example, hot-pressing MgO *in vacuo* has resulted in a highly transparent material of theoretical density. The use of non-graphite dies and high pressures had given a fully densified material of small grain size. In fact, it appeared that the grain size was essentially the same as the particle size of the powder. Unfortunately, these fine-grained high-density materials were weaker than those with coarser grains, contrary to expectation. It was suggested that densification in these materials when formed at ultra-high pressures was entirely the result of plastic deformation and therefore only a weak bond was formed between particles. In general, the authors supported diffusion as the main mechanism of densification. The many questions from the participants showed the great interest in the new techniques, which used pressures up to 750,000 lb./in.<sup>2</sup>.

The densification of kaolin and mixtures of silica and alumina at much more moderate pressures (50,000 lb./in.<sup>2</sup>) and temperatures up to 900° C using dies of heat-resisting metals was discussed by T. G. Carruthers and T. A. Wheat (University of Leeds). Mullite formed at surprisingly low temperatures, and the parts played by chemically bound and physically adsorbed water were discussed.

Two of the papers, by J. F. MacDowell (Corning Glassworks, U.S.A.) and by P. W. McMillan and G. Partridge (English Electric), in the final section, "Other Fabrication Routes", were concerned with the production of dense polycrystalline materials by controlled nucleation of glasses.

The final paper, presented by A. G. Thomas, of the Plessey Research Laboratories, described the coating of very small spheres of UO<sub>2</sub> with Al<sub>2</sub>O<sub>3</sub> in a fluidized bed by the hydrolysis of AlCl<sub>3</sub>.

The meeting, which was organized by Dr. D. T. Livey, of the Metallurgy Division, Harwell, was, in my opinion, an unqualified success. It had a balanced programme which offered something to every worker in the field of ceramics. It was attended by 150 people, of whom half were members of the Society and half guests. The papers presented at this meeting will be published in a separate volume of the *Proceedings of the British Ceramic Society*.

P. POPPER

## NATURE OF ELECTRON EQUILIBRIUM IN HETEROGENEOUS SOLIDS AND THE PROPOSED ELECTRON ENERGY DISTRIBUTION IN DEGENERATE *p-n* JUNCTIONS\*

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IT is well known that the concept of Fermi-level constitutes one of the most important fundamentals of the modern electron theory of solids. According to quantum statistics<sup>1</sup>, this level may be defined to be the highest energy-level occupied by electrons at absolute zero. At higher temperatures, the Fermi-level may be defined to be that for which the probability of occupation by electrons is one-half.

A universally accepted idea, called the 'fundamental principle of statistical mechanics', states that if two or more different solid crystals are brought together to form a contact, then in thermal equilibrium the Fermi-level is constant at every point of the assembly, regardless of the distribution of the electron and hole concentration. Thus, for example, in thermal equilibrium, the Fermi-level in a *p-n* junction is constant at every point of the crystal including the transition region and even the metal ohmic contacts. A statement of this 'principle' may be found in many standard text-books on different subjects such as statistical mechanics, thermo-electricity, and the theory of *p-n* junctions in semiconductors.

It has recently been shown<sup>2</sup> that for the important general class of non-degenerate semiconductors, the validity of the 'principle' described above could not be justified. For simplicity, the argument has been deliberately confined to non-degenerate semiconductors, since for that class of solids, as is well known, the Fermi-Dirac statistics approach the Maxwell-Boltzmann statistics. Thus, electrons and holes may be considered as boltzmann gases, and the well-known classical gas laws may be applied.

This article shows that the arguments and conclusions concerning the electron equilibrium and its energy distribution, arrived at for non-degenerate *p-n* junctions, do

follow from more general new and established quantum-mechanical and quantum-statistical laws. These laws are applicable to heterogeneous metals and semiconductors in general.

In the first two sections we shall review and develop the concepts needed for examining the validity of the 'principle' mentioned earlier. We shall show in section (3) that the basic difficulties with this 'principle' can be directly traced to the conventional mechanism of electron equilibrium in heterogeneous solids. The proposed mechanism of equilibrium and resulting mathematical relations described in section (4) will provide, among other conditions, a basis for some proposed fundamental postulates in quantum statistics later stated in section (5). The postulates pertain to the quantum-mechanical formulation of the concepts of vapour pressure, and the particle diffusion force, all for electron and hole fermion gases in solids. From these postulates and the well-established physics of solids, a number of fundamental relations will be derived. These include the diffusion potentials<sup>3,4</sup>, the diffusion-mobility generalized relationship and, most importantly, the particle diffusion force Fermi-level relationship and the electron energy distribution in degenerate *p-n* junctions with wide and thin transition regions.

From time to time we shall refer to some of the material presented in ref. 2. It is therefore suggested that the reader may do well to acquaint himself thoroughly with the relatively straightforward classical treatment in that reference before attempting to understand the more involved quantum-mechanical theory presented here.

The term *solids* whenever used is meant to include *metals* and *semiconductors*. The latter may be degenerate or otherwise. For all these classes of solids, we shall assume that the volume energy density of quantum states is parabolic.

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In many instances we shall describe a solid as being *heterogeneous*. This will consist of three basic classes. The first is characterized by a spatial variation of the atomic structure. The second by a nearly uniform atomic structure but with a spatial variation of the density or type of impurities. The third by a spatially varying atomic structure as well as density or type of impurities. Examples of the first case include metal-metal, and metal-semiconductor interfaces. Examples of the second include various kinds of  $p$ - $n$  junctions, degenerate or otherwise. Examples of the third are heterojunctions in semiconductors, degenerate or otherwise.

As will become apparent later, the concept of average energy for electrons or holes plays an important part in formulating and understanding the new mechanism of electron equilibrium and the development of the general theory to be presented here. Thus we shall first begin with analysing this important concept.

### (1) Average Energy of Conduction Electrons in Solid Crystals

In this section a general expression will be derived for the average energy of electrons  $\langle \epsilon \rangle$  in the conduction band of a metal or semiconductor. We shall see that  $\langle \epsilon \rangle$  will depend on temperature alone in the case of non-degenerate semiconductors. For metals and degenerate semiconductors,  $\langle \epsilon \rangle$  will depend mostly on the Fermi-level  $\epsilon_f$ .

The average energy  $\langle \epsilon \rangle$  may be defined to be given by:

$$\langle \epsilon \rangle = (1/n) \int_0^\infty \epsilon g(\epsilon) F(\epsilon, \epsilon_f) d\epsilon \quad (1)$$

where  $\epsilon$  is any energy-level in the conduction band measured from its bottom, and  $\epsilon_f$  is the Fermi-level. The latter is considered positive if it lies in the conduction band. The other quantities in equation (1) are described as follows:

$$g(\epsilon) = C_n \epsilon^{1/2} \quad (2)$$

$$F(\epsilon, \epsilon_f) = \{1 + \exp[(\epsilon - \epsilon_f)/kT]\}^{-1} \quad (3)$$

$$n = \int_0^\infty g(\epsilon) F(\epsilon, \epsilon_f) d\epsilon \quad (4)$$

Here  $g(\epsilon)$  is the volume energy density of quantum states in the conduction band and is assumed to be parabolic<sup>6</sup>,  $C_n$  is a constant which depends on the electron effective mass, and Planck's constant. The function  $F(\epsilon, \epsilon_f)$  is the Fermi-Dirac<sup>6,7</sup> probability distribution function,  $k$  is Boltzmann's constant and  $T$  is the absolute temperature. The quantity  $n$  represents the volume concentration of electrons in the conduction band.

In order to make use of the tables of the Fermi-Dirac functions, computed by McDougall and Stoner<sup>8</sup>, to evaluate numerically  $\langle \epsilon \rangle$ , we must introduce in equation (1) two variable transformations. These are:

$$\zeta = \epsilon/kT \quad (5)$$

and:

$$\eta = \epsilon_f/kT \quad (6)$$

In view of all the preceding equations, equation (1) may be written as:

$$\langle \epsilon \rangle = (3/2)kT \rho_\epsilon(\eta) \quad (7)$$

where:

$$\rho_\epsilon(\eta) = (2/3)F_{3/2}(\eta)/F_{1/2}(\eta) \quad (8)$$

and:

$$F_{\kappa}(\eta) = \int_0^\infty \zeta^\kappa d\zeta / [1 + \exp(\zeta - \eta)] \quad (9)$$

Fig. 1 represents the coefficient  $\rho_\epsilon$  as a function of  $\eta = \epsilon_f/kT$ . The quantity  $\rho_\epsilon(\eta)$  may be interpreted as the ratio of the average energy of each conduction electron to the classical average thermal energy  $(3/2)kT$ . It is interesting to notice that  $\rho_\epsilon$  is very nearly 1 whenever  $\epsilon_f$  is below the bottom of the conduction band. The reader will recognize this case to correspond to non-degenerate

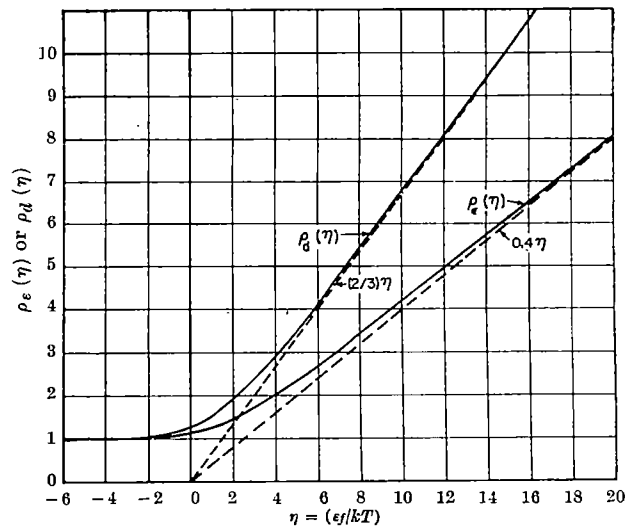


Fig. 1. Dependence on  $\eta = (\epsilon_f/kT)$  of: (a) ratio  $\rho_\epsilon$  of the average energy  $\langle \epsilon \rangle$  of an electron in the conduction band to  $(3/2)kT$ ; (b) ratio  $\rho_{ea}$  of  $(D/\mu)$  to  $(kT/q)$ . The Fermi-level  $\epsilon_f$  is measured from the bottom of the conduction band

$n$ -type semiconductors, or to  $p$ -type semiconductors of any degree of degeneracy or non-degeneracy. The coefficient increases rather rapidly for  $\eta > 0$  and can be shown<sup>9</sup> to approach  $0.4\eta$  asymptotically. This limit is closely reached for strongly degenerate  $n$ -type semiconductors and metals, for which  $\langle \epsilon \rangle \approx 0.6 \epsilon_f$ .

By a similar treatment, it can be shown that the plot of  $\rho_\epsilon(\eta)$  of Fig. 1 may also represent a  $p$ -type semiconductor crystal. For this case the point  $\eta = 0$  would represent the Fermi-level coinciding with the top of the valence-band band, and  $\eta > 0$  would correspond to a degenerate crystal, while  $\eta < 0$  corresponds to a non-degenerate crystal.

It is interesting to notice that for any semiconductor crystal, degenerate or otherwise, the average energy of minority carriers in thermal equilibrium is  $(3/2)kT$  within the respective band, regardless of the carrier concentration. For majority carriers, this conclusion will be true only for a non-degenerate semiconductor crystal.

### (2) Electron Forces and Electron Currents in Heterogeneous Solids

In this section we shall derive from a phenomenological argument some general fundamental relations between the density of electron current in a solid and the forces exerted on the electrons in the conduction band by one or more processes. These relations are important for a clear understanding of the basic questions to be raised and the proposed answers.

Let us consider a solid crystal in which the conduction is only caused by an electrostatic field  $\vec{E}$ , which may be uniform or otherwise. At any point  $P$  in the crystal, the electron current density  $\vec{J}$  may be expressed as:

$$\vec{J} = \mu n(q\vec{E}) \quad (10)$$

Here  $q$  is the magnitude of the electron charge, and  $n$  and  $\mu$  are the electron volume concentration and mobility at point  $P$ , respectively.

Equation (10) may now be interpreted in a more general way. The quantity  $(-q\vec{E})$  represents the average electrostatic force  $\vec{f}_e$  acting on the electron. This force is undoubtedly responsible for the current  $\vec{J}$  under the conditions assumed here. The nature of this force, however, is not really important in so far as the magnitude

and direction of the current  $\vec{J}$ . This will mean that if the conduction electrons were acted on at  $P$  by an equal force  $\vec{f}$  of different nature, then the current  $\vec{J}$  would remain indeed unchanged, provided that  $(\mu n)$  continued to be the same. Therefore, we may now predict the current density  $\vec{J}$  at a point  $P$ , if we know the net average force  $\vec{f}$  acting on each electron at that point. This force may be caused by one or more processes. In general, we may write:

$$\vec{J} = -\mu n \vec{f} \quad (11)$$

and say that  $\vec{f}$  is the resultant of all the average forces exerted by all the processes on the electron at  $P$ .

It should be noticed that equation (11) has been derived without any restriction on the position of the Fermi-level. Hence it may be assumed that the equation is valid for all the solids of interest here.

By a similar argument, it can be reasoned that for a  $p$ -type semiconductor, except for a different sign, an equation identical to that of equation (11) may be obtained.

It can now be shown that equation (11) would lead to a familiar important relationship, usually stated for non-degenerate semiconductors. In such crystals, if the conduction is caused by the diffusion and electrostatic processes, then in view of equation (11), we may write:

$$\vec{J} = -\mu n (\vec{f}_d + \vec{f}_e) \quad (12)$$

where  $\vec{f}_d$  and  $\vec{f}_e$  are the average forces exerted on the electron due to the diffusion and drift processes, respectively.

It has been shown<sup>2</sup> from a pure thermodynamic argument that for a classical ideal boltzon gas, under isothermal conditions:

$$\vec{f}_d = -(kT/n)\nabla n \quad (13)$$

and:

$$D/\mu = kT/q \quad (14)$$

Here  $D$  and  $\mu$  are the diffusion constant and mobility, respectively, for the electron gas considered. Recalling that  $\vec{f}_e = -q\vec{E}$ , then from equations (12), (13) and (14), we have:

$$\vec{J} = qD\nabla n + qn\mu\vec{E} \quad (15)$$

which is a well-known relation. Equation (15) has been used in ref. 2 in discussing the inconsistencies of the conventional energy distribution in non-degenerate  $p$ - $n$  junctions. It will now be noticed that equation (15) is derived on the basis of equation (11) and the ideal gas laws. This will explain why it is justifiable to apply the ideal gas laws to non-degenerate semiconductors, as has been exclusively done in ref. 2.

### (3) Critique of the Conventional Mechanism of Electron Equilibrium in Heterogeneous Solids

We shall next discuss some fundamentally objectionable consequences of the conventional concept of electron equilibrium. The latter is well known to lead supposedly to the familiar 'principle' of constant Fermi-level in heterogeneous solids if they are in thermal equilibrium.

Under thermal equilibrium conditions, it is generally assumed that at every point in a heterogeneous solid, the diffusion current  $\vec{J}_d$  and drift current  $\vec{J}_e$  are equal and opposite, so that:

$$\vec{J} = \vec{J}_e + \vec{J}_d = 0 \quad (16)$$

We shall call this condition *flow equilibrium* to distinguish it from the alternative one to be discussed in the next section.

At first glance, equation (16) appears to be valid, for it seems that if this equation were not satisfied, a current would flow under thermal equilibrium, which clearly would not be possible. Despite this apparent validity of equation (16), the flow equilibrium it describes implies inevitably fundamental subtle but serious contradictions. To explain this statement, consider equation (11), which in view of equation (16) may be written as:

$$\vec{J} = -\mu n \vec{f} = 0 \quad (17)$$

where  $\vec{f}$  is the net average force acting on the electron due to the processes of diffusion and drift. Recalling that equation (17) is supposed to hold at every point in the transition region, and that  $\mu \neq 0$ , and  $n \neq 0$ , then it follows that  $\vec{f} = 0$ .

Thus it can be stated that *the conventional flow equilibrium clearly leads to the conclusion that in thermal equilibrium an electron crossing any solid-solid interface would do so at constant energy*. Some direct consequences of that statement are:

(a) A denial of the existence of the well-known Peltier effect<sup>10</sup>, which only exists because of the possibility that an electron crossing an interface can change its energy, so that the interface thermal energy has to change in such a way as to satisfy the requirements of the first law of thermodynamics<sup>11</sup>.

(b) In the case of a non-degenerate  $p$ - $n$  junction, as shown in section (1) the average majority and minority electron energy is  $(3/2)kT$  above the bottom of the conduction bands everywhere in the crystal. Hence, in thermal equilibrium, the conclusion of a constant electron energy everywhere in the transition region will mean that the conduction bands throughout the crystal should line up. This conclusion, of course, contradicts the conventional concept of the energy diagram which for this simple case would be as represented in Fig. 2. It is clear in that figure that an electron crossing the junction from the  $n$ - to the  $p$ -region, under thermal equilibrium conditions, would gain an amount of energy equal to  $(kT)\ln(n_n/n_p)$ .

It will now be recalled that we have shown earlier that the flow equilibrium leads to the constant electron energy everywhere in a heterogeneous solid crystal. Yet the conventional theory uses the same assumption and concludes that substantial changes do occur in the electron energy under the same conditions. A legitimate question of particular interest at this point would be: how has the conventional theory reasoned on the basis of flow equilibrium the conventional conclusions? We shall

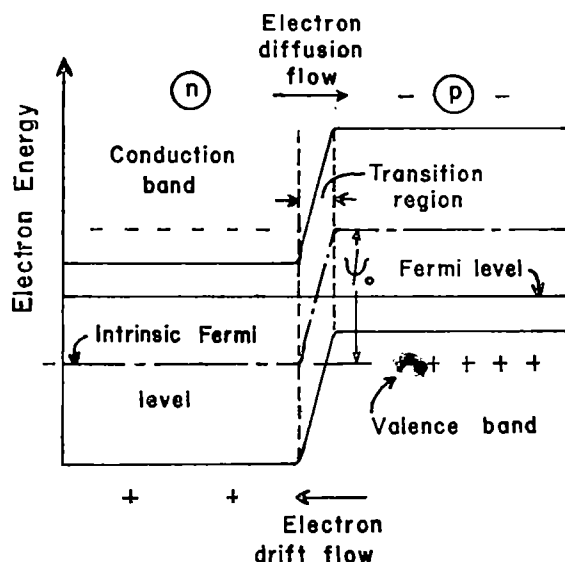


Fig. 2. Conventional electron energy band diagram for a non-degenerate  $p$ - $n$  junction in thermal equilibrium

next summarize and interpret the conventional steps in terms of the electron forces.

The conventional theory begins with assuming equation (16), and (in view of equation (11)) proceeds to consider that at every point of a heterogeneous solid in thermal equilibrium:

$$\vec{J}_e + \vec{J}_d = -\mu n(\vec{f}_e + \vec{f}_d) = 0 \quad (18)$$

Here  $(\vec{f}_e + \vec{f}_d)$  represents the total force acting on the electron at any point due to drift and diffusion. To avoid using the unfortunate consequence of equation

(18) that  $(\vec{f}_e + \vec{f}_d)$  should indeed everywhere be zero, and so would have to be the net change of electron energy, the common procedure is to change the appearance of  $(\vec{f}_e + \vec{f}_d) = 0$  to:

$$\vec{f}_d = -\vec{f}_e \quad (19)$$

It must be noticed here that neither  $\vec{f}_e$  nor  $\vec{f}_d$  represents the total force acting on the electron at any point. Now integrating each side of equation (19) with respect to the distance  $x$ , across, for example, a transition region of width  $w$  in a  $p$ - $n$  junction, provides of course a non-zero answer. This answer has been interpreted to be the built-in potential, considered to be electrostatic in nature, and invariably treated likewise. The integral will be:

$$-\int_0^w \vec{f}_e \cdot d\vec{x} = \int_0^w \vec{f}_d \cdot d\vec{x} \quad (20)$$

It might interest the reader to see further that for a non-degenerate  $p$ - $n$  junction, if we substitute  $\vec{f}_d = (-kT/n)(dn/dx)$ , which has been derived<sup>2</sup> on the basis of the ideal gas laws, equation (20) will become:

$$\begin{aligned} \psi_0 &= -\int_0^w \vec{f}_d \cdot d\vec{x} = \int_0^w \vec{f}_e \cdot d\vec{x} \\ &= -(kT) \int_{n_n}^{n_p} dn/n = (kT) \ln(n_n/n_p) \end{aligned} \quad (21)$$

where  $w$  is the width of the transition region, and  $\psi_0$  is the conventional 'built-in potential'. This result can be shown to require that the Fermi-level should be constant throughout the crystal. For example, it may be explained on the basis of equation (21) by substituting for  $n_n$  and  $n_p$  their values expressed in terms of  $(\epsilon_c - \epsilon_f)$  and  $(\epsilon_f - \epsilon_v)$ , respectively. Here  $\epsilon_c$  is the lowest energy-level in the conduction band of the  $n$ -region, and  $\epsilon_v$  is the highest energy-level in the valence-band of the  $p$ -region. The detailed steps may be found in many standard books on  $p$ - $n$  junction theory.

The reader will now appreciate that the quantity  $\psi_0$ , calculated in equation (21), is not really the total built-in potential. On the contrary, according to equations (19) and (21),  $\psi_0$  represents only the magnitude of either the diffusion or the electrostatic potential. Since according to the assumption of flow equilibrium the two potentials are supposed to be equal and opposite, they should have indeed added up to zero. To avoid facing the conflict of this result with the implications of the Peltier effect, the conventional theory appears as though it has chosen to consider one of the potentials and ignore the existence of the other. Then the chosen potential has been defined to be the electrostatic potential. What has helped conceal these fundamental inconsistencies seems to be the lack of a clear understanding of the nature and properties of the particle diffusion force. So the diffusion process has conventionally been thought to be associated with only a flow, but not a force. It may now be appreciated why it would be essential to understand how the electron equilibrium can be established in a manner compatible with the Peltier effect and other well-established laws of Nature. These fundamental questions will be dealt with in the coming sections.

#### (4) Proposed Mechanism of Electron Equilibrium in Heterogeneous Solids

In section (3) it has been seen how the assumption of flow equilibrium of electrons in solids would present a fundamental conflict with the existence of the Peltier effect. This conflict has arisen over the fact that when it is recognized that the diffusion process exerts an average force on each electron, the assumption of flow equilibrium implies that an electron crossing any heterogeneous region of a solid would do so at constant energy. If we now are permitted to accept as a matter of fact the existence of the Peltier effect, we must inevitably state that: *under thermal equilibrium conditions, the net average force acting on each conduction electron in a heterogeneous solid is not necessarily zero at every point.*

At first glance, this statement appears as though it might not be valid, for it may seem to lead to a net flow of electrons under thermal equilibrium conditions. Such a flow, of course, would be impossible. As will be shown shortly, this statement is only paradoxical, as it does not necessarily require such a current under thermal equilibrium.

To explain this point and its consequences, consider a heterogeneous solid forming a closed loop. Let the whole body be in thermal equilibrium. If the net average force  $\vec{f}$  exerted on each electron is not necessarily zero, then we must conclude that the average value of  $\vec{f}$  around any closed loop  $C$  within the solid must vanish; that is the line integral of  $\vec{f}$  around any closed loop is zero, or:

$$\oint_C \vec{f} \cdot d\vec{s} = 0 \quad (22)$$

If we now assume that  $\vec{f}$  is caused by the electrostatic and diffusion processes, we may replace  $\vec{f}$  by  $(\vec{f}_e + \vec{f}_d)$ . Therefore, equation (22) may be written as:

$$\oint_C (\vec{f}_e + \vec{f}_d) \cdot d\vec{s} = \oint_C \vec{f}_e \cdot d\vec{s} + \oint_C \vec{f}_d \cdot d\vec{s} = 0 \quad (23)$$

According to the well-known laws of electrostatics, the line integral of the electrostatic field  $\vec{E}$ , and  $\vec{f}_e = -q\vec{E}$ , around any closed loop must vanish<sup>12</sup>. Thus, equation (23) reduces to:

$$\oint_C \vec{f}_d \cdot d\vec{s} = 0 \quad (24)$$

In view of Stokes's theorem<sup>13</sup>, we may express the condition for equilibrium described by equation (24) in the microscopic form:

$$\nabla \times \vec{f}_d = 0 \quad (25)$$

which would be valid at any point.

We may summarize the preceding discussions and results in the following statement: *A necessary and sufficient condition for equilibrium of electrons in solids under the action of diffusion alone is that the line integral of the electron diffusion force around any closed loop is zero. This also means that the curl of this force must vanish at every point. Thus the electron diffusion force constitutes a conservative field.*

Equations similar to (24) and (25) are well known to hold for electrostatic and gravitational fields, which are all conservative.

Since each of the possible processes in a heterogeneous solid is associated with a conservative force field, we may state the familiar principle of detailed balancing<sup>14,15</sup> of statistical mechanics in a more detailed way as follows: *Under thermal equilibrium conditions, at any point in a heterogeneous solid, for every electron which is crossing a given area in one direction per unit time due to each process, there is another which is crossing it in the opposite direction.*



Thus, it can clearly be seen that the principle of detailed balancing holds true for all the processes combined.

As an interesting application of equation (24), we shall discuss the equilibrium of the diffusion process in a non-degenerate semiconductor crystal on the basis of the classical formulation of the particle diffusion force of ref. 2. For simplicity let the crystal geometry resemble a ring consisting of any finite number of regions, such as three. Each region has a uniform electron concentration designated as  $n_1$ ,  $n_2$  and  $n_3$ . It is evident that in thermal equilibrium, an electron moving along any closed loop  $C$  within the crystal would be acted on by a non-vanishing

diffusion force  $\vec{f}_d$  only in the transition regions. In this event  $\vec{f}_d$  will be as given by equation (2) of ref. 2. Thus, the average value of  $\vec{f}_d$  over the closed path  $C$  will be proportional to:

$$\oint_C \vec{f}_d \cdot d\vec{s} = kT[\ln(n_1/n_2) + \ln(n_2/n_3) + \ln(n_3/n_1)] = 0 \quad (26)$$

Hence the condition described by equation (24) is satisfied, regardless of the number of different regions in the crystal. We may therefore conclude that the particle diffusion force in its classical form, as defined in ref. 2, does meet the requirements of the new concept of equilibrium.

### (5) Quantum-statistical Formulation of the Particle Diffusion Force

It has been shown that, from a purely thermodynamic argument<sup>2</sup>, the *particle diffusion force* for an ideal classical gas would be  $\vec{f}_d = (-k/n)\nabla(nT)$ . We have used this answer to calculate the *diffusion potentials* in any isothermal non-degenerate  $p$ - $n$  junction. For this class of semiconductors, as is well known, the electrons (and holes) behave very nearly like ideal boltzon gases.

If we now wish to calculate the diffusion potentials in any isothermal heterogeneous solid in general, we must first seek a more general formulation for  $\vec{f}_d$ . This formulation must fulfil the following requirements: (1) preserve the nature of  $\vec{f}_d$ ; (2) obey the laws of quantum mechanics; (3) allow the electrons to behave as fermion gases; (4) tend to the classical formulation, if the fermion gas tends to be a boltzon gas; and (5) lead to a force field, which is conservative, so that equations (24) or (25) are satisfied. We shall see that all these requirements are fulfilled by means of an established law of quantum statistics and three proposed ones. These laws are to be stated and discussed next.

*Vapour pressure of a fermion gas:* This physical quantity is generally defined<sup>16</sup> to be given by:

$$p = (2/3)n\langle\epsilon\rangle \quad (27)$$

Here  $n$  is the volume concentration of the gas particles and  $\langle\epsilon\rangle$  is the average energy of each particle.

Equation (27) is essentially an extension of that derived for a classical gas (ideal or otherwise), which is usually stated as:  $p = mn\langle v^2 \rangle / 3$ . Here  $m$  and  $v$  are the gas particle mass and velocity, respectively, and  $m\langle v^2 \rangle / 2$  has been interpreted to be  $\langle\epsilon\rangle$ .

*Postulate I:* The vapour pressure and the diffusion of a fermion gas constitute different formulations of the same process. This will mean that both formulations are mutually inclusive, so that one uniquely determines the other.

This postulate is again an extension of the classical properties of vapour pressure and diffusion discussed in ref. 2.

*Postulate II:* The preceding postulate suggests the existence of a mathematical link between the concepts of diffusion and vapour pressure. This link may be

reasonably expected to be the *particle diffusion force*. For a fermion gas we postulate it to be given by:

$$\vec{f}_d = - (1/n)\nabla p \quad (28)$$

It will be noticed that equation (28) is a three-dimensional form of equation (1) of ref. 2. The latter equation has been derived for a classical gas, ideal or otherwise. So we merely postulate here that equation (28) holds beyond a classical gas.

*Postulate III:* If there is more than one kind of gas, then equation (27) and the preceding postulates will

still hold, provided that the variables  $\vec{f}_d$ ,  $\epsilon$ ,  $p$  and  $n$  are considered to be for any one kind of gas. Thus, for example, equations (27) and (28) may be applied to either electrons or holes in semiconductors, provided that the respective variable mathematically exists within a closed domain in which the potentials are to be computed.

### (6) Equilibrium Relationship between the Particle Diffusion Force and the Fermi-level in Isothermal Heterogeneous Solids

In this section, we shall derive a fundamental relationship between the (average) particle diffusion force  $\vec{f}_d$  at any point in an isothermal heterogeneous solid and the Fermi-level  $\epsilon_f$  at that point. We shall then discuss the result, which will be of considerable importance in determining the electron energy distribution in any isothermal heterogeneous solid. The basic assumption we shall make here is that the average energy of the electron at every point of interest in the solid corresponds to the electron-lattice equilibrium value at that point. This will mean that the electron has a sufficiently large number of collisions in the immediate neighbourhood of every point of interest so that any energy it has above or below the local equilibrium value is transferred almost entirely to the lattice. Examples, for which this assumption holds, are what we shall call *wide-junction p-n diodes*. These  $p$ - $n$  junctions may be degenerate or non-degenerate. Further discussion of these examples, together with others called *thin-junction p-n diodes*, for which this assumption is not fulfilled, may be found in sections 8 and 9.

In order to relate  $\vec{f}_d$  to  $\epsilon_f$ , at any point of an isothermal heterogeneous solid satisfying the assumption stated here, consider equations (27) and (28), from which it follows that:

$$\vec{f}_d = - (2/3n)\nabla(n\langle\epsilon\rangle) \quad (29)$$

Substituting equation (1) into equation (29), we get:

$$\vec{f}_d = - (2/3n)\nabla\left\{\int_0^\infty \epsilon g(\epsilon) F(\epsilon, \epsilon_f) d\epsilon\right\} \quad (30)$$

Here  $g(\epsilon)$  and  $F(\epsilon, \epsilon_f)$  are as given by equations (2) and (3). Notice that  $n$  and  $\epsilon_f$  in equation (30) are functions of position, whereas  $\epsilon$  is not so. Therefore, equation (30), after some manipulation, will yield:

$$\vec{f}_d = (2C_n/3n)(\nabla\epsilon_f)\int_0^\infty \epsilon^{3/2} \frac{d}{d\epsilon} \{1 + \exp[(\epsilon - \epsilon_f)/kT]\}^{-1} d\epsilon \quad (31)$$

Integrating equation (31) by parts, we get:

$$\vec{f}_d = - (C_n/n)(\nabla\epsilon_f)\int_0^\infty \epsilon^{1/2} \{1 + \exp[(\epsilon - \epsilon_f)/kT]\}^{-1} d\epsilon \quad (32)$$

In view of equations (2), (3) and (4), equation (32) will reduce to:

$$\vec{f}_d = - \nabla\epsilon_f \quad (33)$$

where  $\epsilon_f$  is measured from the bottom of the conduction band.

It must be remembered here that the nature of  $\vec{f}_a$  is not electrostatic. Rather,  $\vec{f}_a$  is entirely caused by the diffusion process, which is generally due to electron-phonon, electron-impurity<sup>17</sup> and possibly electron-electron interactions. This is why  $\vec{f}_a$  after all has been invariably termed the (average) *particle diffusion force*. At any point, this force, according to equation (33), depends only on the gradient of the Fermi-level. Since the curl of any gradient is zero, we may conclude that:

$$\nabla \times \vec{f}_a = 0 \quad (34)$$

Again from equation (33), it follows that the line integral of  $\vec{f}_a$  between any two points depends on the end points rather than the path. Thus  $\vec{f}_a$  constitutes a conservative field, and satisfies the necessary and sufficient conditions for the new mechanism of equilibrium (see equations (24) and (25)).

At this point, the striking significance of equation (33) may not be fully apparent. As an illustration, in the next three sections some particularly important consequences of this fundamental relationship will be discussed.

### (7) The Generalized Diffusion-mobility Relationship in Degenerate Solids

In this section we shall derive a general relationship between the mobility  $\mu$  and the diffusion constant  $D$  for electrons in solids. To do so, we consider the classical definition of  $D$  to be given by Fick's law:  $\vec{J}_a = qD\nabla n$  (ref. 18). Here  $\vec{J}_a$  signifies the electron current density associated with diffusion. By virtue of equation (11) and Fick's law, we may define  $D$  in terms of the particle diffusion force  $\vec{f}_a$  at any point in a solid by:

$$\vec{f}_a = -\vec{J}_a/(\mu n) = -q(D/\mu)(\nabla n)/n \quad (35)$$

Substituting equation (33) into equation (35), and solving for  $(D/\mu)$ , we get:

$$D/\mu = (n/q)(\nabla \epsilon_f)/\nabla n \quad (36)$$

In view of equations (2), (3), (4), (5), (6), (9) and (36), it can be shown that:

$$D/\mu = (kT/q)\rho_a \quad (37)$$

where:

$$\rho_a = [F_{1/2}(\eta)]/\{dF_{1/2}(\eta)/d\eta\} \quad (38)$$

The dependence of the coefficient  $\rho_a$  on the Fermi-level is plotted in Fig. 1. The curve has been computed from the tables of the Fermi-Dirac functions<sup>8</sup>. It can be shown<sup>18</sup> that  $\rho_a$  tends asymptotically to  $(2/3)\eta$ . Thus for metals and strongly degenerate semiconductors, the diffusion-mobility relationship becomes:

$$(D/\mu) \cong (2/3)\epsilon_f/q \quad (39)$$

It is interesting to see that for  $\eta < 0$ , that is, for any non-degenerate semiconductor,  $\rho_a$  approaches 1. Consequently the ratio  $(D/\mu)$  also will approach  $(kT/q)$ , which is the Einstein diffusion-mobility relationship originally derived for ideal boltzon gases.

### (8) Proposed Energy Band Diagrams for Wide-junction Degenerate p-n Diodes

In this section some of the basic general laws established earlier will be applied to determine the energy band diagrams for wide-junction p-n diodes.

Before treating the p-n junction problem, it might be pedagogically desirable to review briefly the important basic properties of the *electron diffusion force*  $\vec{f}_a$ . First,

it must be emphasized that the nature of  $\vec{f}_a$  is not electrostatic. More specifically,  $\vec{f}_a$  is due to the diffusion process, which is the inevitable consequence of the random interaction of a spatially varying density of electron population with the atomic lattice structure. We have furthermore shown that  $\vec{f}_a$ , like the electrostatic electron force  $\vec{f}_e$ , must constitute a conservative field. Thus, it is self-evident that if an electron moves within a solid between any two points, then, regardless of the path, in thermal equilibrium the average work done on the electron due to the processes of diffusion and drift will be:

$$W_{ab} = \int_a^b (\vec{f}_a + \vec{f}_e) \cdot d\vec{s} = \int_a^b \vec{f}_a \cdot d\vec{s} + \int_a^b \vec{f}_e \cdot d\vec{s} \quad (40)$$

It is also self-evident that equation (40) would still hold if thermal equilibrium is disturbed by, for example, a net flow of electrons, provided that the flow does not introduce new processes unaccounted for, such as the electron-hole generation or recombination in semiconductors.

Another relation of considerable importance is described by equation (33). According to this equation, within a heterogeneous solid:

$$\int_a^b \vec{f}_a \cdot d\vec{s} = - \int_a^b \nabla \epsilon_f \cdot d\vec{s} = \epsilon_f(a) - \epsilon_f(b) \quad (41)$$

Here  $\epsilon_f$  is always referred to the bottom of the conduction band. It should be recalled that equation (33), and hence equation (41), are based on the assumption that the electron has sufficient collisions with the lattice atoms in the immediate neighbourhood of points  $a$  and  $b$ , so that its energy may be considered equal to the electron-lattice equilibrium values at either of the two points.

Next, equations (40), (41) and other relations will be used to determine the electron energy distribution in a degenerate wide-junction p-n diode. For such a crystal, let the spatial variation of the Fermi-level  $\epsilon_f$  (referred to the bottom of the conduction band) be as shown in Fig. 3a. In thermal equilibrium, an electron crossing the junction, from the  $n$ - to the  $p$ -region, according to equation (41), will gain from the diffusion process alone an amount of energy equal to the difference between the Fermi-levels at either side of the junction. This energy will be designated as  $\phi_0$  and will be considered to be a positive quantity.

Now consider Fig. 3b, which, as will be shown shortly, represents the energy band diagram corresponding to the process of diffusion alone. The amount of energy  $\phi_0$  must then represent the change in the average electron energy as it crosses the junction from one end to the other. The average values of electron energy in the  $n$ - and  $p$ -region, however, are:  $(3/2)kT\rho_n$  and  $(3/2)kT\rho_p$ , respectively (section 1). Thus from the geometry of Fig. 3b, the bottom of the conduction bands in the  $n$ - and  $p$ -regions must be separated by an amount of energy equal to  $(\phi_0 + \delta)$ , where:

$$\delta = (3/2)kT(\rho_n - 1) \quad (42)$$

Again from the geometry of Fig. 3b, this will mean that in thermal equilibrium the Fermi-level in the  $p$ -region must be above that of the  $n$ -region by an amount of energy equal to  $\delta$ .

The quantity  $\delta$  is not insignificant for strongly degenerate p-n junctions and some metal-semiconductor contacts. This may be seen by referring to section (1) in which we have observed that, for large values of  $\eta$ , the factor  $\rho_n$  tends to 0.4 ( $\epsilon_f/kT$ ). Hence, for strongly degenerate p-n junctions and some metal-semiconductor contacts, in the limit:

$$\delta = 0.6 \epsilon_f \quad (43)$$

Here  $\epsilon_f$  is the energy between the Fermi-level and the bottom of the conduction band.

Now let us consider the electrostatic junction built-in potential associated with the force  $\vec{f}_e$  acting on an electron as it crosses the transition region. This will be done first

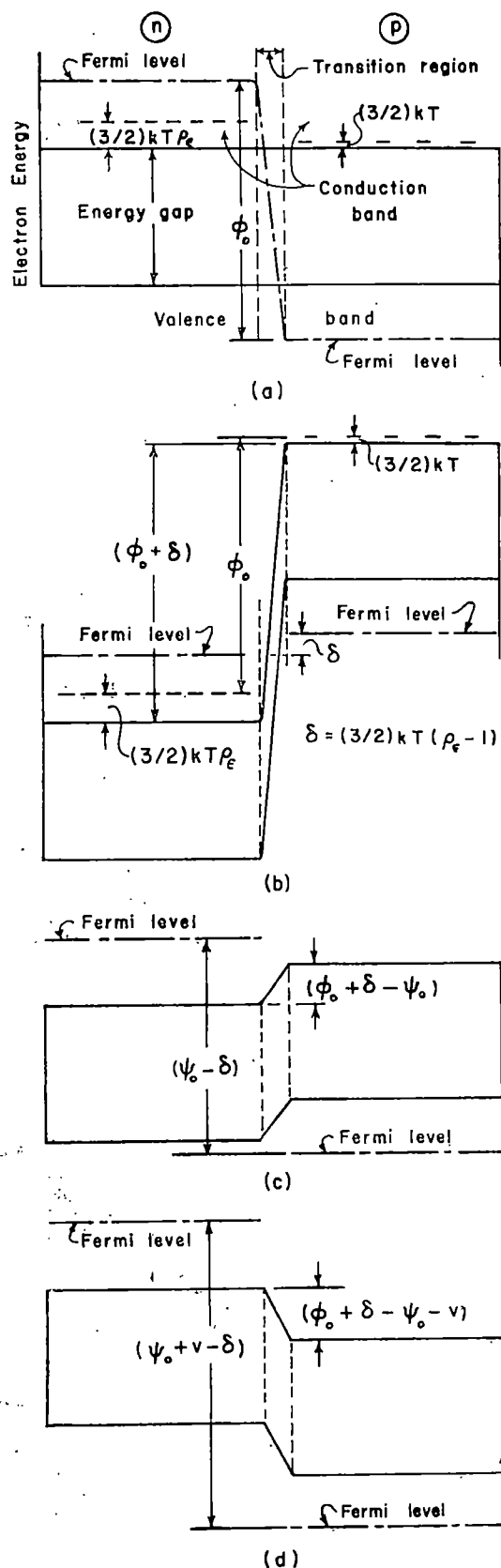


Fig. 3. (a) Spatial variation (referred to the bottom of the conduction band) of the Fermi-level  $\epsilon_f$  and average electron energy  $\langle \epsilon \rangle$  in a degenerate wide-junction p-n diode. Also energy band diagrams for this crystal when it is: (b) in thermal equilibrium and only the diffusion process is accounted for; (c) in thermal equilibrium and both the diffusion and drift processes are accounted for; (d) forward biased and both processes are accounted for. The complete potential  $(\phi_0 + \delta - \psi_0)$  in case (c) has been arbitrarily chosen positive

for a crystal in thermal equilibrium. It should be noticed that in the transition region, the electron forces  $f_d$  and  $f_e$  are always in opposite directions. Since  $f_d$  has been defined in equations (40) and (41) as a positive quantity, to be consistent, we must consider  $f_e$  to be negative. Furthermore, the energy term in equation (40) contributed by  $f_e$  must also be negative. Therefore, we may define the line integral of  $f_e$  across the entire transition region to be  $(-\psi_0)$ , where  $\psi_0$  is positive. Thus the composite built-in potential across the transition region (referred to the conduction bands) will be  $(\phi_0 + \delta - \psi_0)$  as shown in Fig. 3c. So, from the geometry, it can be seen that in thermal equilibrium, the Fermi-levels across the transition region must be displaced by an amount of energy equal to  $(\psi_0 - \delta)$ .

The changes in the energy band diagrams, if thermal equilibrium is disturbed by forward biasing will now be discussed. We shall only treat the case in which no carrier generation or recombination would occur in the transition region.

Under forward-biasing conditions, if minority electrons are injected in a sufficiently long p-region, there will be an excess-charge zone within that region where recombination takes place. It can be shown<sup>20</sup> that the average energy gained by an electron in that zone due to the diffusion process is at most  $kT$ , provided that the injected minority carriers do not reach degenerate proportions. Thus, for convenience, in most practical cases we may completely neglect this quantity.

Consider now the p-n junction, its ohmic contacts and the battery supplying the power. Let it be assumed that the current in the circuit is sufficiently small so that the distribution of electrons in the ohmic contacts does not deviate from thermal equilibrium, and the Joule losses are negligible everywhere. Since the electron energy must be conserved as it goes round the circuit, then the junction built-in potential must change from its thermal-equilibrium value of  $(\phi_0 + \delta - \psi_0)$  to  $(\phi_0 + \delta - \psi_0 - v)$ . Here  $v$  is the forward applied voltage. This change in the junction potential has been arrived at on the basis that, on forward biasing, the electron gains  $v$  electron volts from the battery. Since the diffusion and electrostatic potentials in the ohmic contacts have been assumed to remain constant on forward biasing, the junction potential must then change in such a way as to satisfy the law of conservation of energy. Fig. 3d represents the complete energy distribution in a forward-biased p-n junction.

It is interesting to notice here that the energy band diagram of Fig. 3d and that of Fig. 4c of ref. 2 are compatible. The reason for this is that for non-degenerate semiconductor crystals, according to Fig. 1 and equation (42), the quantity  $\delta = 0$ . Therefore the two diagrams mentioned here become identical.

It should be emphasized that the electrostatic potential  $\psi$  must always satisfy at every point of the transition region the fundamental relation:

$$\nabla^2 \psi = -q(p' - n + N_d - N_a)/\epsilon_0 \quad (44)$$

Here  $p'$  is the density of holes and  $N_d$  and  $N_a$  are the concentrations of the ionized donor and acceptor atoms, respectively, and  $\epsilon_0$  is the dielectric constant.

It should be noticed that the change  $v$  in the built-in junction potential from its thermal equilibrium value is partly electrostatic and partly due to diffusion. The electrostatic part should add up with  $\psi_0$  to make  $\psi$ , which must satisfy the laws of electrostatics as expressed by equation (44). The diffusion part of  $v$  should add up with  $(\phi_0 + \delta)$  to make  $(\phi_0 + \delta)$  which must obey the quantum statistical laws of diffusion. How  $v$  will be divided between the diffusion and electrostatic potentials will depend largely on the situation considered. Further discussion of this point as applied to the injection theory in non-degenerate p-n junctions may be found in section 4 of ref. 2.



(9). Proposed Energy Band Diagrams for Thin-junction Degenerate  $p$ - $n$  Diodes

In section (8), we have seen the part which the  $f_d$  and  $\epsilon_f$  equilibrium relationship plays in determining the electron energy distribution in degenerate wide-junction  $p$ - $n$  diodes. As mentioned earlier, in such  $p$ - $n$  junctions, there are sufficient electron-lattice collisions in the neighbourhood of every point of interest, so that the electron energy corresponds to the equilibrium values at these points.

The object of this section is to investigate the electron energy distribution in an important different class of  $p$ - $n$  junctions. This class is characterized by having space-charge regions so thin that the electron-lattice equilibrium cannot be established within these regions.

It should be recalled that according to section 4, under thermal equilibrium or otherwise,  $f_d$  and  $f_e$  would not necessarily be equal at every point. The validity of this statement may be most clearly seen in Fig. 4. This figure represents the energy band diagrams for a thin-junction  $p$ - $n$  diode, under thermal equilibrium and forward biasing.

To understand these diagrams and how they have been arrived at, consider an electron crossing from the  $n$ - to the  $p$ -region. Let the crystal be first in thermal equilibrium. As the electron crosses the thin space-charge layer, it will be acted on by an opposing electrostatic force ( $-|f_e|$ ). The line integral of this force will transfer to the electron an amount of energy ( $-\psi_0$ ), where  $\psi_0$  is positive. The electrostatic potential  $\psi_0$  must satisfy equation (44).

A simple model is now to be described from which the spatial variation of the diffusion potential in the non-equilibrium region may be determined. It is self-evident that in that region, the electron cannot experience  $f_d$  at all before it makes its first collision. Since collisions are random events, we must only be concerned with the statistical average of these events and their consequences. As the electron passes point A (Fig. 4a) and moves to the right, collisions are bound to occur. These will give rise to an average transfer of energy from the lattice to the electron. When sufficient collisions have happened, the electron energy will attain its equilibrium value. Reaching equilibrium, however, will require time. Since the electron velocity is finite, a certain distance is required in which nearly complete interaction between the electron and the lattice may be accomplished.

To calculate the spatial dependence of  $\phi$  in the junction non-equilibrium diffusion region, consider an electron moving to the right at point A of Fig. 4a. For simplicity, let the width  $w_e$  of the space-charge region be negligibly small. At point  $x = 0$ , before any collisions have occurred, clearly  $\phi = 0$ . At any positive  $x$ , the change in the diffusion potential  $d\phi$  across  $dx$  will be proportional to  $dx$  and to the difference between the equilibrium value of  $\phi$ ; namely  $(\phi_0 + \delta)$ , and  $\phi(x)$ . Thus we may write:

$$d\phi = (\phi_0 + \delta - \phi)dx/L_i \quad (45)$$

Here  $L_i$  may be defined as the *electron-lattice interaction length*. Conceivably this quantity will depend on the mean free path and on the fraction of the energy difference between the lattice and the electron transferred on the average to the latter during each collision. For simplicity, consider  $L_i$  to be constant. This is particularly justified if the impurity concentration outside the space-charge region is constant, as is generally assumed to be the case. Solution of equation (45), subject to the conditions that  $\phi(0) = 0$ , and  $\phi(\infty) = (\phi_0 + \delta)$ , will be:

$$\phi(x) = (\phi_0 + \delta)[1 - \exp(-x/L_i)] \quad (46)$$

Equation (46) is represented in Fig. 4a. In that figure, for simplicity,  $L_i$  has been chosen to be much larger than  $w_e$ .

Let us next consider the energy band diagram corresponding to forward biasing. This is represented in Fig.

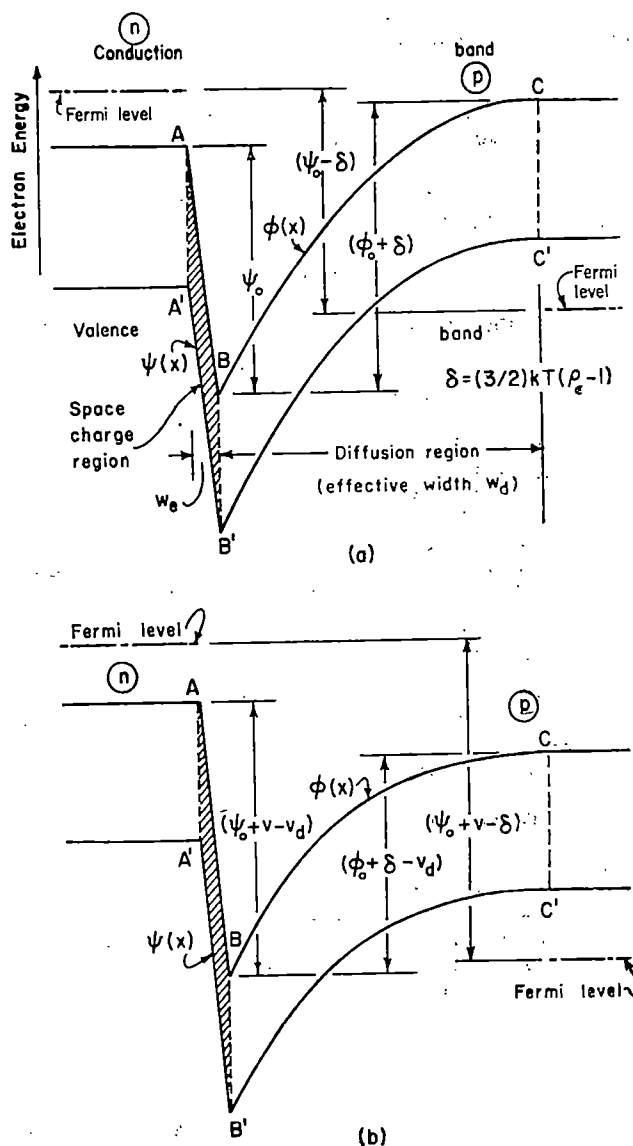


Fig. 4. Complete electron energy band diagrams for a degenerate thin-junction  $p$ - $n$  diode: (a) in thermal equilibrium; (b) under forward biasing

4b. As explained in the previous section, the net change in the composite potential between points A and C must be  $(\phi_0 + \delta - \psi_0 - v)$ . This value follows from the principle of conservation of energy. The new quantity  $v$  must be split into two parts. One must be due to diffusion and the other must be electrostatic. The positive diffusion part  $v_d$  must subtract from  $(\phi_0 + \delta)$  to make  $\phi = (\phi_0 + \delta - v_d)$ , while the electrostatic part  $(v - v_d)$  must add up with  $(\psi_0)$  to make  $\psi = (\psi_0 + v - v_d)$ . Now the quantity  $\psi$  must satisfy equation (44).

It should be noticed that the composite built-in potential across the entire transition region (referred to the conduction bands) is:

$$(\phi_0 + \delta - v_d) - (\psi_0 + v - v_d) = \phi_0 + \delta - \psi_0 - v \quad (47)$$

As expected, the right-hand side of equation (47) is identical with the result obtained for wide-junction  $p$ - $n$  diodes.

The energy band diagrams discussed in this section are believed to be particularly important for all  $p$ - $n$  junctions in which the tunnelling process takes place. It can be shown that these diagrams predict the new mechanism of tunnelling-induced injection of minority carriers<sup>21</sup>. This particular mechanism pertains to the process we believe to be occurring, for example, in  $p$ - $n$  junctions in direct<sup>22</sup>

semiconductors, for which high-efficiency emission of infra-red radiation and the laser action have been reported<sup>23-25</sup>.

For tunnelling non-degenerate  $p$ - $n$  junctions, it can be seen that the diagrams of Fig. 4 would remain substantially the same, except that  $\delta$  may be considered zero.

### (10) Summary and Conclusion

The main object of this article has been to: (1) develop new general equilibrium laws, which electrons should obey in heterogeneous degenerate and non-degenerate solids; (2) apply these general laws to determine new electron energy diagrams for degenerate  $p$ - $n$  junctions, with wide and thin transition regions.

Without loss of generality, the processes considered are mainly diffusion and drift. It has been shown that the mechanism of electron thermal equilibrium in solids cannot be correctly described on the basis of current or current tendencies as generally assumed. Rather, a valid description of this mechanism requires use of the concepts of (average) particle drift and diffusion forces  $f_e$  and  $f_d$ , respectively. The latter force has been formulated quantum statistically for any fermion gas. The new laws require that at every point in a heterogeneous solid in

thermal equilibrium only curl  $(\vec{f}_e + \vec{f}_d) = 0$ . This is in contrast with the conventional assumption, which,

if expressed in terms of these forces, would be  $(\vec{f}_e + \vec{f}_d) = 0$ . The latter condition would not be compatible with the existence of the Peltier effect, although conceivably it would hold for neutral gases. An implication of the new condition is that the electron force  $f_d$ , as is well known for  $f_e$ , constitutes a conservative force field in any heterogeneous solid.

We have furthermore shown that, contrary to general belief, the Fermi-level is indeed far from being constant at various points of any heterogeneous solid in thermal equilibrium. On the basis of the new laws, the relative values of this level have been evaluated for any solid-solid interface. This made it possible to determine new energy

diagrams for degenerate  $p$ - $n$  junctions, under thermal equilibrium and forward-biasing conditions.

In almost all the natural and life sciences, one may expect to find numerous processes and phenomena involving the transport in heterogeneous substances of electrons and/or other charged gases. Thus, as it now seems to be the case for solid-solid interfaces, it might be advisable to re-examine the validity of every foundation, principle and theory pertaining to such processes and phenomena wherever they may be.

I thank E. A. Jarmoc and W. R. Smith, III, for their comments on the manuscript.

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## EFFECT OF TENSILE LOADING ON HYDROGEN MOVEMENT IN STEEL

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THE rate of entry of hydrogen into a steel-walled 6 V6 vacuum tube has been used as an index for hydrogen concentration in steel. The device acts as an ionization gauge. The tube ion current is changed by the presence of hydrogen gas in the tube. This is detected and measured with appropriate electronic equipment.

The Lawrence hydrogen gauge was used to determine the relative rate of hydrogen migration through the walls of a 1010 steel 6 V6 tube when it was placed under tensile loading. The tube was stripped of paint and nickel plate, then hydrogen-charged by making it a cathode in a 1 per cent NaCN and 1 per cent NaOH solution. Current density was 1 amp/ft.<sup>2</sup> for 5 min. It was found that the initial ion current decay rate varied with different tubes (an indication of non-uniform gettering ability of commercial 6 V6 tubes). For this reason, only qualitative effects were anticipated. The charging was carried out in all cases by immersing the tube in the charging solution up to a distance 1/2 in. from the base of the tube.

The tube characteristics were: (1) shell wall, 1010 aluminium-killed steel 0.06 in. thick; (2) tube vacuum— $10^{-8}$  mm mercury; (3) tube type, 6 V6 tetrode; (4) tube shell temperature, 50° C.

The tubes were loaded, and the ion current was recorded before hydrogen charging.

The uncharged tube always gave a slight sharp instantaneous peak on the first loading and afterward showed little or no response. The ion current in all cases shows the gradual drop with time that is due to the gettering action of the tube.

The assembly shown in Fig. 1 was fabricated for tensile loading. The 6 V6 tube was fastened to the jig with low-temperature indium solder to prevent damaging of the tube by overheating. The tubes were loaded, and ion current was recorded before hydrogen charging. A pure tensile load was applied after the tube was hydrogen-charged. The maximum applied load was varied from 0 to 12,000 lb./in.<sup>2</sup>. The rate of loading and unloading was 0.01 in./in./min.

The hydrogen-charged tube was loaded in tension and unloaded with 25 lb. (500 lb./in.<sup>2</sup>) increments at each loading.

No detectable change in ion current occurred until a 300-lb. (6,000 lb./in.<sup>2</sup>) load was applied. After this loading level, an increase in ion current always occurred on loading. On unloading, the ion current returned to

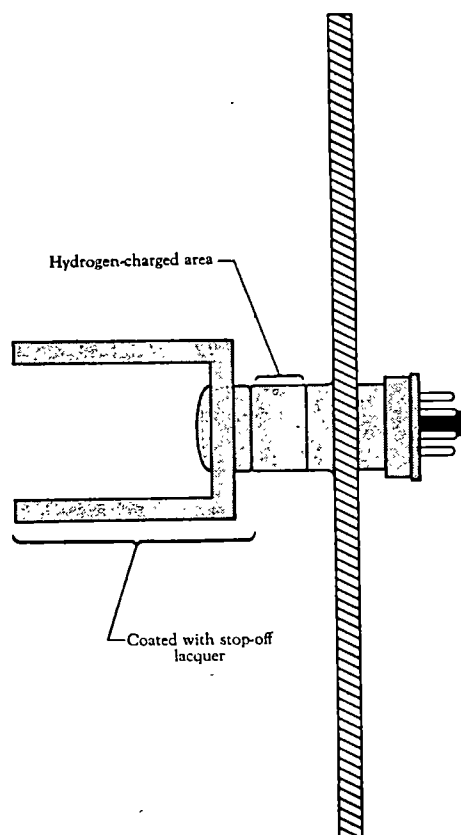


Fig. 1. Assembly used for tensile loading of 6V6 steel-walled tube. The tube is immersed in the hydrogen-charging solution up to the flat base plate. It is then rinsed and dried and loaded in a tensile testing machine

the normal decay curve. Typical loading and unloading curves at loads of 520 (10,380 lb./in.<sup>2</sup>) to 600 lb. (12,000 lb./in.<sup>2</sup>) are shown in Fig. 2. At loads above 450 lb. (9,000 lb./in.<sup>2</sup>), the solder fastening the tube to the jig began to creep. These curves show that the ion current is increased when a tensile load is applied and decreases as it is removed. They show that the amount of increase is greater as the applied load is greater.

The sharp instantaneous peak on tension loading of hydrogen-free tubes looks very similar to the peak obtained on tapping or striking the tube, and is probably the same effect even though the mechanical load is, in our experiments, a pulling effect.

Moving dislocations can carry an atmosphere of impurities along with them. The smaller the impurity atom is, the more easily this can happen. Hydrogen atoms would be easily moved through a metal lattice by moving dislocations. Frank<sup>1</sup>, using a mass spectrometer, detected a rapid increase in hydrogen emitted from a hydrogen-charged steel bar when it was bent in the mass spectro-

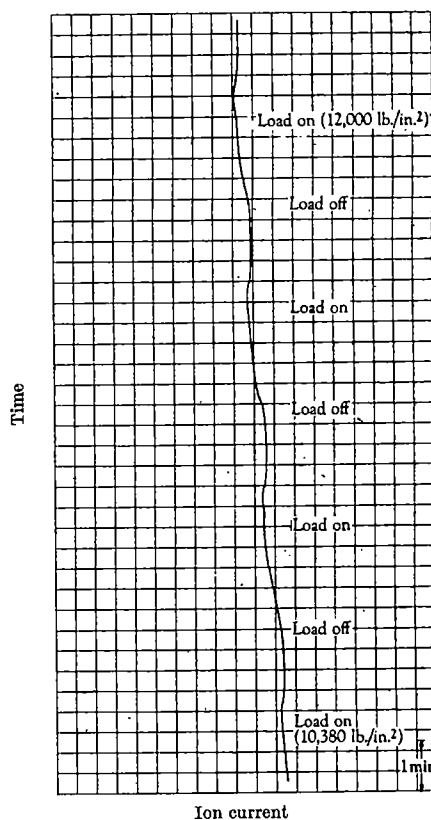


Fig. 2. Ion current versus time recording for a hydrogen-charged tube in tensile loading. Note. 1, Rate of loading is held constant. 2, Tensile load is increased by 25-lb. increments. 3, Gradual drop of ion current with time is due to gettering

meter. His explanation was that the dislocations moved on application of a bending load, carrying their hydrogen atmospheres to the surface where the hydrogen escaped and was registered.

In our experiments, an increase of ion current (hydrogen pressure) in the tube occurs when the tensile load is applied. This levels off as the load is held constant and drops to the normal value when the load is released. For the hydrogen pressure to remain constant (excluding normal gettering effects), a continuous new supply of hydrogen must be carried to the surface. A single rapid spurt of dislocations would cause an increase in ion current and then a rapid decay to the same level as in the unloaded state. This obviously does not occur. Although the load level of 6,000 lb./in.<sup>2</sup> is below the apparent elastic limit of the metal, some dislocations probably move to the surface. The sustained load may keep a relatively constant number of dislocations moving to the surface with their accompanying atmospheres of hydrogen. At present, this appears to be the most logical interpretation.

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## FACTORS INFLUENCING BIOMAGNETIC ENVIRONMENTS DURING THE SOLAR CYCLE

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**D**URING the eleven-year period of the solar cycle there are pronounced and somewhat systematic variations in the flux of both solar and cosmic ray particles reaching the Earth's surface. These two environmental parameters are inversely related; that is, as the

solar activity increases the cosmic ray flux decreases. This article will not be directly concerned, however, with the mechanisms causing these changes in radiation-levels, but instead it examines some of their effects on living organisms subjected to magnetic field environments.



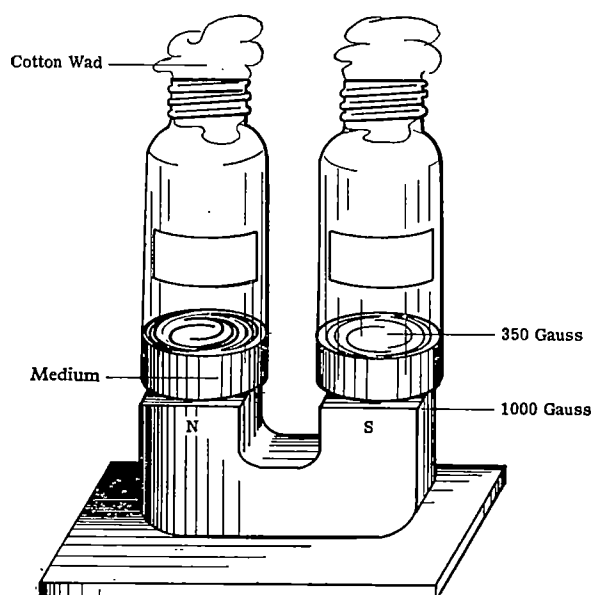


Fig. 1. Sketch of test magnet with culture bottles in position

The reported findings centre around the reproductive responses of the fruit fly, *Drosophila melanogaster*, and involve a continuation of an investigation which demonstrated an inverse relationship between solar flare activity and the progeny yields of flies grown in magnetic fields<sup>1</sup>. This relationship was found just following the 1958 maximum in the solar cycle when the solar activity was still high. The inverse correlations were believed to be caused by the influence of the magnetic fields on incoming ionizing particles which increase during periods of active solar flares. Although it was suggested that these effects were due to the influence of a magnetic field on the path of charged particles, many questions remained to be answered concerning the subtle relationships between the magnetic field conditions and the changing environmental parameters.

Presented also are speculations concerning the mechanism producing the experimentally observed trends. Emphatically it is not intended that these hypotheses are the only explanations for the observed effects; however, in many cases the variations in the results are consistent with known or inferred fluctuations in geophysical or astrophysical conditions. In the formulation of the suggested relationships between reproductive responses and radiation effects, summarized data from the International Geophysical Year have been utilized. In one section, for example, satellite results are presented which indicate a possible influence of the outer Van Allen radiation belt on the reproductive response effects.

With only a few exceptions the experimental procedure was as previously given<sup>1</sup>: culture bottles designated as *N* and *S* were placed on opposite poles of permanent magnets with pole faces 4.5 cm<sup>2</sup> and an 8-cm separation. This experimental arrangement provides two magnetic fields similar in strength but with opposing orientation. The field strengths decrease from the pole regions and a measure of the divergence of flux is indicated in Fig. 1, showing a pair of bottles in position. The culture bottles were 5 cm in diameter and 11.5 cm high; medium (cornmeal-molasses type with propionic acid mould inhibitor) was placed in the bottom to a depth of about 2.5 cm. The laboratory temperature was 22° ± 2° C, and both barometric pressure and relative humidity were monitored.

Each magnet was placed in one of four geomagnetic orientations, and this designated orientation was maintained throughout an entire series of generation matings. For example, east (*N*)—west (*S*) means the *N* pole was oriented geomagnetic east and the *S* pole geomagnetic

west. The *N* and *S* culture bottles were compared as pairs in each generation, and after the *F*<sub>1</sub> repeated filial generation crosses were made using six adult pairs removed from the preceding *F* generation. The parents were left ten days in the bottles. Countings were made over an 8-day interval, starting with the day the new generation flies were seen. Cultures hereafter designated as 'controls' were grown out of the magnetic fields, and all cultures were initiated, as well as removed and counted, on the same dates.

As in the previous investigations, the environmental parameters were averaged over a 72-h period covering the day before, the day of, and the day after the matings in the new culture. During this specific 72-h period in the life-cycle, these organisms disclose a high sensitivity to environmental changes.

Values for solar flare activity may disclose day-to-day fluctuations of several orders of magnitude. Therefore, it has been found advantageous<sup>1</sup> to use the factor *S'*<sub>*F*</sub> given by:

$$S'_F = \log (S_F + 1) \quad (1)$$

where *S*<sub>*F*</sub> is the daily value issued by the High Altitude Observatory, Boulder, Colorado<sup>2</sup>. The (*S*<sub>*F*</sub> + 1) factor not only provides logarithmic comparisons but also allows on a logarithmic basis a data point for the occasional sequence of zero values for *S*<sub>*F*</sub> over the 72-h period of interest.

Cosmic ray data were obtained through the courtesy of Prof. J. A. Simpson, Enrico Fermi Institute of Nuclear Studies, University of Chicago. These were reduced by a constant factor to give more convenient numbers in the analyses; the *C'*<sub>*R*</sub> or 'reduced factor' is:

$$C'_R = (C_R - 2,000) \times 10^{-3} \quad (2)$$

where *C*<sub>*R*</sub> is the bi-hourly count of neutron flux corrected for barometric pressure.

The previously used statistical correlation coefficient *r* gives a measure of the degree of linear correlation in the data being compared. If the correlation is only approximately linear and follows some higher order function one cannot evaluate or examine the relationship by simply determining the correlation coefficient. The present work was therefore examined using a method of grouped or cell frequency data analysis<sup>3</sup>. A dependent variable such as progeny yield (*F*<sub>*t*</sub>) was grouped according to variations in an environmental parameter or independent variable. Progeny data which were chronologically the same and grown on magnets with the same orientations were grouped according to interval changes in a given environmental parameter; for example, let us say in the interval change of *C'*<sub>*R*</sub> from 0.95 to 0.96 all the progeny (total counts designated as — *F*<sub>*t*</sub>) from generations located within this interval were tabulated and an average taken. This system of grouping may be shown by the general expression:

$$\bar{M}_1 = \frac{\sum [(F_t)_A \dots (F_t)_B]}{n}; \quad \bar{M}_2 = \frac{\sum [(F_t)_C \dots (F_t)_D]}{n}; \quad (3)$$

where  $\bar{M}_1$  is the mean value of the environmental parameter and *n* is the number of progeny generations in the interval [(*F*<sub>*t*</sub>)<sub>*A*</sub> ... (*F*<sub>*t*</sub>)<sub>*B*</sub>], etc. Intervals of  $\bar{M}$  were chosen so that generally at least four *F*<sub>*t*</sub> values were averaged. In many cases there were 10–20 progeny generations within the interval. If an  $\bar{M}$  interval contained less than four *F*<sub>*t*</sub> values the data were incorporated into the next nearest bracket of  $\bar{M}$  values. It appeared that over intervals containing 15–16 generations of flies the  $\bar{M}$  values disclosed sufficient variation to make this method of data analysis feasible. It should also be pointed out that the possibility of the variations in progeny with the  $\bar{M}$  values being caused by genetic drift is negligible. Each  $\bar{M}$  interval contained a selection of *F* generations which

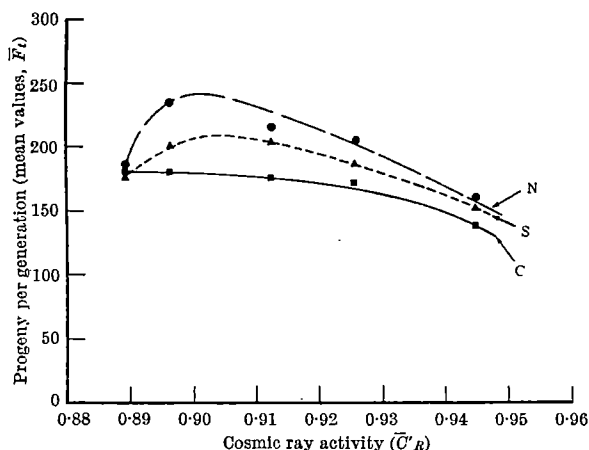


Fig. 2. Grouped cosmic ray-progeny data for sixteen generations [east (N)-west (S) orientation], 1962 data

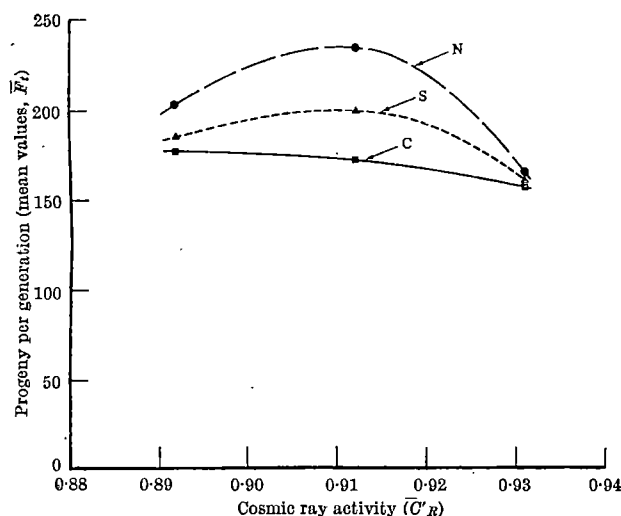


Fig. 3. Reproductive response in  $F_1$  generations grown out of field (removed from one magnet in series shown in Fig. 2)

depended on the level of the random fluctuations in the environmental parameter, and this greatly reduces the possibility of the trends being caused by a sequential change.

Within each frequency cell, data points are presented for the biomagnetic conditions  $N$  and  $S$  as well as the controls or  $C$  values. Since data points for each  $F$  generation in a test series correspond chronologically the differences between individual  $C$  and the  $N$  or  $S$  progeny generation points may be analysed using statistical comparisons of the population means within a given  $M$  cell. The standard deviation of the mean of the paired differences  $-\sigma_d$  is given by

$$\sigma_d = \left[ \frac{\sum (d_i - \bar{d})^2}{n(n-1)} \right]^{1/2} \quad (4)$$

where  $d_i$  represents the difference  $N_1 - C_1$  or  $S_1 - C_1$  depending on whether comparisons are being made between the  $N$  or  $S$  pole data. The value of  $\bar{d}$  is the mean of the differences and  $n$  the number of pairs in the  $M$  interval. The  $t$  test for significance is then calculated from:

$$t_N = \frac{(\bar{N} - \bar{C})}{\sigma_d} \text{ or } t_S = \frac{(\bar{S} - \bar{C})}{\sigma_d} \quad (5)$$

The values of  $t_N$  or  $t_S$  were compared with the values in a  $t$ -table of significant differences<sup>4</sup>. The discussions of the presence or lack of significance of specific data in the following sections are based on this method of analysis.

Results from grouped data are given for the most recent tests as well as a re-examination of experiments conducted over the past several years. By examining all this information the period of coverage extends from the initial test series when the solar activity was still moderately high into the present condition of the "quiet sun". The general approach is to examine the more recent tests (1962), then discuss results extending back towards the 1958 solar maximum. Finally, some of the perplexing aspects of the early environmental investigations are considered in the light of the recent findings.

### Cosmic Ray Effects (1962)

In Fig. 2 are the grouped cosmic ray-progeny averages for 16 generations from each of two magnets oriented east ( $N$ )-west ( $S$ ) and two separate control cultures grown at the same time and for the same number of generations. The standard variation in  $F_t$  for each point on this curve is approximately 50. There is a steady, non-linear decline in the grouped control progeny with increasing cosmic ray activity, whereas the data from the  $N$  and  $S$  magnetic field conditions demonstrate a different reproductive response effect. There appears to be a maximum in both magnetic field curves at a cosmic ray  $C_R$  value of around 0.90 (equation 2). It should also be noted in Fig. 2 that the average progeny or  $F_t$  values for the magnetic field data are higher than the control points. The  $t$  test values in Table 1 show that with the exception of the data points at the high and low end of the curves the  $N$  and  $S$  data are significantly higher than the controls.

Table 1. STATISTICAL EVALUATION OF MAGNETIC FIELD DATA SHOWN IN Fig. 2

$C_R$	$N$ -pole			$S$ -pole			Significance $P < 0.05$	Degrees of freedom
	$t_N = \frac{(\bar{N} - \bar{C})}{\sigma_d}$	Std. dev $\sigma_d$		$t_S = \frac{(\bar{S} - \bar{C})}{\sigma_d}$	Std. dev $\sigma_d$			
0.889	0.23	23.6		0.44	12.6	No		7
0.896	1.80	32.8		2.15	11.3	Yes		7
0.912	3.59	11.8		3.10	10.0	Yes		15
0.925	2.12	18.8		1.86	11.7	Yes		15
0.944	0.66	30.1		0.83	21.0	No		7

The effect producing the maxima in the progeny response curves for the magnetic field data shown in Fig. 2 appeared to be transferred to a succeeding generation. From one of the test magnets six pairs of flies were removed and an  $F_1$  generation was grown out of the field for the entire generation sequence. The results of the grouped analysis for this series are shown in Fig. 3 along with the regular control data. Since the flies from one magnet were used, only three data cells could be obtained. The maxima appear in the curves for the progeny grown out of the field conditions, whereas the regular control data appear as in Fig. 2 with the steady decline in progeny with increasing cosmic ray activity. The  $N$  and  $S$  curves also lie above the control curve and in the same relative positions as the parent curves in Fig. 2, and again only the central points on the  $N$  and  $S$  curves are significantly higher than the control values. It appears that the response effects produced by the magnetic field environments are transferred to the first generation offspring; as yet, flies have not been mated beyond the  $F_1$  generation taken out of the field.

The data shown in Fig. 2 were grouped according to total progeny. In Fig. 4 the sexes are grouped separately for the three environmental conditions. It appears that there is a subtle difference between sex ratio changes in the magnetic field and control cultures, particularly at low  $C_R$  levels.

Within the same general time span as covered by the data in Fig. 2, two other tests were also made on different mating dates and on magnets with different orientations: one magnet oriented east ( $S$ )-west ( $N$ ) and one north ( $N$ )-south ( $S$ ). The results of the grouped analysis (combining data from both magnets) are given in Table 2.

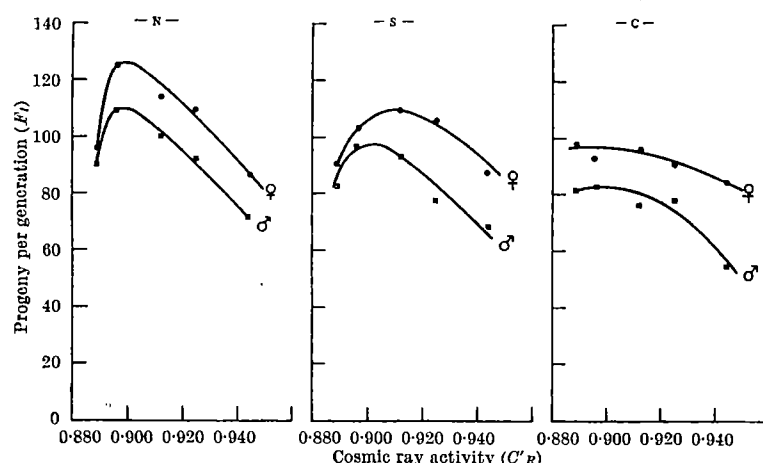


Fig. 4. Sex grouped data from two magnets (Fig. 2 series)

The maxima, although not so pronounced, are again observed when these data are plotted and the *N* pole curve lines above the *S* pole curve.

Table 2. GROUPED  $C'_R$ -PROGENY ANALYSIS FROM TWO MAGNETS WITH DIFFERENT GEOMAGNETIC ORIENTATIONS; 1962 DATA ( $F_1$  IS NUMBER OF PROGENY PER GENERATION)

$C'_R$ av.	$N$ pole	$F_t$	$S$ pole	No. of generations
0.882	211	168		6
0.906	234	189		6
0.914	246	210		6
0.934	196	178		4
0.949	191	174		5

### Solar Effects (1962)

The average solar flare  $S'_F$  values (equation 1) were much lower during the 1962 period of examination. The grouped  $S'_F$  averages for the progeny shown in Fig. 2 are listed in Table 3, and it may be seen in these data that there are no consistent trends or correlations; in fact, the progeny yields are virtually independent of solar flare activity.

Table 3. GROUPING OF 1962 SOLAR FLARE DATA SHOWING THE ABSENCE OF SMOOTH TRENDS

$\bar{S}'_F$	$F_1$ progeny per generation		
	<i>N</i>	<i>S</i>	<i>C</i>
0.013	194	192	170
0.151	205	153	125
0.175	207	204	185
0.232	201	184	169

### Solar and Cosmic Ray Effects (1961)

In the 1961 analysis neither the  $C'_R$  nor  $S'_F$  grouped data show smooth trends. In Table 4 are  $S'_F$  data averaged for three magnets oriented east (*N*)-west (*S*), and although in the magnetic field there is a general inverse trend or decrease in progeny with increasing solar flare activity, the data show pronounced fluctuations. In the grouped progeny data for the  $C'_R$  function (also listed in Table 4) maxima are indicated; however, the trends are not smooth. Compared with the data in Fig. 2 one cannot discuss the information obtained during the 1961 period with regard to making any comparisons or predicting trends. Taken as an isolated group, the data during 1961 disclose rather erratic variations with the extra-terrestrial environmental parameters. The lack of smooth variations during 1961 may be explained by the fact that this is

Table 4. GROUPED SOLAR FLARE AND COSMIC RAY DATA FOR THE 1961 PERIOD

$S'_F$	$F_1$ progeny per gen.			$C'_R$	$F_1$ progeny per gen.		
	<i>N</i>	<i>S</i>	<i>C</i>		<i>N</i>	<i>S</i>	<i>C</i>
0.044	113	102	125	0.769	81	70	75
0.097	64	77	87	0.849	112	98	100
0.152	84	100	72	0.865	81	86	102
0.217	96	54	76	0.881	57	58	82
0.250	57	55	112	0.988	71	49	55
0.317	67	49	58				

approximately the midpoint between solar maximum and minimum and the response of the organisms to environmental changes may be a period of transition. This transition possibility is further indicated by examining data previous to the 1961 test interval.

### Solar and Cosmic Ray Effects (1959-60)

During this period the solar activity was still high, and this appeared to be the predominant influence on the biomagnetic response effects. In Fig. 5 are the grouped  $S'_F$  data for two magnets oriented west (*N*)-east (*S*), and it may be seen that there is a regular trend in the *N* and *S* pole data with a maximum indicated at  $S'_F$  values around 2.2. The control data do not indicate this same trend, that is, the reproductive response of the flies in the magnetic field

to solar flare activity appears to be markedly different from the response in control cultures out of the field. The control data for the results given in Fig. 5 are listed in Table 5, and the absence of the smooth trend is apparent. In general, all the 1960 data (high solar flare activity) indicate that the organisms are responding to different environmental parameters depending on whether the flies are grown in or outside the magnetic field. In a later section, we will speculate about this finding in greater detail.

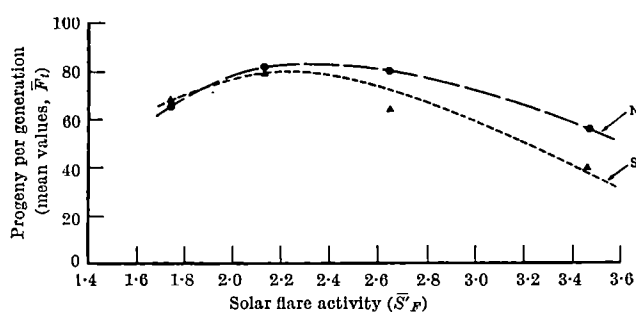
Table 5. GROUPED CONTROL DATA FOR THE 1959-60 SERIES SHOWN IN FIG. 5 (SOLAR FLARE ACTIVITY)

$\bar{S}'_F$	$F_1$ progeny per generation
0.175	63
0.212	73
0.264	36
0.346	86

### Influence of Van Allen Radiation Belt

It has been shown by Forbusch, Pizzella and Venkatesan<sup>6</sup> that there is an indication of a correlation between the equatorial ring current surrounding the Earth and the radiation from the outer Van Allen belt as detected by the satellite *Explorer 7*. These authors published a series of daily measurements of both the ring current  $U$  and the counting rate  $R_b^*$  from the belt (at 4.1 Earth radii). These data extended from November 1, 1959, to August 27, 1960, or during the period when the solar activity was still high; in fact, correlations were shown between solar events and the  $U$ - $R_b^*$  variations.

During this same period of observation, progeny data were available from two magnets oriented west (*N*)-east (*S*). If the equatorial ring current  $U$  modulates the incoming radiation, then one might look for a relation between the progeny and the  $U$  parameter; that is, under the assumption that the ring current modulates at least a portion of the radiation which ultimately reaches the fields of the test magnets. This proposed relationship

Fig. 5. Reproductive response from two magnets during period of high solar activity [west (*N*)-east (*S*)]. 1959-60 data



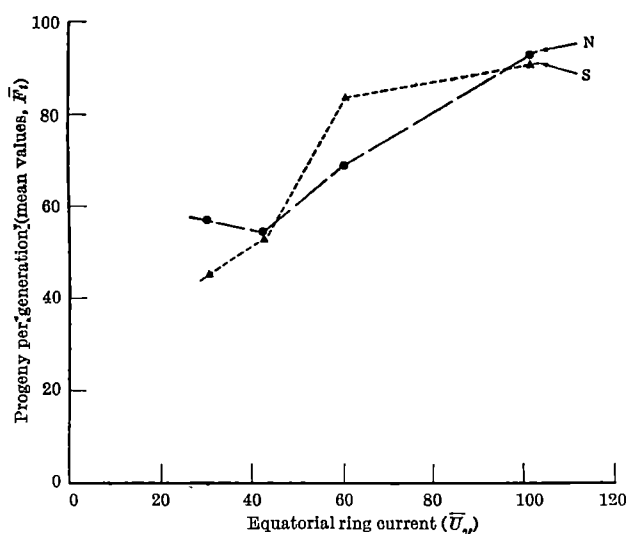


Fig. 6. Indicated relationship between magnetic field progeny and equatorial ring current. 1959-60 data

is indicated in the grouped analysis in Fig. 6, and although a correlation is suggested many more data would have to be obtained before this influence could be stated with a high degree of certainty.

#### Barometric Pressure Influence

One of the first observations made during the initial investigations of environmental effects was the fact that there was an apparent correlation between progeny yields— $F_1$  and  $P$ —barometric pressure<sup>6</sup>. It was realized that these effects were not due to barometric pressure alone, since placing the culture bottle in an electric field reduced the  $P$  correlation and produced higher progeny yields even though the changes in pressure were the same as in control cultures. This finding was believed to be due to the influence of the electric field on the ion density within the culture bottles. Outside the electric field the primary factor would be barometric pressure; air acts as an absorber of incoming ionizing particles and high pressure would reduce the particle number. Inside the electric field the total ion density would be reduced and the variations in ion level would be less sensitive to  $P$ -changes in the environment. These experiments were all conducted during the period of high solar activity.

Beginning in 1961 and extending into the more recent analysis the high correlations between control progeny and barometric pressure were no longer obtained. This may be explained by the fact that as both the cosmic ray flux and energy increase, the influence of local barometric pressure changes becomes less significant in controlling the local ion concentration. The greatest absorption effects in the atmosphere would occur when the cosmic ray energy was low or during the observed period of high solar activity.

During the period of high correlation between progeny and barometric pressure it was found that flies grown in the magnetic field disclosed a much reduced correlation; this may be explained by the fact that the magnetic field reduced the cosmic ray component during this test period. Thus, the 1960 barometric pressure observations support the hypothesis of a combined cosmic ray-solar flare effect in cultures outside the magnetic field with the major influence on the reproductive responses of flies in the magnetic fields being solar flares. An empirical relationship was worked out<sup>1</sup> to take into account both the environmental parameters of  $P$  and  $S_F$ ; however, it now appears that this equation is only applicable during the active period in the solar cycle and should be further modified to account for the  $C_R$  parameter. If one considers all the test data examined, it may be seen that

the barometric pressure effect is in general a secondary influence and only plays a significant part during high solar and low cosmic-ray activity.

#### Tentative Mechanisms

An attempt will be made to integrate the various findings presented in the preceding paragraphs into a composite view of the changes taking place during the solar cycle. As previously pointed out, much of what will be said is based on suggested relationships and indicated trends in data which, to be well established, will have to be followed through several solar cycles. Although the trends are not always simple, the data demonstrate that the reproductive responses of *Drosophila* disclose variations which are related to changes in the terrestrial environment.

For example, in Fig. 2 the maximum points and the higher progeny of the cultures in the magnetic field may be explained in part by considering the influence of a magnetic field on cosmic ray particles. It has been shown, for example, by L. F. Curtiss<sup>7</sup> that a considerable fraction of the cosmic ray flux ( $\sim 30$  per cent) can be deflected by a magnetic field. It will be assumed that the magnets used here also deviate a portion of the cosmic ray particles out of the field region covered by the culture bottles. The amount of deflexion will depend both on the kinetic energy and on the charge of the particle, given by:

$$mv^2/\rho = Hev \quad (6)$$

where  $\rho$  is the path radius of a particle with charge  $e$ , mass  $m$ , and velocity  $v$  moving in a magnetic field of strength  $H$  gauss. For high-energy particles in the relativistic range:

$$pc/e = 300 H \rho \quad (7)$$

where  $c$  is the velocity of light and  $p$  is the so-called magnetic rigidity of the particle. When  $p$  is given in units of  $eV/c$ , the path radius of the particle in cm is given by:

$$\rho = p/300 H \quad (8)$$

These equations apply to the two-dimensional case and are, therefore, useful only when considering simplified conditions. We can, however, obtain some idea of the order of magnitude of the deflexion effect by considering charged particles arriving along the equatorial plane of the test magnets.

The cosmic ray data used in these investigations represent neutron counts produced by the local interaction of primaries; the majority of the interaction particles are probably protons and electrons. In the case of the cosmic ray data presented in Fig. 2, the deflexion of these primaries may not be great; for example, a 100-MeV particle would have a radius of curvature of approximately 3 m in a 350-gauss field which would represent the field strength at the region of the medium surface shown in Fig. 1. Such a particle approaching the magnet might be deflected slightly by the first pole and as a consequence could completely miss the test region of the adjacent bottle on the opposite pole of the magnet. A greater deviation of particles at certain energy-levels and charges could account for the maxima in the progeny response curves shown in Fig. 2, that is, through an energy selection process within the magnetic field regions.

It does appear, however, that the magnetic field 'conditions' the flies to the cosmic ray environment. The maxima in the progeny response curves were transferred to the first generation offspring; therefore, whatever conditioning occurs in the magnetic field appears to be genetically transferred. It has been shown by Reddi and Rao<sup>8</sup> that cosmic rays produce chromosomal breakage in *Drosophila melanogaster*. It is suggested that the magnetic field in some manner reduces the frequency of this chromosomal breakage by cosmic radiation. Thus, in the case of the results obtained during the recent cosmic ray period,

there exist both the deflexion effect of the magnetic fields on the cosmic ray particles as well as a possible genetically transferred conditioning effect of the magnetic field on the organism.

During the periods of high solar activity, the incoming particles are of lower energy, and it can be shown by equation 8 that the radius of curvature of a charged particle in the magnetic field is considerably smaller. For example, a 1 MeV particle approaching the equatorial plane of the magnet at the medium surface would have approximately a 3-cm radius of curvature, and as a result of its lower energy there is a greater probability that it would be 'captured' by the magnetic field and spiral into the test region of the magnet. The situation in the magnetic field is quite different during high solar activity from that during the period of high cosmic radiation. This could explain the lack of correlation of the 1960 control data (Table 5) with the  $S'_F$  parameter. Thus in the case of the control data, during the high solar flare activity the effects of both cosmic ray and solar flare radiation are acting on the organism. When encountering the magnetic field conditions, a portion of the cosmic ray component may be deflected out of the field region (as indicated for the 1962 data) and the magnetic field progeny are subjected to a lower cosmic ray flux but, by the same token, a higher solar flare flux. The arrival directions of the solar particles are such that they may first be trapped by the magnetic field of the Earth, then by the fields of the test magnets, and finally by this energy selection process exert a greater influence on the reproductive responses of flies in the magnetic field than in control cultures.

An example of the changing trends and correlations over the two periods of the solar cycle is indicated in a

Table 6. SIMPLIFIED REPRESENTATION OF PROGENY TRENDS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Environmental condition		In magnetic field ( $N$ and $S$ )	Controls
Cosmic ray period	$\begin{matrix} \overline{C_R} \\ P \\ \overline{S_F} \end{matrix}$	$\begin{matrix} (-) \\ (0) \\ (0) \end{matrix}$	$\begin{matrix} (-) \\ (0) \\ (0) \end{matrix}$
Solar flare period	$\begin{matrix} \overline{C_R} \\ P \\ \overline{S_F} \end{matrix}$	$\begin{matrix} (0) \\ (0) \\ (-) \end{matrix}$	$\begin{matrix} (+) \\ (+) \\ (0) \end{matrix}$

very simplified form in Table 6. In this table a definite trend is indicated by a plus or minus sign depending on whether the curve has a positive or negative slope, and if the trend is uncertain or erratic it is indicated by a zero. Table 6 shows neither differences in the level of the progeny responses nor detailed variations in the curves, simply the general trends in the data.

When considering only the factors of trends in data the conditions are similar for both the magnetic field and the control data during the cosmic ray period. The trends are, however, reversed for the two environmental conditions during the period of high solar flare activity.

<sup>1</sup> Levengood, W. C., and Shinkle, M. P., *Nature*, **195**, 967 (1962).

<sup>2</sup> Solar Activity Summaries prepared by Trotter, D. E., and Robers, W. O., High Altitude Observatory, University of Colorado, for the International Geophysical Corporation.

<sup>3</sup> Freund, J. E., *Modern Elementary Statistics* (Prentice-Hall, Englewood Cliffs, N.J., 1952).

<sup>4</sup> Dixon, W. J., and Massey, jun., F. J., *Introduction to Statistical Analysis* (McGraw-Hill, Inc., New York, 1957).

<sup>5</sup> Forbusch, S. E., Plizzella, G., and Venkatesan, D., *J. Geophys. Res.*, **67**, 3651 (1962).

<sup>6</sup> Levengood, W. C., and Shinkle, M. P., *Science*, **132**, 34 (1960).

<sup>7</sup> Curtiss, L. F., *J. Res. Nat. Bur. Stand.*, **9**, 815 (1932).

<sup>8</sup> Reddi, O. S., and Rao, M. S., *Nature*, **201**, 96 (1964).

## IMMUNOGENICITY OF ANTIGEN-CONTAINING RIBONUCLEIC ACID PREPARATIONS FROM MACROPHAGES

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ATTENTION has been directed recently to the possibility that macrophages play an intermediate part in antibody formation. Over the years, evidence has accumulated that phagocytic cells in lymphoid tissues and spleens take up foreign proteins and retain them for several months (see reviews by Haurowitz<sup>1</sup> and Campbell and Garvey<sup>2</sup>). Flagellar antigen labelled with iodine-131 persists for four weeks in the dendritic macrophages of lymphoid follicles<sup>3</sup> and human serum albumin persists in such phagocytes in chick spleen<sup>4</sup>. Lysosomal fractions from spleens of animals several days after injection of red blood cells<sup>5</sup> have proved to be immunogenic, and immunogenic preparations have been obtained from spleen and liver three weeks after injection of *Shigella*<sup>6</sup> and bovine serum albumin<sup>7</sup>.

Garvey and Campbell<sup>8,9</sup> have reported that the antigen which persists in liver is complexed with nucleic acids. Fishman and Adler<sup>10</sup> found that RNA extracted with phenol from macrophages after contact with bacteriophage induced phage-neutralizing antibodies when added to lymphoid cells in tissue culture or transplantation chambers. The inducing factor was species-specific, ribonuclease sensitive, and appeared to be present in low-molecular-weight RNA fractions<sup>11</sup>.

It is an open question whether antigen can stimulate potential antibody-forming cells directly or whether passage of antigen through an intermediate cell is a necessary step in the induction of antibody globulin syn-

thesis. Macrophages may simply take up and store antigen in close proximity to immunologically reactive cells, or they may have to process antigen in some way before induction of antibody formation can occur. We have attempted to analyse this problem further. Haemocyanin was used as an antigen because it is a large molecule which can conveniently be characterized by centrifugation on sucrose density gradients. Using haemocyanin labelled with iodine-131, it has been possible to follow the fate of antigen in peritoneal macrophages and extracts made from them.

Small amounts of antigenic material were found in the aqueous phase with RNA extracted by phenol treatment of macrophages previously exposed to haemocyanin *in vivo*. The <sup>131</sup>I-label gave quantitative information about the amount of antigenic material present in the extracts, and the immunogenicity of antigen associated with RNA could be compared with that of free labelled antigen. Extracts of RNA were also made immediately after addition of <sup>131</sup>I-haemocyanin to macrophages. Such RNA preparations contained a smaller percentage of macromolecular <sup>131</sup>I-material than extracts made some time after macrophages had been in contact with haemocyanin. However, provided enough haemocyanin was added immediately before phenol extraction, the extracts were similarly immunogenic.

The results suggest that macrophages which have taken up antigen do not form a specific new informational RNA but may process antigen in some way so as to make it highly immunogenic. Whether this is a necessary step in

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antibody formation is not clear. Some of these results have been presented at the symposium on the "Molecular and Cellular Basis of Antibody Formation" held in Prague in May 1964 (ref. 12).

**Iodination of haemocyanin.** Haemocyanin from *Maia squinado* blood was purified in columns of DEAE ion-exchange cellulose using gradient elution (0.05 M NaCl–0.05 M potassium phosphate pH 6.7 to 0.4 M NaCl in 0.05 M potassium phosphate) (Humphrey, unpublished results). It was labelled with carrier-free iodine-131 by the method of Hunter and Greenwood<sup>13</sup>. Specific radioactivities of 5–40  $\mu\text{Ci}/\mu\text{g}$  gave satisfactory preparations, but with higher radioactivities haemocyanin underwent partial degradation and loss of precipitability with specific antisera to haemocyanin.

**Test for immunogenicity.** All the experiments were carried out with inbred mice (*C<sub>3</sub>H/He*). Since soluble haemocyanin does not give a good primary response in mice, and we wished to detect small amounts of immunogenic material, we resorted to a cell transfer system<sup>6</sup> into primed mice. Normal mouse spleen cells ( $3 \times 10^7$  cells/mouse) were incubated with <sup>131</sup>I-haemocyanin or RNA preparations for 1 h at 37° C in Gey's medium and then transferred into mice that had been primed in the footpads with 5–10  $\mu\text{g}$  alum precipitated haemocyanin at least two months previously. At that time, the sera of the recipient mice had a very low antigen-binding capacity. Peak titres appeared about nine days after cell transfer. 5–6 mice were used for each group.

**Antibody assay.** The antigen-binding capacity of the mouse sera was estimated by an indirect antigen-binding test<sup>14</sup>, using <sup>131</sup>I-haemocyanin and antiserum and precipitating the antigen/antibody complex with antibody to mouse  $\gamma$ -globulin. Each tube contained 0.1 ml. saline, 0.04  $\mu\text{g}$  <sup>131</sup>I-haemocyanin (5  $\mu\text{Ci}/\mu\text{g}$ ) and 5  $\mu\text{l}$ . antiserum (or 5  $\mu\text{l}$ . of 2- and 6-fold dilutions in normal mouse serum). The reaction mixture was incubated at 37° C for 30 min and then an excess of anti-mouse  $\gamma$ -globulin was added. After incubating at 37° C once more, the volumes were made up to 1 ml. with saline. The samples were left in the cold overnight, centrifuged at 4° C and the <sup>131</sup>I-haemocyanin bound by the precipitate counted. All Ag-binding capacities were corrected for non-specific binding of <sup>131</sup>I-haemocyanin by normal mouse serum. The precipitability of each <sup>131</sup>I-haemocyanin preparation was tested with a known antiserum. Antigen-binding capacity (ABC) of the mouse sera was expressed as  $\mu\text{g}$  haemocyanin bound per ml. of antiserum at serum dilutions, at which 30 per cent of the antigen was bound. It was observed, using antiserum from mice stimulated by a secondary injection of antigen, that the plot of percentage antigen bound against log (antiserum concentration) was approximately linear between 80 and 30 per cent of antigen bound. A standard curve was used to correct antigen-binding capacity of the higher titre sera, which bound more than 30 per cent of the <sup>131</sup>I-haemocyanin at the dilutions used.

The results of the indirect antigen-binding test were also checked with a modified direct radioprecipitin test<sup>15</sup> combining binding of <sup>131</sup>I-haemocyanin by antiserum and autoradiography<sup>12</sup>.

An indirect antigen-binding test was also used to test for antigenic properties of <sup>131</sup>I-material in macrophages and RNA preparations characterized on sucrose gradients. Anti-haemocyanin from a hyperimmunized rabbit was added to the gradient fractions. After incubating for 1 h at 37° C, a slight excess of antibody to rabbit  $\gamma$ -globulin was added, and the mixture was re-incubated at 37° C for 1 h. After standing overnight at 4° C the proportion of <sup>131</sup>I-radioactivity bound by the antigen/antibody precipitate was determined. Normal rabbit serum served as a control for non-specific binding of <sup>131</sup>I-material.

**Phenol treatment of macrophages.** Three days after an intraperitoneal injection of 10 per cent proteose peptone (Difco), peritoneal cells were collected by washing out the

peritoneum with 3 ml. 1 : 20,000 heparin containing 5 per cent normal rabbit serum. Each mouse yielded about  $10^7$  cells, at least 80 per cent being macrophages, that is, cells which rapidly phagocytose particulate matter. The cells were washed twice with Gey's medium, homogenized in 0.025 M sodium phosphate buffer pH 7.0 with a 'Teflon' homogenizer, and shaken for 15 min with an equal volume of washed, re-distilled phenol either at +20° C or in an ice bath. All subsequent procedures were performed in an ice bath. The aqueous phase was re-extracted twice for 5 min with an equal volume of phenol. Phenol was removed by six extractions with washed ether. Nitrogen was then bubbled through the solution to remove ether. The ratio of the absorption density at 260 and 280 m $\mu$  was 2.

RNA was extracted from macrophages either after contact of the peritoneal cells with <sup>131</sup>I-haemocyanin *in vivo* for 2.5 h (preparation I) or after addition of <sup>131</sup>I-haemocyanin to normal macrophages immediately before homogenization (preparation II) (Table 1).

Table 1. RNA PREPARATIONS

Preparation I	Preparation II
<sup>131</sup> I-HCY i.p. → 20 mice (80 $\mu\text{g}$ )	20 mice
↓ 2.5 h	↓
Macrophages (2–3 × 10 <sup>6</sup> )	Macrophages
↓ homogenize	↓ <sup>131</sup> I-HCY, 0.3 $\mu\text{g}$ or 3.2 $\mu\text{g}$ homogenize immediately
↓ Extract with phenol 3 times	↓
↓ Extract aqueous phase with ether 6 times	↓
↓ RNA preparation	↓

**RNA preparation I obtained from macrophages after contact with haemocyanin *in vivo*.** Preliminary experiments had demonstrated that <sup>131</sup>I-haemocyanin injected intraperitoneally into mice is taken up rapidly by peritoneal cells (peak radioactivity on macrophages occurs within 1 h). More than half the material taken up is rapidly degraded intracellularly, iodine-131 is lost from the cells, but after 2–3 h the remaining <sup>131</sup>I-radioactivity (about 0.1–0.4 per cent of the injected dose) persists in the cell for more than 24 h (ref. 12). A time-interval of 2.5 h between haemocyanin injection and collection of peritoneal cells was therefore chosen for the preparation of macrophage RNA. At that stage about two-thirds of the <sup>131</sup>I-material in the cells appears to be degraded (TCA soluble and not precipitable with anti-haemocyanin)<sup>12</sup>. One-third will be referred to as macromolecular <sup>131</sup>I-material, precipitable with trichloroacetic acid.

A typical experiment will be described in detail. Peritoneal cells were collected from 20 mice 2.5 h after intraperitoneal injection of 4  $\mu\text{g}$  <sup>131</sup>I-haemocyanin/mouse. 0.45 per cent of the injected radioactivity was then present in the washed cells. The macrophages were treated with phenol at +4° C (as described previously), yielding 750  $\mu\text{g}$  RNA containing 3 per cent of the total <sup>131</sup>I-radioactivity on the macrophages. The aqueous phase selects free iodine-131, but 10 per cent of the radioactivity was in macromolecular form (precipitated with 10 per cent TCA and with specific anti-haemocyanin serum). The presence of macromolecular iodine-131 is confirmed when the RNA is analysed on a sucrose gradient (4–20 per cent sucrose pH 7.4). Fig. 1 shows the pattern of distribution of <sup>131</sup>I-radioactivity on the gradient and the absorption density at  $D_{260}$  of the RNA. The RNA shows the usual-sized components (30 S, 16 S and small-molecular-weight material). The major proportion of the radioactive counts stays on top of the gradient (tubes 12 and 13). However, there is always some radioactivity associated with S values of the original haemocyanin preparation (tube 7) and also some <sup>131</sup>I-material with higher and lower molecular weight (tubes 3, 4, and 9, 10), which may represent partly degraded, complexed or original haemocyanin. The sedimentation behaviour of the original <sup>131</sup>I-haemocyanin



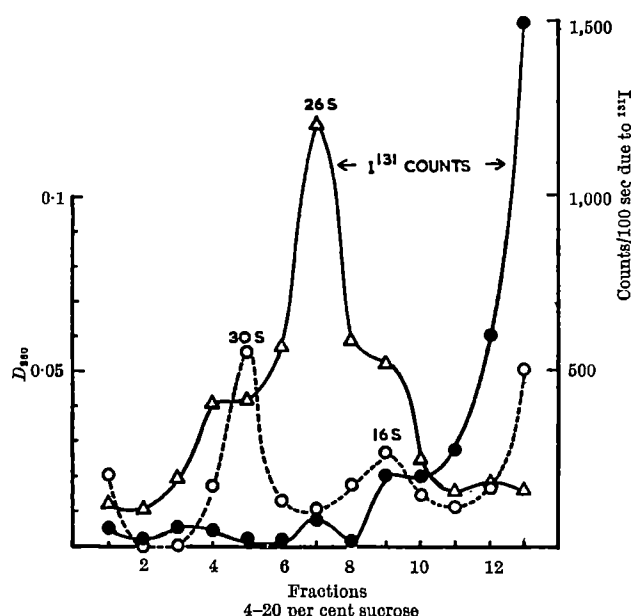


Fig. 1. Phenol-extracted macrophage RNA (Series I) after ultracentrifugation in a density gradient. O, Absorption density of RNA at 260  $m\mu$ ; ●, counts due to  $^{131}\text{I}$ , RNA preparation I; Δ, counts due to  $^{131}\text{I}$ -HCY added to normal macrophage RNA. RNA solution containing bentonite was layered on a 4-20 per cent sucrose gradient, containing 0.05 M KCl, 0.05 M *tris* buffer pH 7.4, and 0.001 M  $\text{MgCl}_2$ . Tube 13 represents the top of the gradient after centrifuging for 3.5 h at 34,000 r.p.m. in a *SW* 39 rotor in the Spinco model *L* ultracentrifuge.

mixed with normal mouse RNA can also be seen in Fig. 1. The positions of the  $^{131}\text{I}$ -peaks coincide well with sedimentation values kindly determined by Dr. P. Charlwood on an analytical ultracentrifuge. At the same pH (7.4) haemocyanin had an  $S_{20,w}$  of 26 with small amounts of aggregated and smaller molecular weight material ( $S_{20,w}$  of 36 and 15). The RNA thus contains in macromolecular form about 0.3 per cent of the  $^{131}\text{I}$ -radioactivity in the macrophages, or 0.9 per cent of the macromolecular iodine-131 present in the macrophages at 2.5 h. In the assay of immunogenicity each mouse received 1/15 of this material.

Another RNA preparation, left to stand overnight in the cold, is shown in Fig. 2. The RNA is partly degraded, probably due to traces of ribonuclease, which appears to contaminate most RNA preparations. Nevertheless, the macromolecular  $^{131}\text{I}$ -peaks are still present.

Immunogenicity of the RNA extract was tested immediately after preparation by incubation of aliquots with normal mouse spleen cells and subsequent intraperitoneal transfer into 5-6 primed mice per group (as described). Each mouse received approximately 50  $\mu\text{g}$  RNA containing macromolecular  $^{131}\text{I}$ -material of the order of 0.00004  $\mu\text{g}$  (calculated on the basis of radioactivity of the injected  $^{131}\text{I}$ -haemocyanin). The mouse sera were assayed for antibody nine days after the intraperitoneal injection. Table 2 shows results of this particular experiment; mice in the control group had titres averaging 0.73  $\mu\text{g}$  ABC/ml.; three out of five animals in the group receiving the RNA had increased serum antibody titres with a geometric mean of 17.8  $\mu\text{g}$  ABC/ml. The conditions of our assay make this difference highly significant. The second antiserum dilutions bind none or only a small percentage of the  $^{131}\text{I}$ -haemocyanin in the control mice, whereas mice in the experimental group with the higher titres bind all, or a high proportion of, the  $^{131}\text{I}$ -haemocyanin present. Pre-treatment of the RNA with 4  $\mu\text{g}$  RNase/50  $\mu\text{g}$  RNA for 30 min at 37° C inhibited the increase in serum antigen-binding capacity of the recipient mice.

Seven similar experiments were carried out, extracting RNA from cells 2.5 h after *in vivo* injection of haemocyanin or in some cases 4 h after *in vitro* incubation of

Table 2. IMMUNOGENICITY OF RNA PREPARATION I  
Indirect  $^{131}\text{I}$ -Ag binding test

Spleen cells	$\mu\text{g}$ RNA/mouse	RNase $\mu\text{g}/\text{mouse}$	$\mu\text{g}$ HCY bound/ml. mouse serum	Individual titres	Mean (geometric)
+	—	—	3.7	0.5 1.6 2.0 0.0	0.73
+	60	—	0.0	0.0 0.8 1.4 4.0	
+	60	4	3.0	3.1 81.0 61.6 38.5	17.8
+			3.6	1.6 0.0 5.6 3.6	1.64

RNA preparation I obtained from macrophages 2.5 h after injection of  $^{131}\text{I}$ -haemocyanin (HCY) *in vivo*. Spleen cells ( $3 \times 10^7$  cells/mouse) were pre-incubated with RNA preparation I 1 h at 37° C before intraperitoneal injection into mice. The recipient mice had been primed 2 months previously with 5  $\mu\text{g}$  alum-HCY in the foot-pads. In the last group 300  $\mu\text{g}$  RNA were treated with 20  $\mu\text{g}$  RNase for 30 min at 37° C before incubation with the spleen cells. The mice were bled 8 days after the transfer of spleen cells.

macrophages with  $^{131}\text{I}$ -haemocyanin when similar amounts of haemocyanin had been taken up. In most cases the cells were treated with phenol at 20° C for 20 min for the first extraction, since 20° C appeared to yield more messenger-type RNA in liver and plasma cell tumours<sup>16</sup>. Only half the RNA preparations I proved to be significantly immunogenic in the recipient mice. No visible differences in the molecular size of RNA or macromolecular  $^{131}\text{I}$ -radioactivity could be correlated with any success or failure in antibody induction.

Phenol treatment of macrophages immediately after addition of  $^{131}\text{I}$ -haemocyanin. It was important to ascertain whether active uptake by macrophages is required for the formation of an immunogenic RNA-antigen complex. As control,  $^{131}\text{I}$ -haemocyanin in amounts equivalent to those taken up by peritoneal cells *in vivo* (0.2-0.8  $\mu\text{g}$ ) was added to peritoneal cells from 20 normal mice before phenol treatment. A much lower percentage of the macromolecular  $^{131}\text{I}$ -counts was found in the aqueous RNA phase, and preparations made in this way were not detectably immunogenic. When 10-25 times the amount of  $^{131}\text{I}$ -haemocyanin taken up by macrophages *in vivo* was added to normal peritoneal cells, the absolute amount of macromolecular  $^{131}\text{I}$ -material present in the phenol-extracted RNA preparations was similar to that found in the series I RNA preparations—but only 0.03 per cent of total  $^{131}\text{I}$ -haemocyanin was recovered in macromolecular form in the phenol-extracted RNA preparation. Table 3 shows that such an RNA preparation was also immunogenic in three out of five mice. When the RNA was treated with RNase, no increase in serum antigen-binding capacity was produced.

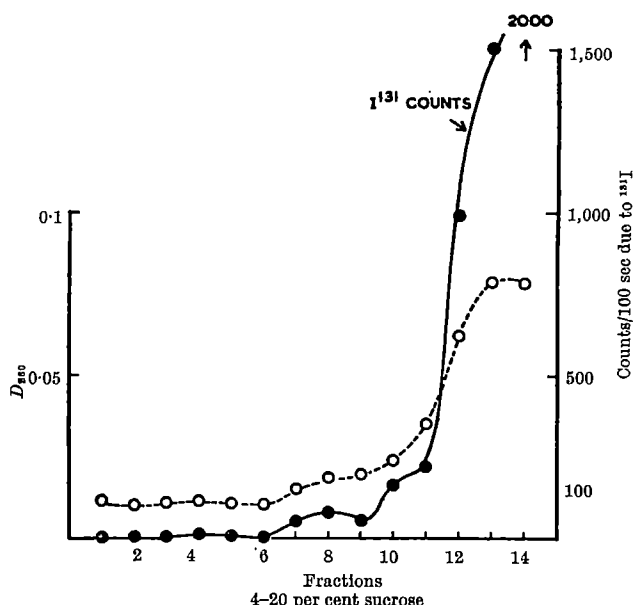


Fig. 2. Degradation of RNA preparation I at 4° C. O, Absorption density of RNA at 260  $m\mu$ ; ●, counts due to  $^{131}\text{I}$ , RNA preparation. Sucrose gradient and conditions of centrifugation as in Fig. 1. The RNA preparation had been left to stand for 1 day at + 4° C.

Table 3. IMMUNOGENICITY OF RNA PREPARATION II  
Indirect  $^{125}\text{I}$ -Ag binding test

Spleen cells	$\mu\text{g}$ RNA/mouse	RNase $\mu\text{g}/\text{mouse}$	$\mu\text{g}$ HCY bound/ml. mouse serum					Mean (geometric)
			Individual titres*					
+	—	—	0.6	3.0	2.9	1.2	0.9	1.69
+	70	—	17.0	4.0	38.5	24.4	4.0	12.3
+	70	4	1.2	2.7	2.7	2.3	0.8	1.74

RNA preparation II obtained from macrophages immediately after addition of  $3.2 \mu\text{g}$   $^{125}\text{I}$ -HCY.

\* Antigen-binding capacity of antisera of recipient mice 9 days after transfer of spleen cells.

Other conditions as in Table 2.

**Comparison of RNA preparations I and II.** Table 4 shows a comparison of the amounts of macromolecular  $^{125}\text{I}$ -material associated with RNA extracts. By macromolecular iodine- $^{131}\text{I}$  we mean material the sedimentation-coefficient of which is larger than  $6S$  on a sucrose gradient, which precipitates with TCA, and which retains the capacity to combine specifically with rabbit anti-haemocyanin as measured by secondary precipitation with antibody to rabbit  $\gamma$ -globulin. Normal rabbit serum served as a control. The results of typical experiments are illustrated in Table 4. The differences have been strikingly consistent in numerous experiments.

Table 4. COMPARISON OF RNA PREPARATIONS FROM MACROPHAGES AFTER *in vivo* CONTACT WITH  $^{125}\text{I}$ -HCY (I) AND IMMEDIATELY AFTER ADDITION OF  $^{125}\text{I}$ -HCY (II)

	RNA prepn. I	RNA prepn. II	
$\mu\text{g}$ $^{125}\text{I}$ -HCY before phenol treatment		0.8	3.2
Macromolecular $^{125}\text{I}$ -material in macrophages (approx. $\mu\text{g}$ )*	0.13	—	—
Per cent of macromolecular $^{125}\text{I}$ obtained in RNA preparation	0.9	0.016	0.03
Total macromolecular $^{125}\text{I}$ -material in RNA prepn. (approx. $\mu\text{g}$ )*	0.0012	0.00012	0.0009
$\mu\text{g}$ RNA injected/mouse	60	70	70
Macromolecular $^{125}\text{I}$ -material injected per mouse (approx. $\mu\text{g}$ )*	$4 \times 10^{-5}$	$1 \times 10^{-5}$	$5 \times 10^{-5}$
Immunogenicity	+	—	+

\* Calculated on basis of the specific radioactivity of  $^{125}\text{I}$ -HCY used.

**Immunogenicity of small amounts of haemocyanin.** The immunogenicity in our assay system of small amounts of  $^{125}\text{I}$ -haemocyanin *per se* was compared with that of the RNA preparations. Table 5 shows that the minimum amount of haemocyanin giving a significant antibody response above control levels is  $0.001 \mu\text{g}/\text{mouse}$  in the spleen cell transfer system. Lower amounts did not give any significant rise in antigen-binding capacity in mice.  $0.001 \mu\text{g}$  represents an amount 20 times greater than the amount of macromolecular  $^{125}\text{I}$ -material in the RNA preparations (calculated from the  $^{125}\text{I}$ -radioactivity of the added protein). The haemocyanin was diluted in the presence of small amounts of bovine serum albumin to ensure that the haemocyanin did not stick to the glass containers, and the efficacy of this procedure was tested by measuring the radioactivity of the injected solutions. Incubation with spleen cells before transfer into primed mice is a more sensitive method for detecting antigen, since  $0.001 \mu\text{g}$  haemocyanin injected alone intraperitoneally into these mice did not increase the antigen-binding capacity of the recipients.

The use of inbred mice gave us reproducible responses. In several series of experiments it was found that  $0.001 \mu\text{g}$  haemocyanin + spleen cells was the minimum dose to

raise the serum antigen-binding capacity of recipient mice. With this low dose generally three or four out of five recipients responded (Tables 5 and 6). Doubling the dose to  $0.002 \mu\text{g}$  haemocyanin induced higher titres in all recipients.

Pre-incubation of haemocyanin and spleen cells with ribonuclease up to levels of  $8 \mu\text{g}$  RNase per mouse did not affect the response appreciably (Table 6).

Table 6. EFFECT OF RIBONUCLEASE ON IMMUNOGENICITY OF SMALL AMOUNTS OF HCY  
Indirect  $^{125}\text{I}$ -Ag binding test

$^{125}\text{I}$ -HCY $\mu\text{g}/\text{mouse}$	RNase $\mu\text{g}/\text{mouse}$	$\mu\text{g}$ HCY bound/ml. mouse serum					Mean (geometric)
		Individual titres*					
0.001	—	3.6	10.7	2.4	7.5	22.0	7.95
0.001	2	3.2	8.0	4.8	50.0	4.8	6.3
0.001	4	13.5	30.0	9.0	21.0	4.1	12.3
0.001	8	1.2	10.5	2.0	5.3	21.0	5.0
—	—	1.2	1.5	1.2	1.6	2.2	1.5

\* Antigen-binding capacity of recipient mice 8 days after transfer of spleen cells. Spleen cells ( $3 \times 10^7/\text{mouse}$ ) were pre-incubated with  $^{125}\text{I}$ -HCY 1 h at  $37^\circ$  before injection into primed mice. In the groups with RNase, the  $^{125}\text{I}$ -HCY was treated with the stated amount of RNase for 30 min at  $37^\circ$  C before incubation with the spleen cells.

## Discussion

It has been suggested<sup>10</sup> that phenol-extracted RNA from rat peritoneal cells by inducing phage neutralizing activity, contains information for the synthesis of specific antibody. Similarly, RNA from rabbit lymph glands has been reported to transfer transplantation immunity<sup>17</sup>. We found that RNA preparations obtained by phenol treatment of mouse peritoneal cells 2.5 h after contact with  $^{125}\text{I}$ -haemocyanin *in vivo* can be immunogenic in primed mice, but we obtained evidence of the presence of antigenic  $^{125}\text{I}$ -material in the RNA preparations. Furthermore, RNA preparations made immediately after the addition of  $^{125}\text{I}$ -haemocyanin to peritoneal cells also proved to be immunogenic, although it was necessary to add about 20–30 times as much  $^{125}\text{I}$ -haemocyanin to obtain similar amounts of macromolecular  $^{125}\text{I}$ -material in the aqueous RNA phase (Table 4). Thus, at least in our system the immunogenicity of the RNA preparations is not due to the formation of a specific new informational RNA by macrophages following contact with antigen.

The finding that small amounts of protein contaminate phenol-extracted RNA agrees with the experience of other workers, who found, for example, trace amounts of enzymes and virus proteins associated with phenol-extracted RNA. None the less, it was striking that a far greater percentage of macromolecular  $^{125}\text{I}$ -material was found in the macrophage RNA preparations after cellular uptake of haemocyanin *in vivo* than after addition of  $^{125}\text{I}$ -haemocyanin to peritoneal cells just before phenol treatment (Table 4). This difference has been consistently observed in many experiments. The other point which emerges is that both types of RNA-Ag preparation can be highly immunogenic compared with haemocyanin alone. The minimum amount of haemocyanin eliciting similar increases in serum antigen-binding capacity in recipient mice is  $0.001 \mu\text{g}$  haemocyanin, about 20 times the amount calculated to be present in the immunogenic RNA preparations.

In the control RNA extracts (II) it seems probable that antigen is bound by RNA, and RNA simply enhances uptake of antigenic material by the relevant cells. This would explain the inhibition of antibody response after pre-treatment of the RNA preparation with ribonuclease. The results after *in vivo* uptake of haemocyanin suggest that, in addition, an active process may take place in the macrophages.

It is thus still not possible to decide whether macrophages are essential for the induction of antibody formation. By taking up foreign material in lymphoid tissues they may bring the antigen close to potential antibody forming cells in the tissues. Several possible functions may be cited. Macrophages may simply serve as a storage

Table 5. IMMUNOGENICITY OF SMALL AMOUNTS OF HCY  
Indirect  $^{125}\text{I}$ -Ag binding test

Spleen cells	$^{125}\text{I}$ -HCY $\mu\text{g}/\text{mouse}$	$\mu\text{g}$ HCY bound/ml. mouse serum					Mean (geometric)
		Individual titres					
+	0.001	60.0	28.8	4.8	45.0	51.0	28.0
+	0.0005	1.6	3.6	3.0	4.0	1.4	2.5
+	0.0001	0.3	1.6	0.9	1.8	1.8	1.07
+	0.00005	1.5	1.3	0.3	0.3	0.4	0.59
+	—	0.5	4.0	1.0	0.75	2.5	1.30
—	0.001	0.3	1.6	3.0	2.5		1.32

Spleen cells ( $3 \times 10^7$  cells/mouse) were pre-incubated with  $^{125}\text{I}$ -HCY for 1 h at  $37^\circ$  C before intraperitoneal injection into primed mice. The recipient mice had been primed 2 months previously with  $10 \mu\text{g}$  alum-HCY and were bled 9 days after cell transfer.

depot, have nothing to do with antibody formation or, alternatively, be responsible for an essential step in inducing synthesis of antibody globulin. It is probable that antigens on whole cells, particulate antigens, or bacteria have to be processed or partly degraded in macrophages to release and make available appropriate antigenic determinants to the reactive lymphoid cells. Our evidence suggests that processing of a soluble protein (haemocyanin) is not necessary to obtain immunogenic material (RNA preparation II), but the yield of immunogenic material from macrophages that had actively taken up haemocyanin is much higher (RNA preparation I). Further characterization of the immunogenic material is necessary to decide whether an active complexing or binding of antigen or its fragments with RNA (or other substances present in aqueous phenol extracts) takes place, perhaps as a mechanism of protecting antigen against lysosomal digestive enzymes. It is striking that although the major proportion of haemocyanin taken up by peritoneal cells is rapidly degraded, a small amount of antigen persists for many days in macrophages (Askonas and Rhodes, to be published), suggesting some binding or protective mechanism. Dutton and Harris<sup>20</sup> also presented evidence that spleen cells, irradiated and killed after incubation with serum albumin, liberate a factor stimulating a secondary antibody response *in vitro* (as measured by DNA synthesis). Ten times the amount of HSA as such was without effect. Possible transfer of material between macrophages and lymphocytes has been discussed by a number of workers<sup>11,17-19</sup>.

Our conclusions are that RNA preparations extracted with phenol from mouse peritoneal cells after uptake of <sup>125</sup>I-haemocyanin *in vivo* are immunogenic in primed mice. The RNA contained macromolecular <sup>125</sup>I-radioactivity with properties compatible with the presence of antigen or fragments thereof. RNA extracts prepared from macro-

phages immediately after addition of <sup>125</sup>I-haemocyanin were also immunogenic, provided larger amounts of <sup>125</sup>I-haemocyanin had been added to the macrophages. Pre-treatment of the RNA preparations with RNase in each instance diminished the antibody response.

The minimum amount of <sup>125</sup>I-haemocyanin eliciting increases in the serum antigen-binding capacity in primed recipient mice was 0.001 µg/mouse. This is 20 times the amount calculated as being present in the immunogenic RNA preparations. This response was not susceptible to low levels of ribonuclease, which inhibited the immunogenicity of RNA preparations.

We thank Mr. Brian Wright and Mrs. Frances Gotch for their assistance.

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## Staphylococcus PHAGE-BACTERIUM IN VIVO INTERACTION

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*STAPHYLOCOCCUS aureus* has remained a constant threat to man by virtue of its ubiquity, physiological versatility, and ability to cause disease. Recent experience with antibiotic-resistant strains has led to the frank recognition of our uncertain position and has stimulated the search for a better understanding of this microbe, its pathogenic nature and its reliable control.

The clinical use of *Staphylococcus* bacteriophage lysate has been disappointing<sup>1</sup>, and its use appears to have been reserved for those situations in which all else has failed and little hope remains. Despite this circumstance, favourable results have been achieved<sup>2</sup>. Unfortunately, it has not been possible to recognize a satisfactory *modus operandi* which might account for success or failure in the use of these preparations. Therefore, it is our purpose to investigate the use of bacteriophage lysate in staphylococcal infection, to determine its potential and to establish its limitations as a lytic and/or immunoprophylactic agent.

In a previous report<sup>3</sup> we presented evidence to indicate that *S. aureus*, Type 80/81 and bacteriophage 81 were capable of *in vivo* interaction, resulting in 81 per cent survival of infected mice. This article describes certain factors which appear to influence the degree of interaction and extent of subsequent protection of infected mice.

The experimental system under investigation consisted of male Swiss mice (14-16 g) which were infected intra-

peritoneally with  $1.5 \times 10^8$  *S. aureus*, Type 80/81 suspended in 5 per cent gastric mucin (Wilson granular mucin, type 1701-W). Phage 81 was administered into the opposite side of the peritoneum, and plasma-levels of phage were established by bleeding groups of 5 mice from the tail at certain intervals of time. Blood was collected in cold heparinized saline (50 µ/ml.) and was centrifuged at 1,230 relative centrifuged force for 10 min, and the supernates were titrated according to procedures outlined by Adams<sup>4</sup>.

Features of the *Staphylococcus* phage-bacterium *in vivo* interaction are presented in Fig. 1, which shows the plasma-levels of phage 81 at various intervals of time after administration to infected and uninfected mice. In the uninfected group (broken line), approximately  $10^{5.5}$  p.f.u./ml. (plaque forming units/ml.) plasma were present in the 30-min sample, and this level persisted without significant change throughout the 2-h observation period. In contrast, infected mice (solid line) did not show evidence of circulating phage at 30 min. However, by 90 min the levels of phage had risen to  $10^{6.5}$  p.f.u./ml., exceeding the levels seen in uninfected mice by some 90 per cent. These results permit the interpretation that the administration of *Staphylococcus* bacteriophage to infected mice results in: (1) adsorption of phage to the infecting bacterial cell, followed by (2) a rise period and significantly increased levels of circulating phage. Distinction of this series of



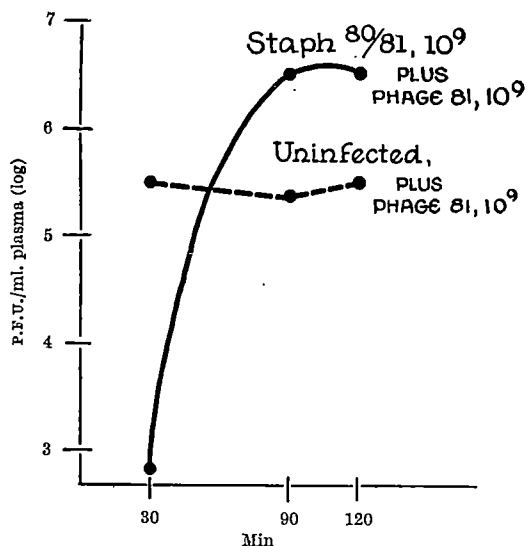
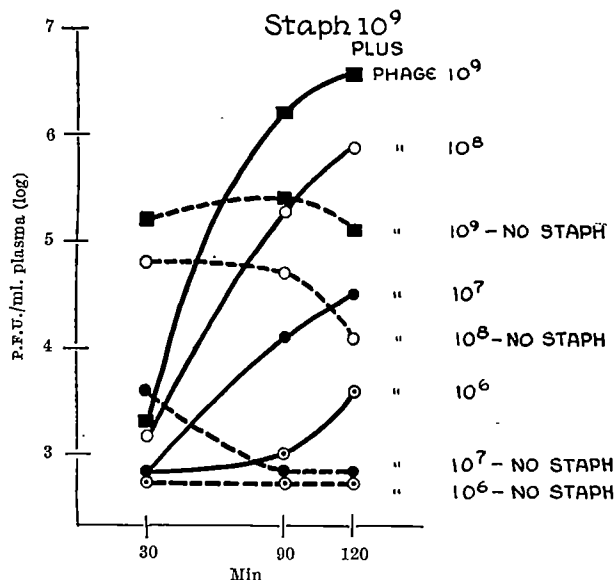
Fig. 1. *Staphylococcus* phage-bacterium in vivo interaction

Fig. 2. Influence of input multiplicity on phage-bacterium interaction

events has made it possible to follow the interaction under various conditions and, further, to correlate the features of this event with subsequent protective activities observed in mice.

The effect of treating staphylococcal-infected mice with various concentrations of phage has been determined. In this experiment various groups of mice were infected and treated with decreasing numbers of phage particles so that the input multiplicity was varied from 1 to 0.001. The results are illustrated in Fig. 2. A comparison of phage plasma-levels in infected (solid line) and corresponding uninfected groups (broken line) indicates that interaction occurred at all the multiplicities tested. This was made evident by a period of adsorption followed by a rise period and significantly increased levels of phage. It will also be observed that, as input multiplicity was decreased, the rate of phage appearance in the blood decreased, and within the time limit imposed, lower levels of circulating phage were evident at the end of 2 h. This probably indicates that as input multiplicity was decreased, the proportion of bacteria initially infected by phage was also decreased, resulting in a more gradual development of the rise period.

When intraperitoneal infection of mice was initiated as already described here, the immediate administration

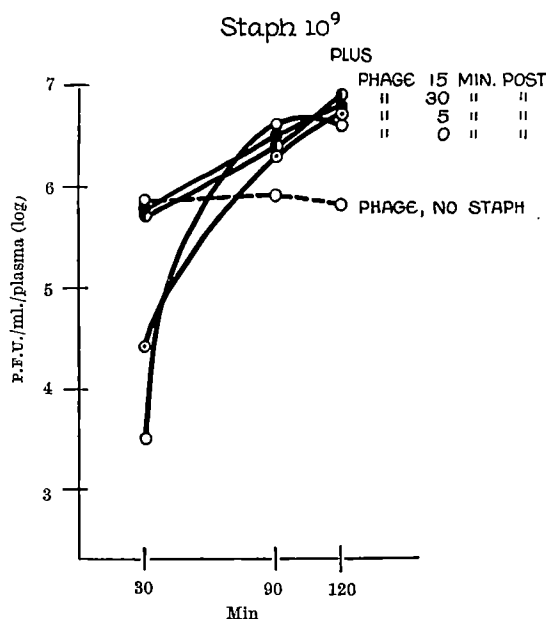


Fig. 3. Influence of time of phage administration on phage-bacterium interaction

of lysate containing an equal number of phage particles resulted in 80 per cent protection. The degree of protection was found to be strongly dependent on the time of phage administration; the greater the time-interval between infection and phage administration, the less the protective activity. Therefore, it was of interest to determine if reduced protective activity was directly related to a diminished interaction between phage and infecting bacteria, possibly during the period of adsorption. Groups of mice were infected and phage was administered at 0, 5, 15 and 30 min post-infection. The results are presented in Fig. 3. When phage was administered immediately after the infecting organism, the resultant curve indicated a period of adsorption followed by increased phage-levels. Phage administered 5 min after infection did not appear to be as completely adsorbed as indicated by the higher phage-levels present in the 30-min plasma sample. With further delay of 15 and 30 min before phage administration, there was little evidence of adsorption. However, in contrast to the uninfected control groups, these groups did eventually show increased phage-levels that may be suggestive of phage-bacteria interaction at some site other than the peritoneum.

Table 1. CORRELATION OF PHAGE-BACTERIUM INTERACTION AND MOUSE SURVIVAL

Phage admin. after Staph. infection min.	Relative phage adsorption (%)	Mouse survival (%)
0	99.9	80
5	90	65
15	6	10
30	0	0

The correlation which was found to exist between phage-bacterium interaction and mouse survival is shown in Table 1. It is evident that when phage was administered without delay adsorption was greatest, and that the degree of adsorption was rapidly diminished with delayed administration of phage. It is also apparent that the greater the degree of adsorption the higher the percentage survival of mice, and conversely, when adsorption was low or absent, the survival of mice was also correspondingly low or absent.

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## AMINO-ACID CONTROL OF STREPTOMYCIN ACTION ON MAMMALIAN CELLS

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**S**TREPTOMYCIN in relatively high concentration is toxic for some strains of mammalian cells<sup>1</sup>. Cells resistant to streptomycin were detected in cultures of sensitive cells and maintained their resistance on repeated sub-culture. Although sensitivity and resistance could be demonstrated under a given set of conditions, variations in the culture media affected the response of the cells to streptomycin. For example, a sensitive strain of cells manifested various degrees of resistance when different lots of horse serum were used in the media<sup>1</sup>.

We recently noted that all our strains of cells began to respond to streptomycin in an identical manner; they remained healthy and there was but a slight inhibition of growth (Fig. 1). All attempts to elicit the former pattern of sensitivity and resistance utilizing horse sera from different sources failed. Inasmuch as the sensitivity of the cells in our early observations was apparently due to a property of the serum used at that time, an examination of nutritional factors affecting sensitivity was initiated.

The conditions for determining the effect of streptomycin on cells were those described previously<sup>1</sup>. Synthetic medium 1066 (ref. 2) was utilized unless otherwise stated, streptomycin was added in a concentration of 3 mg/ml., and a clone culture of strain *L* mouse fibroblasts was used in the tests.

The cells were killed by streptomycin when medium 1066 was supplemented with 10 per cent 'Agamma' horse serum (a commercial preparation from which all the  $\gamma$ -globulin and half the  $\beta$ -globulin were removed) (Fig. 1). The cells were also sensitive when the  $\beta$ - and  $\gamma$ -fractions of horse serum (Fractions II and III) were added to the 'Agamma' horse serum supplement. These fractions apparently were not involved in the lack of sensitivity of the cells in whole horse serum, and the sensitive reaction in 'Agamma' horse serum was probably due to the removal of other components or an alteration in the serum components during processing.

The strain of cells utilized was able to grow in medium 1066 or in Eagle's amino-acid and vitamin mixture<sup>3</sup> containing 10 per cent beef peptone dialysate. Although the cells were killed by streptomycin in the 1066-peptone medium, they remained healthy in Eagle's-peptone medium plus streptomycin (Fig. 1).

Medium 1066 is much more complex than Eagle's mixture, and there is a variety of compounds present in medium 1066, and not present in Eagle's mixture, that may have caused the differences in the reactions of the cells to streptomycin. One outstanding difference in the two media is the presence of 260 mg/l. of cysteine in medium 1066 and none in Eagle's mixture. When this amount of cysteine was added to Eagle's-peptone medium the cells were killed by streptomycin in it; as little as 100 mg/l. had the same effect.

The concentration of cysteine utilized had no effect on the growth of the cells in the absence of streptomycin. Cysteine would probably be oxidized to a great extent under the test conditions, and tests with cystine gave identical results. The addition of larger amounts of other individual amino-acids in Eagle's mixture caused some enhancement of the action of streptomycin, but this occurred at levels of 400 mg/ml., at which concentration the amino-acids themselves were slightly toxic to the cells.

The cells were not killed by streptomycin in Eagle's mixture plus 'Agamma' horse serum, and there was less inhibition of growth with Eagle's mixture than with medium 1066 with horse serum. The addition of 200 mg/l. of cysteine to the Eagle's mixture caused the cells to respond in a manner identical to that observed with medium 1066 with the respective serum supplements (Fig. 1).

Cells sensitive to the action of streptomycin have been observed to be unaffected by dihydrostreptomycin<sup>4</sup>. The addition of cysteine to Eagle-peptone medium or an excess of cysteine to 1066-peptone medium did not affect the cells' reaction to dihydrostreptomycin.

These results indicate that the quantity of a single amino-acid in the growth medium is a determining factor in the response of mammalian cells to streptomycin. The fact that a sufficient amount of cyst(e)ine is requisite for streptomycin to act when 'Agamma' horse serum is used as a medium supplement suggests that the prime role of the serum supplement is to provide for the growth of the cells and that it has no special role in the action of streptomycin on the cells. It has been observed that serum binds half-cystine residues from medium<sup>5</sup>, and differences in the response of cells to streptomycin with different samples of horse serum may perhaps be explained on the basis of variations in the ability of the respective serum samples to bind cyst(e)ine or other amino-acids.

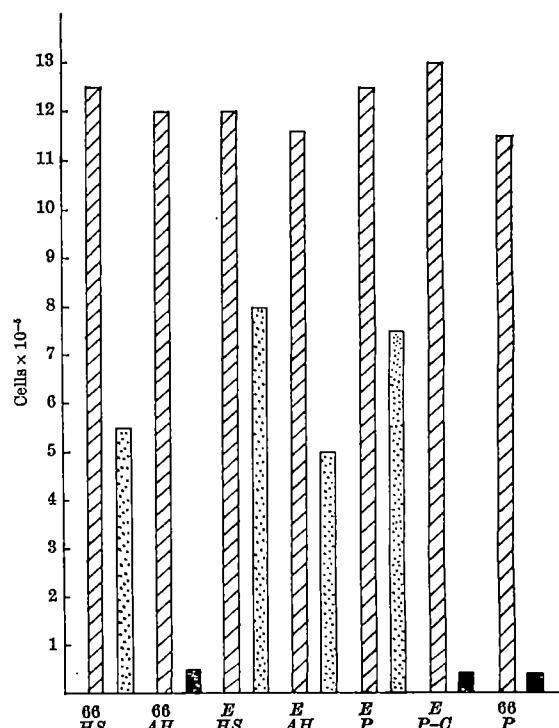


Fig. 1. Cell counts after seven days growth. Inoculum — 100,000 cells. Dead cells were killed within 48 h. Lined bars, no streptomycin; dotted bars, streptomycin added, cells healthy; black bars, streptomycin added, cells dead. E, Eagle's mixture; 66, medium 1066; HS, horse serum; AH, 'Agamma' horse serum; P, peptone; C, cysteine

Variations in the amino-acid content of a growth medium can affect the permeability, RNA synthesis, and other regulatory mechanisms of cells<sup>6</sup>. Alterations such as these in the behaviour of the cells by the added cyst(e)ine may be involved in their sensitivity to streptomycin; however, recent findings on the mode of action of streptomycin on bacteria suggest yet another hypothesis as to its possible role.

Streptomycin causes misreading of the genetic code in an *in vitro* system and may be toxic for cells by causing the production of large amounts of abnormal or non-functioning proteins<sup>7</sup>. There appear to be streptomycin-activated suppression mechanisms in bacteria, and with some strains of *E. coli* streptomycin may cause 'over-suppression', leading to the production of lethal amounts of faulty protein<sup>8</sup>. Perhaps streptomycin alters the coding properties of mammalian cells in a manner such that an abnormal protein containing a relatively large amount of a particular amino-acid is specified. If this amino-acid is present in sufficient quantity to permit the

production of lethal amounts of this protein, the cell is killed. The hypothesis assumes that the abnormal protein formed by the cell strain utilized in this work has a high cystine content; if other strains of cells are stimulated to produce abnormal proteins with a high content of a different amino-acid, that amino-acid may be required in relatively large quantities in the medium for streptomycin to be toxic for them. This possibility is being investigated.

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## RELATIONSHIP OF BARR BODIES, NUCLEAR SIZE AND DEOXYRIBONUCLEIC ACID VALUE IN CULTURED HUMAN CELLS

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THE results of recent work have greatly clarified our knowledge as to the origin of the Barr body. There can no longer be any doubt that a Barr body represents a single X-chromosome in a heavily condensed state. It is much less clear why tissues of female origin should contain a proportion of cells in which Barr bodies cannot be seen<sup>1</sup>. Evidence has previously been presented that, in human cell cultures, nuclei containing a Barr body are on an average somewhat smaller than those without one<sup>2,3</sup>. It has now been possible to obtain more exact measurements relating to this problem, by removing the variation in nuclear size due to polyploidy. The results indicate that nuclear size may be a major factor in determining whether a Barr body is to be formed or not.

The cells originated from skin biopsies, which were cultured according to the method of Harnden<sup>4</sup>. Two different cultures were used, both of which had apparently normal XX sex chromosomes. Culture A, which had been growing for 9 weeks and had been sub-cultured 9 times, had an apparent trisomy (with translocation) of chromosomes in the 13-15 group (Dr. J. H. Edwards, private communication); culture B, which had been growing for 6 weeks and had been sub-cultured 5 times, had an apparently normal karyotype.

After trypsinization, the cells were allowed to grow on cover slips for 25 h, when they were immediately fixed in 95 per cent ethanol for 0.5 h. They were stained by the Feulgen reaction, following hydrolysis in N hydrochloric acid at 60°C for 10 min. DNA measurements were carried out using a Barr and Stroud integrating microdensitometer GN2, which is based on the instrument designed by Deeley<sup>5</sup>. The assumption underlying this technique is that the amount of light absorbed by Feulgen-stained nuclei bears a direct relationship to the amount of DNA present. The actual technique has recently been described by Burks and Bakken<sup>6</sup>. The

cells were mounted on glass slides and no attempt was made to squash them, it having previously been shown that such preparations are suitable for assessing the ploidy of interphase nuclei<sup>7</sup>.

For each nucleus, the following particulars were recorded: (1) the presence or absence of Barr bodies (in culture B, which contained a considerable proportion of cells with multiple chromocentres, this fact was also recorded); (2) the length of the two main axes, as measured with a micrometer eye-piece; (3) the amount of light absorbed, as recorded by the integrating microdensito-

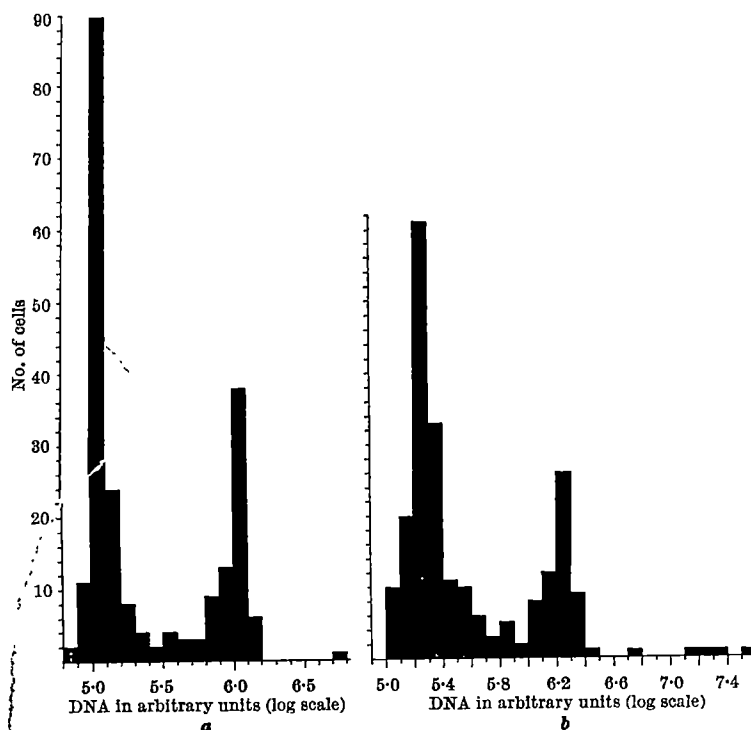


Fig. 1. Frequency distribution of DNA values in cultured cells. a, culture A; b, culture B



meter. From both cultures, 250 nuclei were recorded, but for the purpose of the present investigation nuclei which were in mitosis were excluded.

Nuclear areas were computed according to the formula  $\pi ab$ , where  $a$  and  $b$  are half axes, and the products converted into square micra, using a stage micrometer. For the purpose of plotting DNA values, the voltage readings of individual nuclei were converted into logarithms and multiplied by the reciprocal of log 2, resulting in a log scale in which any value is separated from double its value by one unit.

The DNA values are shown in Fig. 1. The two peaks in both cultures are separated by exactly one unit, and, although the values obtained are arbitrary, it is reasonable to assume that the distributions on the left and the right represent the diploid and tetraploid values respectively. The cells in the intermediate range are presumed to be in the process of synthesis. The tetraploid cells probably represent a heterogeneous group, consisting of at least three populations: (1) cells which have completed DNA synthesis prior to mitosis; (2) cells which, having completed DNA synthesis, nevertheless do not enter mitosis<sup>8,9</sup>; (3) cells which will eventually enter into tetraploid mitosis.

The mean nuclear areas, classified according to their DNA values and the presence or absence of sex chromatin, are given in Tables 1 and 2. For each culture, the difference between any two means in the diploid range is significant at the 1 per cent or a lower level. The size distributions of the diploid nuclei in culture *B* are shown in Fig. 2. It is apparent that nuclei which lack a Barr body tend to be larger than those which contain one. Previously the average difference was estimated at about 6 per cent<sup>2,3</sup>, but it now appears that for any given DNA range the difference is larger. The results presented here further indicate that nuclei with multiple chromocentres are considerably smaller than those either with or without Barr bodies.

The difference in size between nuclei without a Barr body, with one Barr body and with chromocentres appears to hold also in cells with higher than diploid DNA values (Tables 1 and 2), but the available results are too small to be significant. The average size of tetraploid nuclei with two Barr bodies was strikingly large. The relationship of Barr bodies to nuclear size in cells with polyploid DNA values requires further investigation.

The larger size of nuclei without Barr bodies in diploid cells is in agreement with the suggestion by Austin and Amoroso<sup>10</sup> that the lack of Barr bodies in early embryos<sup>11</sup>

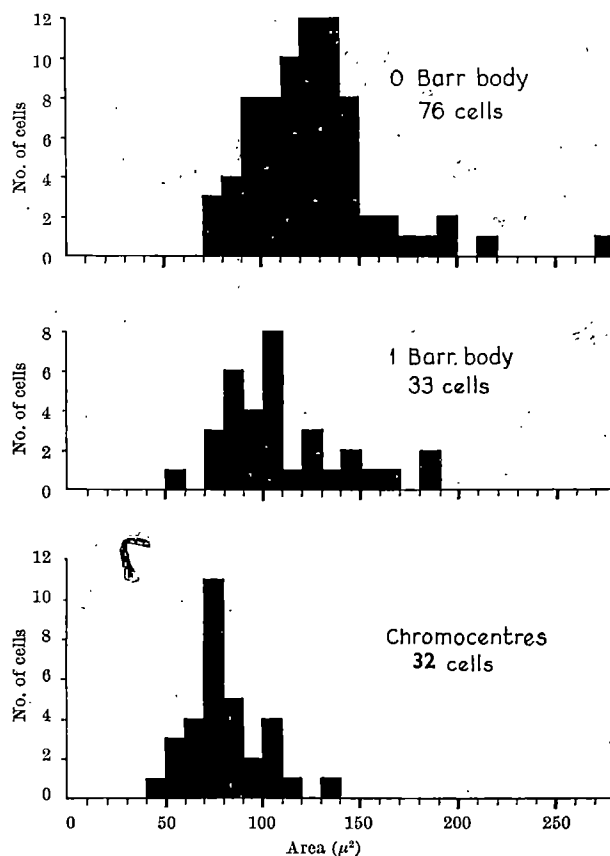


Fig. 2. Frequency distribution of nuclear areas of diploid cells from culture *B*.

is connected with the large size of their nuclei and that sex chromatin is not formed until the nuclei become sufficiently small.

It now appears that in the largest diploid nuclei neither X-chromosome has a tendency to condense, while in the smallest nuclei parts of chromosomes other than the X are likely to condense and thus obscure the presence of a Barr body; but in nuclei of intermediate size, one of the X-chromosomes tends to condense preferentially and forms a Barr body.

Commoner<sup>12</sup> has left open the question whether the difference between euchromatin and heterochromatin is due either to nucleotide composition, or to the macromolecular configuration (for example, coiling) of the polynucleotide. Although certainly not conclusive, the evidence available from the sex chromatin of mammals appears to favour the hypothesis of coiling, which is essentially a reversible process and therefore more likely to account for the presence or absence of sex chromatin in different nuclei. According to this assumption, a Barr body would represent an X-chromosome which is more tightly coiled than other major parts of a chromosome in a particular nucleus, an occurrence which is itself dependent on the macromolecular configuration of the rest of the nuclear material. The explanation that the heterochromatin of sex chromosomes may be due to differential spiralization is in accordance with the view of White<sup>13</sup>.

Any evaluation of the effect of nuclei other than those containing a visible Barr body with regard to the hypothesis of inactivation of the X-chromosome, as proposed by Lyon<sup>14-16</sup>, will have to await more detailed knowledge of the specific activity of the X-chromosomes, and the genes borne on them, in these cells.

We thank Dr. J. H. Edwards and Dr. J. Jancar for supplying the skin biopsies and Prof. L. S. Penrose for advice. The integrating microdensitometer was provided by the Central Research Fund, University of London.

Table 1. NUCLEAR AREAS, CLASSIFIED ACCORDING TO DNA VALUES AND SEX CHROMATIN STATUS  
Culture *A*

DNA value	Sex chromatin status	No. of cells	Mean nuclear area ( $\mu^2$ )	S.D.
Diploid	$\{B_0$	49	141.28	42.42
	$\{B_1$	86	119.62	37.22
Intermediate (triploid)	$\{B_0$	4	177.00	35.52
	$\{B_1$	10	128.00	32.98
Tetraploid	$\{B_0$	8	179.75	52.52
	$\{B_1$	50	157.12	52.17
	$\{B_2$	9	248.00	52.71

$B_0, B_1, B_2 = 0, 1, 2$  Barr bodies, respectively.

Table 2. NUCLEAR AREAS, CLASSIFIED ACCORDING TO DNA VALUES AND SEX CHROMATIN STATUS  
Culture *B*

DNA value	Sex chromatin status	No. of cells	Mean nuclear area ( $\mu^2$ )	S.D.
Diploid	$\{B_0$	76	126.32	32.67
	$\{B_1$	33	103.82	30.10
	Chromocentres	32	80.85	18.63
Intermediate (triploid)	$\{B_0$	11	137.83	37.52
	$\{B_1$	6	136.67	29.34
	Chromocentres	2	91.50	0.71
Tetraploid	$\{B_0$	14	243.93	102.37
	$\{B_1$	38	178.26	46.69
	Chromocentres	5	143.20	7.12
Higher than tetraploid	$\{B_0$	0	—	—
	$\{B_1$	2	517.00	268.70
	Chromocentres	3	357.33	76.12

$B_0, B_1 = 0, 1$  Barr bodies respectively.

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## CYTOTOXIC ACTIVITY COMPARED WITH PHOTOMETRIC MEASUREMENTS OF SELECTED ALKYLATING AGENTS

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It has long been desired to be able to measure chemically the fate of anti-tumour compounds following injection. It has also been desired to attempt to correlate anti-tumour activity, as measured by biological or cytotoxic activity, with what we may call chemical concentration of a particular compound or its presumed active group. Without resorting to rather expensive isotopic techniques, this has not been practical. Epstein *et al.*<sup>4</sup> and Klatt *et al.*<sup>6</sup> reported that the reaction of  $\gamma$ -(4-nitrobenzylpyridine) with various ethylenimines or alkylating agents produces a blue colour after further treatment with base. The method depends on the replacement of one Cl<sup>-</sup> by *p*-nitrobenzylpyridine and the subsequent development of blue colour in triethylamine. Linford<sup>7</sup> has measured the hydrolysis rates of three alkylating agents and their reactions with constituents of human blood serum. He found that 'Chlorambucil', chemically very similar to phenylalanine mustard (PAM), was absorbed to serum proteins *in vitro* more rapidly than 'Cytosan', and concluded that such serum protein condensation may be a source of waste of administered drug.

In our laboratory a series of experiments was designed in order to determine the chemical and biological fates of selected alkylating agents after injection into rabbits, and preliminary results have been published<sup>10</sup>.

The four compounds examined were nitrogen mustard (HN<sub>2</sub>) NSC 762, alanine mustard (AlHN<sub>2</sub>) NSC 17663, phenylalanine mustard (PAM) NSC 8806 and 'Cytosan' NSC 26271. (These compounds were received from the Cancer Chemotherapy National Service Center of the National Cancer Institute.)

The colorimetric procedure utilizing nitrobenzylpyridine and triethylamine has been described by Klatt *et al.*<sup>6</sup> and will be referred to hereafter as the NBP-TEA test. Alcohol precipitation was accomplished according to the methods of Linford<sup>7</sup> and was used whenever photometric measurements were to be done. The cytotoxic metabolic inhibition (CMI) test was used to determine biological activity and is described elsewhere<sup>8</sup>.

For *in vivo* investigations, male albino rabbits weighing about 3 kg were given a single intravenous injection of one of the compounds being examined. The rabbits were assumed to have an extracellular fluid volume of about 500 ml., and the compounds were injected in concentrations such that 1 ml. of serum might be expected to have maximal cytotoxicity exerted by the minimal amount of compound. All animals were bled by cardiac puncture 5 min, 30 min and 60 min after injection. The blood was chilled immediately and the separated serum frozen in dry ice and alcohol and stored at -20° C until tested. The clotted blood was homogenized in a Waring blender, treated with absolute ethyl

alcohol and centrifuged, and the chemical concentration of each compound was determined in the supernatant fluid.

Preliminary investigations using the NBP-TEA test showed linearity between optical density at 565 m $\mu$  and drug concentration over a range of 2-100  $\mu$ g/ml. for HN<sub>2</sub>, AlHN<sub>2</sub> and PAM when the compounds were dissolved in either 0.85 per cent saline or normal rabbit serum. Freshly prepared 'Cytosan' was not consistently measurable by the NBP-TEA test.

In order to effect a comparison of *in vitro* and *in vivo* biological and chemical activity and to determine the stability of these four compounds as they might be used clinically, each was dissolved in 0.85 per cent saline at 1 mg/ml. and allowed to stand at room temperature over a period of 6 days. Samples were taken at intervals, shell frozen in dry ice and alcohol and stored at -20° C until all the samples had been accumulated. Colorimetric assays and cytotoxicity tests were carried out on each sample.

The results of duplicate titrations are given in Fig. 1. There was a decrease both in apparent molarity by the NBP-TEA test and in cytotoxicity by the CMI test for AlHN<sub>2</sub>, HN<sub>2</sub> and PAM, which decrease was in proportion to the respective apparent molar concentrations of each of the compounds. With time, 'Cytosan' increased by both parameters.

Fig. 2 presents the mean values in serum samples for eight animals each given a single intravenous injection of each of the compounds so that the serum at time 0 would contain the apparent molar concentrations shown on the left ordinate. Neither AlHN<sub>2</sub> nor HN<sub>2</sub> had any significant activity by either assay 5 min after injection. There was no increase in the apparent molarity of 'Cytosan' (as determined by optical density) in contrast to the three-fold increase in apparent molarity seen *in vitro* (Fig. 2). In the face of this stability of apparent molarity measured by optical density, the cytotoxicity increased 3- to 4-fold. PAM showed a decrease in apparent molarity similar to AlHN<sub>2</sub> and HN<sub>2</sub> but without a concomitant decrease in cytotoxicity. Indeed, an increase in cytotoxicity was apparent. Serial dilutions of the 30-min serum samples from four of the animals administered PAM were made simultaneously with serial dilutions of PAM freshly prepared in normal rabbit serum. These animals had received 50 mg of PAM, and, with an extracellular fluid volume of 500 ml., the serum samples were expected to contain 0.1 mg/ml.

The results of the cytotoxicity tests of these preparations are shown in Fig. 3. It is readily apparent that a PAM concentration of 10<sup>-5</sup> mg/ml. in serum 30 min after injection retained full toxic activity, while the freshly prepared samples began to lose toxicity in concentrations of < 10<sup>-2</sup> mg/ml. Thus, it seemed that the toxicity of PAM was not related to the presence of intact chloroethyl groups alone.

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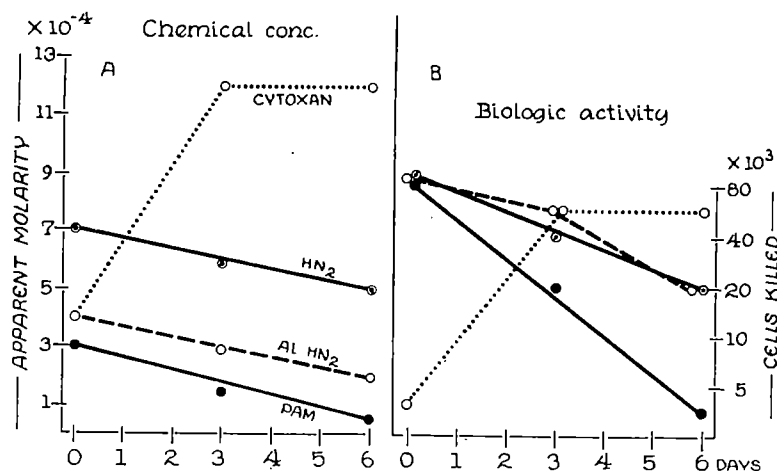


Fig. 1. Comparison of chemical concentration as measured by the NBP-TEA test with cytotoxic activity with respect to time in solution at room temperature. The time 0 points on B reflect the cytotoxic activity of the apparent molar concentrations of each of the compounds at time 0 in A.

$$\text{Apparent molarity} = \frac{\text{O.D. at time } t}{\text{O.D./mole at time 0}}$$

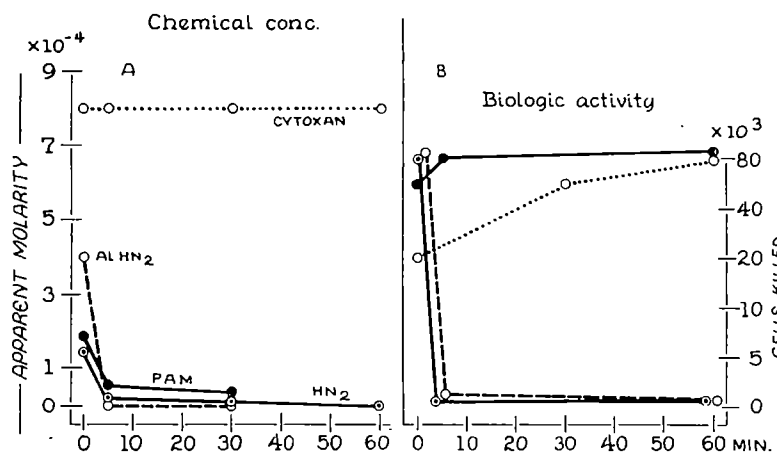


Fig. 2. Comparison of chemical concentration as measured by the NBP-TEA test with cytotoxic activity with respect to time *in vivo*. The time 0 points on B reflect the cytotoxic activity of the apparent molar concentrations of each of the compounds at time 0 in A.

$$\text{Apparent molarity} = \frac{\text{O.D. at time } t}{\text{O.D./mole at time 0}}$$

Since Linford<sup>7</sup> found that 'Chlorambucil' had a characteristic absorption spectrum with a maximum at 258  $\text{m}\mu$ , it was felt that perhaps PAM might exhibit some change in its absorption spectrum which could account for the increased cytotoxicity *in vivo*. PAM was dissolved in normal rabbit serum. The serum was deproteinized with ethyl alcohol according to Linford<sup>7</sup> and the absorption spectrum determined against the normal rabbit serum-ethyl alcohol blank. PAM was found to have an absorption maximum at 258  $\text{m}\mu$  using a Beckman model DU spectrophotometer. Five and 30 min after the intra-

Table 1. AVERAGE AMOUNT OF PAM RECOVERED IN BLOOD SAMPLES TAKEN FROM 3 RABBITS 5 AND 30 MIN AFTER INJECTION OF 50 MG/RABBIT TO GIVE AN ESTIMATED SERUM-LEVEL OF 0.1 MG/ML.

Time	Sample	mg./ml.-258 $\text{m}\mu$	mg./ml.-NBP-TEA
5 min	Clot	$7 \times 10^{-3}$	$4.2 \times 10^{-3}$
	Serum	$2.9 \times 10^{-3}$	$1.4 \times 10^{-3}$
	Total	$9.9 \times 10^{-3}$	$5.6 \times 10^{-3}$
30 min	Clot	$7 \times 10^{-3}$	0
	Serum	$2.6 \times 10^{-3}$	0
	Total	$9.6 \times 10^{-3}$	0

Table 2. STABILITY OF COMPOUNDS STUDIED IN POOLED NORMAL RABBIT SERUM AT 37° C FOR 0.5 H

Compound	Molarity $\times 10^{-4}$	O.D. (565 $\text{m}\mu$ )	O.D. (565 $\text{m}\mu$ ) 0.5 h
$\text{HN}_2$	7	0.67	0.67
Al $\text{HN}_2$	4	0.90	0.22
PAM	3	0.69	0.72
'Cytozan'	5	0.01	0.01

venous injection of 50 mg of PAM into each of three rabbits, blood was withdrawn by cardiac puncture and allowed to clot in ice. After separation, the clots were homogenized in water in a Waring blender at 4° C, and both sera and the supernatant fluid from the blood clots were deproteinized with ethyl alcohol. Optical densities were determined at 258  $\text{m}\mu$  and parallel NBP-TEA tests were carried out on portions of the same samples.

The results, given in Table 1, show that 100 per cent of the PAM was recovered from the blood at both 5 and 30 min after injection when assayed at 258  $\text{m}\mu$ . However, only 56 per cent of the PAM was recoverable in 5 min and none was recoverable in 30 min when the NBP-TEA test was used. Of additional interest was the observation that approximately three times as much PAM was found in the blood clot as in the serum, regardless of the test used to measure it. No shift was seen in the absorption spectrum of PAM in the serum samples which might account for the consistently observed increase in cytotoxicity.

It might be suggested that these compounds alkylate serum proteins and that the loss in apparent molarity (Fig. 2A) after injection was due to this alkylation. To test this possibility, each of the compounds was dissolved at 1 mg/ml. in pooled normal rabbit serum and allowed to stand at 37° C for 30 min. The results of the NBP-TEA tests made on these samples, given in Table 2, show that only Al  $\text{HN}_2$  exhibited a decrease in optical density during this period. These results are in general agreement with those of McAllister *et al.*<sup>8</sup>, who found that  $\text{HN}_2$  retained about 25 per cent of its cytotoxic activity when measured by the CMI test after storage for 4 h at 37° C in human serum. Thus, it would appear that the loss in apparent molarity of  $\text{HN}_2$  and PAM *in vivo* is due not to protein binding but rather to removal from the blood in the

case of  $\text{HN}_2$  or to some as yet unknown mechanism.

'Cytozan' is considered to require modification of the molecule by processes which occur *in vivo* before the terminal chlorine atoms become active<sup>1,5</sup>. Foley *et al.*<sup>5</sup> have postulated an enzymatic hydrolysis of 'Cytozan' to cytoxyl alcohol or cytoxyl amine primarily by enzymes of the mammalian liver. 'Cytozan' has also been reported

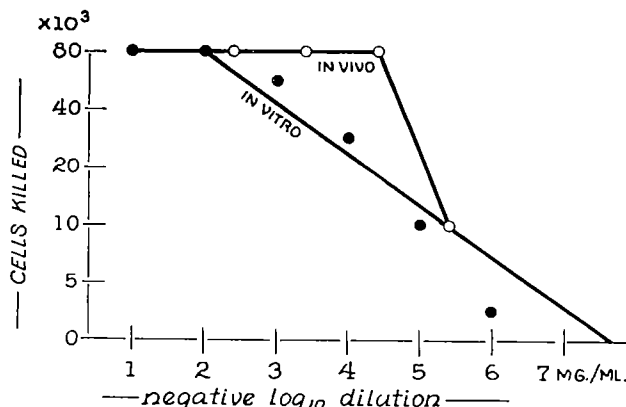


Fig. 3. Comparison of cytotoxicity of PAM freshly prepared in normal rabbit serum with 30-min serum samples of rabbits injected with PAM



to be inactive in mammalian cell cultures<sup>2</sup> when the culture method of Eagle and Foley was used<sup>3</sup>. However, when the CMI test was used, a consistent low level of cytotoxicity was observed<sup>9</sup>. Quite possibly, two mechanisms may obtain for the activation of 'Cytozan'. Conceivably, prolonged incubation (as used in the CMI test) could cause the formation of NOR-HN<sub>2</sub>, showing a cytotoxicity directly proportional to the active terminal chlorine atoms (Fig. 1). On the other hand, the cytotoxicity could be increased *in vivo* as postulated by Foley *et al.*<sup>5</sup> with no relationship to an increase in reactive chloroethyl groups as measured by the NBP-TEA (Fig. 2).

A similar mechanism of action cannot be postulated for PAM. Following injection, there was a sharp decrease in reactive chloroethyl groups, but a thousand-fold increase in toxicity (Figs. 2 and 3). Linford<sup>7</sup> showed that the alkylation reaction between 'Chlorambucil' and serum proteins occurring *in vitro* was about 50 per cent complete in 12 h at 37° C. In our hands, the alkylation reaction between PAM and serum proteins occurring *in vivo* was complete in 30 min as evidenced by the negative results with the NBP-TEA test (Table 1). If PAM and 'Chlorambucil' can be considered to react in similar ways, the full recovery of PAM 30 min after injection by absorption measurements at 258 mμ can only mean that PAM was hydrolysed to a compound

with the same absorption maximum as PAM and to a compound which alkylates serum proteins within 30 min. Thus, the latter substance could conceivably be more cytotoxic than PAM itself. The rapid alkylation of PAM with serum proteins may not constitute a source of waste of administered drug as postulated by Linford for 'Chlorambucil', but rather provide a drug of considerably increased clinical value.

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## TAXONOMIC RELATION OF ERYTHROCYTE COUNT, MEAN CORPUSCULAR VOLUME, AND BODY-WEIGHT IN MAMMALS

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ERYTHROCYTE counts and sizes have been published<sup>1-3</sup> for many species of mammals, but, so far as we have been able to ascertain, a correlation of red blood cell count and mean corpuscular volume with taxonomic status of the various species has not been enunciated. Red blood cell count and mean corpuscular volume vary widely among vertebrates, but haematocrit percentages show relatively little variation<sup>3</sup>. It follows, of course, that if haematocrit percentages do not vary greatly, then red blood cell count and mean corpuscular volume must vary in relation to each other. The publications containing information about comparative haematology commonly present values for red blood cell count and cell size in various species of mammals without attempting to relate differences within or between taxa<sup>1-3</sup>. This article will discuss an intrafamily relation of erythrocyte number and mean corpuscular volume to body-weight.

Haematological investigations of the peripheral blood of native small mammals were initiated at Oak Ridge National Laboratory in order to establish baseline values before research was begun on the effects of ionizing radiation on the blood of mammals living in environments contaminated with radionuclides. Haematopoietic tissues are relatively radiosensitive<sup>4</sup>, and sampling of peripheral blood affords an opportunity to examine effects in blood without killing animals, which is of decided advantage in research concerned with population dynamics. In addition, haematology should be of considerable diagnostic value in detecting radiation-induced pathological conditions in other tissues in much the same way that clinical haematology is utilized<sup>5</sup>.

Most of the mammals sampled were from our laboratory colonies or were live-trapped during different seasons of the year on the U.S. Atomic Energy Commission reservation at Oak Ridge, Tennessee (Table 1). Mammals from other areas are listed in Table 2. Individuals were excluded if they exhibited pathological conditions known to cause

changes in the peripheral blood. The average weights listed for each species are for 'adult' and 'sub-adult' animals, because red blood cell count and haematocrit percentages are lower in very young mammals than in older mammals<sup>3,6</sup>.

Blood was taken by tail slice from the smaller rodents and from either the tail or an amputated toe of larger animals. Blood samples were extracted from shrews by cardiac puncture and from bats by amputation of a wing. Blood from all species was collected in 2-dram vials coated on the inside surface with ~1 mg of disodium ethylenediamine tetraacetate powder, which was used as an anticoagulant. A Coulter model B electronic particle counter was utilized to obtain red blood cell counts; counter error was <2 per cent. Haematocrit percentages were obtained with an International Micro-capillary centrifuge, model B, and reader, model CR. Haematological techniques adapted or developed during this work are described in detail elsewhere<sup>6</sup>.

The number of erythrocytes per mm<sup>3</sup> is inversely related to the body-weights of the species in each of the families listed in Table 1. Mean erythrocyte volume is directly related to body-weight within each rodent family examined. Erythrocyte number is plotted against body-weight in Fig. 1. Curves are provided only for the Cricetidae and Sciuridae since only two species are available in each of the other two families. However, it appears that, when more species of appropriate size in the Soricidae and Muridae are sampled, the resultant curves will not coincide with the curves for the cricetids and sciurids, at least in the region where the lightest species occur.

In the Cricetidae, rate of decrease in erythrocyte number is relatively rapid from *P. leucopus* to *S. hispidus* (Fig. 1). The flexions at each end of the sigmoid curve indicate that counts much greater than 12.5 cells × 10<sup>6</sup>/mm<sup>3</sup> or much less than 6.0 cells × 10<sup>6</sup>/mm<sup>3</sup> are not to be expected for members of the Cricetidae in the Oak Ridge area. Also,

Table 1. WEIGHTS, ERYTHROCYTE COUNTS (RBC), HAEMATOCRIT PERCENTAGES (HCT), AND MEAN CORPUSCULAR VOLUMES (MCV) FOR MAMMALS FROM THE OAK RIDGE AREA

Values are means  $\pm 1$  S.E. Numbers in brackets indicate number of samples

Taxon	Weight (g)	RBC ( $\times 10^6/\text{mm}^3$ )	HCT (%)	MCV ( $\mu^3$ )
Insectivora				
Soricidae				
Least shrew, <i>Cryptotis parva</i>	5.4 $\pm$ 0.55 (7)	14.66 $\pm$ 0.62 (7)	49 $\pm$ 0.99 (5)	35 $\pm$ 1.9 (5)
Short-tailed shrew, <i>Blarina brevicauda</i>	13.7 $\pm$ 0.47 (17)	13.96 $\pm$ 0.51 (17)	45 $\pm$ 1.63 (15)	33 $\pm$ 1.0 (15)
Rodentia				
Sciuridae				
Southern flying squirrel, <i>Glaucomys volans</i>	64.4 $\pm$ 2.96 (13)	10.86 $\pm$ 0.33 (13)	46 $\pm$ 0.96 (12)	43 $\pm$ 0.62 (12)
Eastern chipmunk, <i>Tamias striatus</i>	80.9 $\pm$ 12.31 (36)	9.55 $\pm$ 0.18 (34)	42 $\pm$ 0.70 (26)	46 $\pm$ 0.81 (26)
Grey squirrel, <i>Sciurus carolinensis</i>	412 $\pm$ 29.32 (13)	7.31 $\pm$ 0.10 (13)	43 $\pm$ 1.12 (12)	58 $\pm$ 1.01 (12)
Woodchuck, <i>Marmota monax</i>	3,811 $\pm$ 136.02 (68)	5.27 $\pm$ 0.09 (64)	39 $\pm$ 0.54 (64)	75 $\pm$ 0.90 (63)
Cricetidae				
Eastern harvest mouse, <i>Reithrodontomys humulis</i>	7.7 $\pm$ 0.25 (48)	12.34 $\pm$ 0.19 (47)	48 $\pm$ 0.70 (41)	39 $\pm$ 0.66 (41)
White-footed mouse, <i>Peromyscus leucopus</i>	20.1 $\pm$ 0.24 (204)	12.31 $\pm$ 0.09 (196)	46 $\pm$ 0.31 (164)	38 $\pm$ 0.36 (163)
Golden mouse, <i>Peromyscus nuttalli</i>	20.9 $\pm$ 0.54 (57)	12.23 $\pm$ 0.19 (55)	46 $\pm$ 0.65 (47)	38 $\pm$ 0.50 (47)
Pine vole, <i>Microtus pinetorum</i>	26.3 $\pm$ 0.33 (166)	10.92 $\pm$ 0.10 (161)	44 $\pm$ 0.32 (138)	41 $\pm$ 0.32 (138)
Marsh rice rat, <i>Oryzomys palustris</i>	51.2 $\pm$ 1.95 (62)	7.82 $\pm$ 0.11 (62)	45 $\pm$ 0.68 (47)	58 $\pm$ 0.85 (47)
Hispid cotton rat, <i>Sigmodon hispidus</i>	101 $\pm$ 1.38 (466)	6.89 $\pm$ 0.03 (465)	44 $\pm$ 0.18 (436)	65 $\pm$ 0.21 (436)
Muskrat, <i>Ondatra zibethicus</i>	1,232 $\pm$ 29.74 (58)	6.02 $\pm$ 0.09 (57)	43 $\pm$ 0.58 (54)	72 $\pm$ 0.58 (54)
Muridae				
House mouse, <i>Mus musculus</i>	15.6 $\pm$ 0.35 (79)	11.16 $\pm$ 0.14 (77)	48 $\pm$ 0.43 (70)	44 $\pm$ 0.62 (70)
Norway rat, <i>Rattus norvegicus</i>	209 $\pm$ 19.56 (14)	8.89 $\pm$ 0.17 (14)	45 $\pm$ 0.88 (14)	50 $\pm$ 0.68 (14)

Table 2. WEIGHTS, ERYTHROCYTE COUNTS, HAEMATOCRIT PERCENTAGES, AND MEAN CORPUSCULAR VOLUMES FOR MAMMALS OUTSIDE THE OAK RIDGE AREA  
Values are means  $\pm 1$  S.E. Numbers in brackets indicate number of samples

Taxon	Location and average post-removal time at bleeding	Weight (g)	RBC ( $\times 10^6/\text{mm}^3$ )	HCT (%)	MCV ( $\mu^3$ )
Chiroptera					
Vespertilionidae					
Eastern pipistrelle	Norris, Tenn.				
<i>Pipistrellus subflavus</i>	1 day	5.1 $\pm$ 0.24 (11)	14.57 $\pm$ 0.52 (10)	47 $\pm$ 0.82 (10)	34 $\pm$ 1.40 (10)
Red bat	Clinch River, Tenn.*				
<i>Lasiurus borealis</i>	1 day	9.2 $\pm$ 0.50 (5)	19.61 $\pm$ 0.43 (5)	44 $\pm$ 1.36 (5)	22 $\pm$ 0.24 (5)
Big brown bat	Spring Hill and Andersonville, Tenn.				
<i>Eptesicus fuscus</i>	1 day	16.4 $\pm$ 3.60 (2)	11.96 $\pm$ 0.18 (2)	46 $\pm$ 5.05 (2)	38 $\pm$ 4.50 (2)
Rodentia					
Sciuridae					
Least chipmunk	Beartooth Mtns., Wyo.†				
<i>Eutamias minimus</i>	1 month	50.5 $\pm$ 4.38 (3)	10.15 $\pm$ 0.17 (2)	47 $\pm$ 2.50 (2)	46 $\pm$ 1.00 (2)
	3 months	49.6 $\pm$ 3.58 (3)	11.42 $\pm$ 0.27 (3)	52 $\pm$ 1.30 (3)	46 $\pm$ 0.33 (3)
Red squirrel	Alaska				
<i>Tamiasciurus hudsonicus</i>	1 week	264 $\pm$ 4.91 (10)	11.31 $\pm$ 0.21 (10)	50 $\pm$ 0.52 (10)	44 $\pm$ 0.47 (10)
	1 month	266 $\pm$ 8.19 (10)	10.07 $\pm$ 0.23 (10)	45 $\pm$ 0.60 (10)	45 $\pm$ 0.79 (10)
	2 months	265 $\pm$ 8.28 (8)	9.77 $\pm$ 0.38 (8)	44 $\pm$ 1.22 (8)	46 $\pm$ 0.88 (8)
	Smoky Mtns., Tenn.‡				
	2 days	200 $\pm$ 5.48 (11)	9.23 $\pm$ 0.19 (11)	46 $\pm$ 1.23 (11)	50 $\pm$ 1.36 (11)
Fox squirrel	Michigan				
<i>Sciurus niger</i>	3 days	631 $\pm$ 55.00 (4)	7.33 $\pm$ 0.35 (4)	38 $\pm$ 3.27 (4)	52 $\pm$ 4.61 (4)
	1 month	708 $\pm$ 63.69 (4)	7.07 $\pm$ 0.43 (4)	39 $\pm$ 2.15 (4)	55 $\pm$ 2.28 (4)
Cricetidae					
Northern pygmy mouse	Texas				
<i>Baiomys taylori</i>	1 week	7.9 $\pm$ 0.24 (11)	11.81 $\pm$ 0.23 (11)	45 $\pm$ 0.33 (11)	38 $\pm$ 0.96 (11)
	2 months	7.8 $\pm$ 0.25 (11)	11.74 $\pm$ 0.37 (11)	42 $\pm$ 1.28 (10)	36 $\pm$ 1.28 (10)
Oldfield mouse	Georgia				
<i>Peromyscus polionotus</i>	1 week	14.1 $\pm$ 0.54 (13)	12.07 $\pm$ 0.20 (13)	42 $\pm$ 0.77 (7)	35 $\pm$ 0.65 (7)
	Born in laboratory	12.4 $\pm$ 0.29 (14)	12.20 $\pm$ 0.24 (14)	41 $\pm$ 0.69 (8)	34 $\pm$ 1.03 (8)
Deer mouse	Smoky Mtns., Tenn.‡				
<i>Peromyscus maniculatus</i>	2 days	17.5 $\pm$ 0.68 (3)	12.20 $\pm$ 0.22 (3)	42 $\pm$ 3.70 (3)	35 $\pm$ 3.38 (3)
	2 months	19.7 $\pm$ 1.94 (3)	13.55 $\pm$ 0.20 (3)	45 $\pm$ 1.85 (2)	34 $\pm$ 1.00 (2)
Gapper's red-backed mouse	Smoky Mtns., Tenn.‡				
<i>Clethrionomys gapperi</i>	2 days	23.3 $\pm$ 1.14 (8)	13.87 $\pm$ 0.42 (8)	48 $\pm$ 1.72 (7)	34 $\pm$ 0.74 (7)
	1 month	31.5 $\pm$ 3.55 (2)	16.56 $\pm$ 0.36 (2)	52 $\pm$ 0.5 (2)	35 $\pm$ 0.50 (2)
	2 months	28.7 $\pm$ 2.45 (3)	13.51 $\pm$ 0.43 (3)	50 $\pm$ 1.85 (2)	36 $\pm$ 3.00 (2)
Meadow vole	Virginia				
<i>Microtus pennsylvanicus</i>	2 days	39.9 $\pm$ 3.60 (3)	11.19 $\pm$ 0.69 (3)	43 $\pm$ 1.94 (3)	39 $\pm$ 2.40 (3)
Hispid cotton rat	Georgia				
<i>Sigmodon hispidus</i>	1 week	112 $\pm$ 7.67 (11)	7.08 $\pm$ 0.11 (11)	47 $\pm$ 1.09 (11)	66 $\pm$ 1.29 (11)
	Texas				
	1 week	89 $\pm$ 5.16 (3)	6.59 $\pm$ 0.49 (3)	39 $\pm$ 1.56 (3)	60 $\pm$ 0.80 (3)
	2 months	122 $\pm$ 8.62 (3)	7.01 $\pm$ 0.19 (3)	40 $\pm$ 1.27 (3)	58 $\pm$ 2.88 (3)
Round-tailed muskrat	Florida				
<i>Neofiber alleni</i>	1 week	224 $\pm$ 7.61 (8)	4.98 $\pm$ 0.16 (8)	41 $\pm$ 0.82 (8)	73 $\pm$ 1.60 (8)
	2 months	268 $\pm$ 4.22 (7)	5.57 $\pm$ 0.19 (7)	39 $\pm$ 1.01 (7)	70 $\pm$ 2.64 (7)
Eastern wood rat	Wartburg, Tenn.				
<i>Neotoma floridana</i>	1 day	279 $\pm$ 28.84 (5)	7.74 $\pm$ 0.43 (5)	40 $\pm$ 1.84 (5)	56 $\pm$ 2.84 (5)
	30 days	323 $\pm$ 31.80 (5)	8.58 $\pm$ 0.54 (5)	42 $\pm$ 2.29 (5)	49 $\pm$ 1.95 (5)
	Virginia				
	1 day	339 $\pm$ 18.92 (9)	7.68 $\pm$ 0.24 (9)	42 $\pm$ 5.45 (9)	55 $\pm$ 1.13 (9)
	30 days	348 $\pm$ 19.89 (6)	7.44 $\pm$ 0.16 (6)	41 $\pm$ 0.92 (6)	55 $\pm$ 2.68 (6)

\* Migratory species. † From elevation of  $\sim 8,000$  ft. ‡ From elevations ranging from 4,500 to 6,300 ft.

judging from the data of Table 1, it appears that mean corpuscular volume much greater than  $74 \mu^3$  or much less than  $38 \mu^3$  will not be found in this family. The rapid increase in mean corpuscular volume from *M. pinetorum* to *O. palustris* is interesting; in the transition from 'mouse size' to 'rat size' in this family it may be that the surface-to-volume ratio of the erythrocyte becomes less critical in the maintenance of blood equilibria.

The red blood cell values for the lightest sciurids are relatively high, in comparison with the cricetids, but it appears that when a more definitive curve can be drawn for this family, the curve will converge with, or cross

under, the curve for the cricetids in the area where the values for the heaviest species of these families are located. Similarly, mean corpuscular volume in the lightest sciurids is smaller than in cricetids of comparable weights, but the mean corpuscular volumes of *M. monax* and *O. zibethicus* are similar.

The rate of decrease for red blood cell count and the rate of increase for mean corpuscular volume may be relatively low in both the Muridae and Soricidae within the weight ranges spanned between the two species examined for each family. Actually, the differences between red blood cell count and mean corpuscular

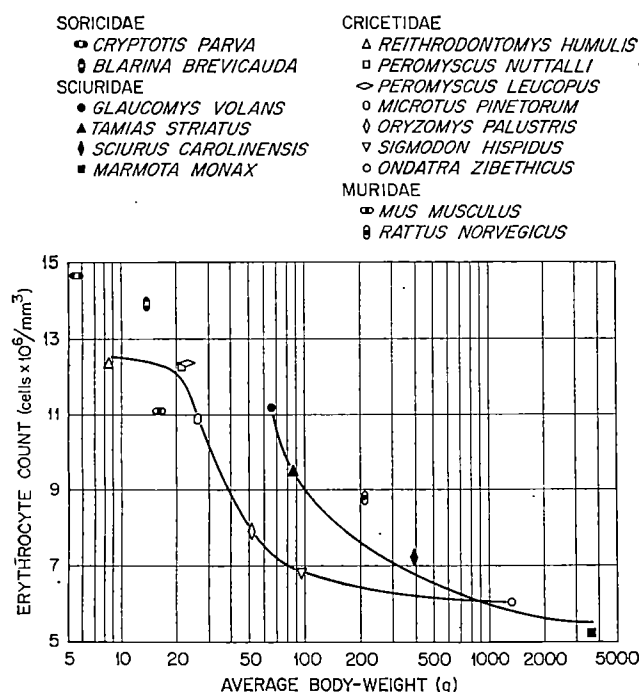


Fig. 1. Relation of erythrocyte count to body-weight in the Soricidae, Sciuridae, Cricetidae, and Muridae of the Oak Ridge area

volume in the two shrews are not significant, and it may be that both these species are in the weight range where relatively little change in these values occurs in the Soricidae. The least shrew (*C. parva*), despite its name, is not the lightest soricid, and *B. brevicauda* qualifies only as a 'medium-sized' member of the family. Similarly, *M. musculus* is not the lightest murid, and *R. norvegicus* is not one of the heaviest. More species of appropriate weights in these families must be examined before more can be said as to the interrelation of red blood cell count, mean corpuscular volume, and weight.

Haematocrit percentages ranged from about 39 to 49 in the mammals represented in Table 1. There appeared to be a tendency for the lightest species of each family to have relatively high haematocrit percentages and for the heaviest species of each family to have relatively low percentages.

The effects of altitude on blood apparently vary according to the species of animal involved. Red cell counts and haematocrit percentages are increased in rabbits, dogs and sheep acclimated to high altitude, but blood counts in the vicuña (*Lama vicugna*) and the llama (*L. glama*) are much the same at sea-level and at high altitudes<sup>7</sup>. Kalabuchov<sup>8</sup> reported that the wood mouse (*Apodemus sylvaticus*) and the yellow-necked mouse (*A. flavicollis*) were able to increase the number of erythrocytes in response to decreased atmospheric pressure, but that the field mouse (*A. agrarius*), the bank vole (*Clethrionomys glareolus*), and the grey hamster (*Cricetulus migratorius*) were not able to do so. Furthermore, the red blood cell count in *A. sylvaticus* transferred from a mountain (1,600 m) to a plain (150 m) dropped only slightly after a month, and by two months was even slightly elevated over the previous value for the mice at the high altitude.

We sampled four species from relatively high altitudes (Table 2). The erythrocyte count of the *E. minimus* after one month at a low altitude may have been temporarily depressed, as found by Kalabuchov<sup>8</sup> for *A. sylvaticus*, because the count of each *E. minimus* increased after three months. The *T. hudsonicus* from the Smoky Mountains had to be returned immediately because of an agreement with the U.S. National Park Service, but it is evident the red blood cell count for these squirrels was

considerably higher than the red blood cell-weight curve for sciurids on the Oak Ridge area (Fig. 1). The count for each *P. maniculatus* after two months at the low elevation increased at least a million over the count two days after removal. Of the eight *C. gapperi* sampled two days after removal, four died within one month. Satisfactory blood samples were taken from only two of the surviving four mice at one month; but the count for each was more than one million higher than the highest count recorded for any of the eight animals sampled two days after removal from the high altitude. However, the counts after two months for three of the *C. gapperi* were similar to the values for the eight mice two days after removal.

It seems that *E. minimus*, *T. hudsonicus*, *P. maniculatus*, and *C. gapperi* may be examples of species which possess the capacity to withstand relatively great changes of oxygen pressures. Hall<sup>9</sup> proposed the term 'eurybaric' for such species and 'stenobaric' for those species that are incapable of adjusting to great differences in altitude. Kalabuchov<sup>8</sup> speculated that the degree of plasticity as regards physiological adjustment to changes in altitude may be an important factor in the vertical distribution of mammals.

Latitude, as well as altitude, may be important in considerations of comparative haematology. The *T. hudsonicus* from Alaska, *S. niger* from Michigan, and the *M. pennsylvanicus* from Virginia had higher red blood cell counts than might be expected from the red blood cell-weight curves for the respective families in the Oak Ridge area (Fig. 1). Furthermore, the counts for the *B. taylori* from Texas, *P. polionotus* from Georgia, and *N. alleni* from Florida were lower than might be expected for similarly-sized cricetids in the Oak Ridge area. The values for the Alaska *T. hudsonicus* and the Florida *N. alleni* are particularly interesting. The average red blood cell count for the Alaska *T. hudsonicus* at first sampling was more than two million higher (18 per cent) than the count for the Smoky Mountain squirrels in spite of the fact that the latter were from an altitude of from 4,500 to 6,300 ft. and the former were living at a low altitude (~450 ft.). The average red blood cell value for the *N. alleni* at first sampling was about one million less than for the red blood cell count for the *O. zibethicus*, which weighed more than five times as much as its smaller relative. It may be seen that the erythrocyte count decreased in the Alaska *T. hudsonicus* and increased in the *N. alleni* while these animals were in our laboratory.

The red blood cell and mean corpuscular volume values for the *S. hispidus* from both Texas and Georgia are very similar to the counts for the local cotton rats. This species apparently has extended its geographical range in the central part of the United States northward within a relatively short time. For example, Cockrum<sup>10</sup> estimated that this rat extended its range about 100 miles northward in Kansas during 1933-47. Perhaps the northward movement of this species has been too rapid for evolution of the haematopoietic system to 'keep pace'.

Two species did not fit into this theory of a taxonomic relation of red blood cell count and mean corpuscular volume to body-weight. The *N. floridana* from a valley in the Cumberland Mountains were trapped only about 20 miles west of Oak Ridge, and the elevation was almost exactly the same. However, the red blood cell count for these wood rats and the ones from Virginia was almost a million higher than for the *S. hispidus*, which weighed only about one-third or one-fourth as much as the *N. floridana*. Perhaps other species in the genus *Neotoma* (particularly in the sub-genera *Teonoma*, *Hodomys* and *Teanopus*) should be examined in order to ascertain whether high red blood cell count and small cell size are peculiar only to *N. floridana* (and perhaps closely related species) or are characteristic of the entire genus. Erythrocyte count of the *E. fuscus* was less than that of the lighter *P. subflavus*, but the *L. borealis*, almost twice as heavy



as the *P. subflavus*, exhibited red blood cell counts more than five million more than for the smaller bat. The *L. borealis* is a migratory bat; *E. fuscus* and *P. subflavus* hibernate in caves or similar shelters. During favourable weather, all three species feed at night; but during the day *L. borealis* hangs in trees and the other two species retire to protected sites. It is obvious that bats which take refuge in trees and similarly exposed places must be subjected to greater vicissitudes of weather than are bats that live in the equable environment of such places as caves. Perhaps the haematopoietic system of *L. borealis* has evolved to meet the severe metabolic demands that must arise at times, particularly during cold weather and migration. However, the changes in the peripheral blood of bats induced by daily torpor or winter hibernation probably should be examined in detail before evaluation of normal values is attempted.

Inspection of the literature indicates that a relation between red blood cell count, mean corpuscular volume, and size of mammal may exist in families other than those discussed in this article. For example, Ponder *et al.*<sup>10</sup> examined the blood of captive camelids and reported red blood cell counts (cells  $\times 10^6/\text{mm}^3$ ) of 19.40 for the alpaca (*Lama pacos*), 11.30 for the llama (*Lama glama*), 10.80 for the Arabian camel (*Camelus dromedarius*), and 10.45 for the Bactrian camel (*Camelus bactriens*). Average erythrocyte counts (cells  $\times 10^6/\text{mm}^3$ ) have been summarized<sup>3</sup> for bovids such as domesticated goats (16.0), sheep (10.3), and cattle (8.1). Gulliver, in his intensive examination of the sizes (diameters) and shapes of red blood cells of vertebrates<sup>11</sup>, concluded that, within an order or even some families of mammals, erythrocytes with the greatest diameters were found in large species and the smallest cells in small species. On the other hand Emmons wrote: "What determines the size of the red cell for different species of mammals is yet unknown; certainly there is no relation between the size of the animal and that of its erythrocyte"<sup>12</sup>.

Several writers have touched on haematological and physiological aspects that may be involved in a taxonomic interrelation between animal size and erythrocyte number, shape and size. Gulliver was interested in the sizes of red corpuscles in relation to taxonomy, as discussed earlier, but he speculated also on the possible physiological significance of the different sizes and number of these cells in the various species<sup>11</sup>. Wintrobe stated: "Differences in number, size, and morphology of the red

blood corpuscles of the lower vertebrates and those of the mammalia furnish further evidence of the efficiency attained through the evolutionary process"<sup>3</sup>. Jordan suggests that, in general, erythrocyte count, size and shape are related to the metabolism of animals<sup>13</sup>. It has been demonstrated that metabolic rate is inversely related to the size of mammal<sup>14-16</sup>. Consequently, if metabolic rate is related to size of mammal, if red blood cell number and size are related to metabolism, and if erythropoietic systems have evolved along diverging lines, it seems likely that correlations between red blood cell count, mean corpuscular volume, and species weights also may exist (but at differing rates) throughout the various natural taxa of the Mammalia.

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## VISUAL RECEPTOR POTENTIAL OBSERVED AT SUB-ZERO TEMPERATURES

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THE recent discovery of the early receptor potential in the eye of the Cynomolgus monkey by Brown and Murakami<sup>1</sup> seems to provide a very useful system for the examination of visual excitation. The latency of the early receptor potential is extremely short<sup>1,2</sup> and, as Cone<sup>3</sup> has shown, the amplitude of the signal is linear with respect to the amount of rhodopsin bleached, strongly suggesting that the early receptor potential is closely linked to the initial events in the bleaching of rhodopsin. Since then it has been shown that the early receptor potential consists of two independent receptor potentials, the positive peak and the negative peak<sup>3</sup>. The positive peak has the shortest latency of all known receptor potentials<sup>3</sup>, and its extraordinary resistance to cooling<sup>3</sup> seems to open up a new avenue of approach in visual excitation research. In order to establish the

relationship between the positive peak and visual excitation, it is important to ask by what mechanism the positive peak is generated. Elucidation of this question will probably have important consequences in the understanding of visual excitation itself. Moreover, it is not unlikely that the positive peak is generated by one of the initial steps of visual excitation. The question before us is, then, at what stage in the bleaching of rhodopsin does the generation of the positive peak occur?

As is well known, the bleaching of rhodopsin to a mixture of all-trans retinal and opsin proceeds over several transient intermediates in the following sequence: pre-lumirhodopsin, lumirhodopsin, metarhodopsin I, and metarhodopsin II<sup>4-6</sup>. Presumably the initial step, rhodopsin  $\rightarrow$  pre-lumirhodopsin, results from the photoisomerization of the 11-cis retinal to all-trans<sup>4,7</sup>. All the other steps are thermal, dark reactions which follow as a consequence of this initial, photochemical event<sup>8</sup>. All

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these transient intermediates can be stabilized by cooling<sup>4</sup>. Cattle pre-lumirhodopsin in solutions is stable below about  $-140^{\circ}\text{C}$  (refs. 4 and 7), and lumirhodopsin and metarhodopsin I, below about  $-40^{\circ}\text{C}$  and  $-15^{\circ}$  to  $-20^{\circ}\text{C}$ , respectively<sup>5,6,8,9</sup>.

Wald and his colleagues have long felt that the hydrolysis of metarhodopsin to retinal and opsin is much too slow a process to have a direct role in visual excitation<sup>4,5,8,10,11</sup>. On the other hand, Hagins<sup>12</sup> showed that a process strongly suggestive of the hydrolysis of metarhodopsin occurs in rabbit eyes long before the electroretinographic response to the stimulus flash can be recorded. He therefore concluded that the release of retinal from opsin may well be responsible for the excitation. Abrahamson *et al.*<sup>13</sup> have also concluded that not only is the bleaching of metarhodopsin fast enough to be involved in the excitatory process but is likely to be so because of the large positive changes of entropy involved in this step. In the light of the recent evidence that the absorption spectrum of metarhodopsin II is very similar to that of retinal and opsin, what these workers observed may have been the conversion of metarhodopsin I to II rather than the hydrolysis of metarhodopsin II (ref. 6). Although the hydrolysis of metarhodopsin II is a slow process, the transformation of metarhodopsin I to II is likely to be very rapid at the physiological temperature<sup>6</sup>. Thus, even if the hydrolysis of metarhodopsin II were to be excluded from considerations of speed, the reaction, metarhodopsin I  $\rightarrow$  II, cannot be discarded *a priori* as a possible source of excitation.

As the foregoing discussion suggests, pertinent information relating to the question of visual excitation is still rather scarce. In the work recorded here we sought to localize the step or steps in the bleaching of rhodopsin which might be responsible for the production of the positive peak of the early receptor potential. Our technique has been to observe the behaviour of the positive peak in the excised eye of the albino rat at low temperatures at which one or more of the transient stages in the bleaching of rhodopsin is known to be stable.

The essential elements of the experimental arrangement are similar to those described previously<sup>2,3</sup>. The positive peak of the early receptor potential was observed in the electroretinogram (ERG) elicited from the excised eye of the albino rat using a 65-joule strobe lamp having less than a 0.7-msec flash duration. Unlike the later retinal potentials the early receptor potential displays an extraordinary resistance to anoxia<sup>1-3</sup>. Therefore, the positive peak of the early receptor potential could be investigated on excised eyes, making it relatively simple to vary the temperature of the eyes. On the other hand, since the visual pigment does not regenerate in the excised rat eyes, each set of observations had to be made on a freshly excised, fully dark-adapted eye. The electrodes consisted of cotton wicks leading to silver wires coated with silver chloride. The cotton wicks were soaked in a 2:1 mixture of glycerol and saline to prevent them from freezing and to help maintain good contact with the eye at low temperatures while retaining their conductivity. In order to minimize artefacts the electrodes were carefully shielded from the flash, exposing only the tips of the cotton wicks, the strobe lamp was housed in a soft steel box, and the entire equipment was housed in an electrostatically shielded cage. The remaining artefacts were negligible during the time-interval of interest. The eye could be kept at any selected temperature by placing it on top of a small copper cylinder, kept partly submerged in a thermal bath. The thermal bath consisted of an aqueous solution of calcium chloride maintained at its freezing temperature by adding dry ice. The temperature of the bath was varied by changing the calcium chloride concentration.

All the work recorded here was carried out at temperatures below about  $5^{\circ}\text{C}$ . We have previously reported<sup>3</sup> that the positive peak could be observed at temperatures

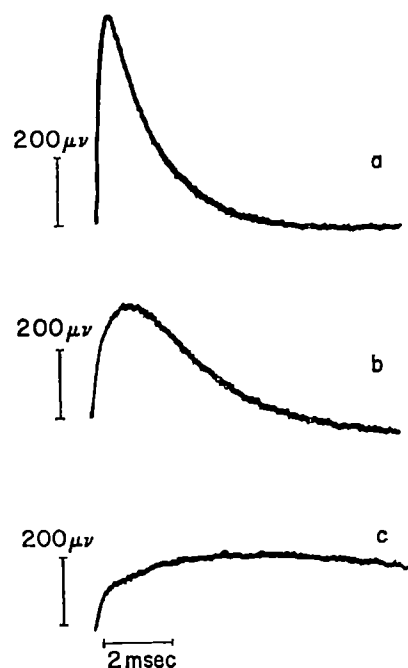


Fig. 1. Dependence on temperature of the positive peak of the early receptor potential. The positive peak of the early receptor potential observed in the ERG of the dark-adapted albino rat at various temperatures: a,  $0^{\circ}\text{C}$ ; b,  $-10^{\circ}\text{C}$ ; c,  $-20^{\circ}\text{C}$ .

as low as  $-10^{\circ}\text{C}$ . We have now extended the measurements to about  $-35^{\circ}\text{C}$ . The positive peak can still be seen at this low temperature. The signals observed at  $0^{\circ}$ ,  $-10^{\circ}$ , and  $-20^{\circ}\text{C}$  are presented in Figs. 1a, 1b and 1c, respectively. Although the signal amplitudes vary from one eye to another, there is a general tendency for the amplitude to decrease with lowering of temperature. At  $0^{\circ}\text{C}$  the amplitude of the positive peak varies roughly between  $300\ \mu\text{V}$  and  $500\ \mu\text{V}$ , and, at  $-15^{\circ}\text{C}$ , between  $100\ \mu\text{V}$  and  $300\ \mu\text{V}$ . The signals obtained at  $-35^{\circ}\text{C}$  varied between  $50\ \mu\text{V}$  and  $100\ \mu\text{V}$ .

As may be seen in Fig. 1, the amplitude maximum of the signal shifts to later and later times as the temperature is lowered. We have measured the time-interval between the start of the flash and the arrival of the amplitude maximum of the signal (peak time) at five different temperatures,  $5^{\circ}$ ,  $0^{\circ}$ ,  $-5^{\circ}$ ,  $-10^{\circ}$  and  $-15^{\circ}\text{C}$ . At  $5^{\circ}\text{C}$  the amplitude maximum is reached in about 0.4 msec, and at  $-15^{\circ}\text{C}$  the peak arrives in about 3.3 msec, giving a temperature coefficient ( $Q_{10}$ ) of about 3.

Since the duration of the stimulus flash is about 0.7 msec, there is a considerable temporal overlap between the flash and the observed response, particularly at  $0^{\circ}$  and  $5^{\circ}\text{C}$ . Therefore, the shape of the leading edge of the positive peak is probably modulated by the shape of the stimulus flash. For this reason no attempt was made to calculate the rate constants from the shape of the signal; rather, the reciprocal of the peak time was taken as a measure of the rate of the production of the positive peak. The results are shown in Fig. 2 as an Arrhenius plot. As may be seen in Fig. 2, the experimental points fall on a straight line with very good accuracy, yielding an activation energy of  $16 \pm 3\ \text{kcal/mole}$ .

As the temperature is lowered, the resistance of the cotton wick electrodes is expected to change, and possibly contact resistances and stray capacitances and inductances may change. We have investigated the possible effects of the electrodes on the measured temperature dependence of the peak time. A small piece of metal was put in contact with electrodes instead of the eye deliberately to produce a measurable photo-artefact. The time of the arrival of the peak of the artefact was measured as a function of temperature. Although the peak time of the artefact

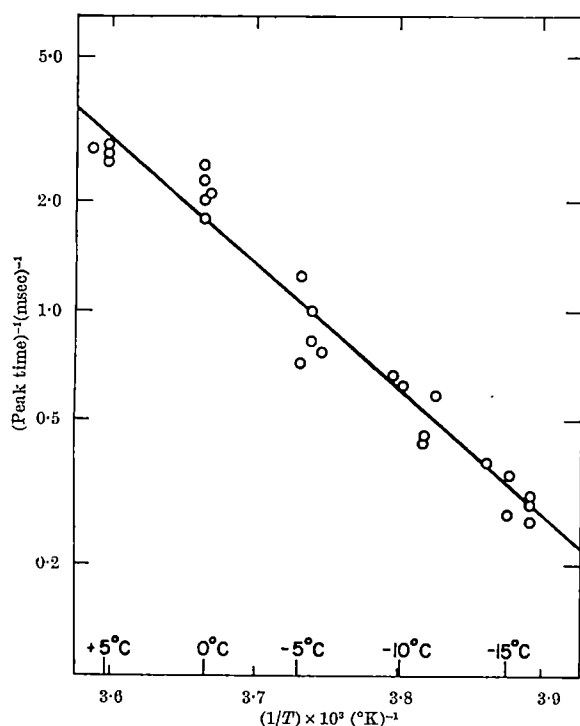


Fig. 2. Arrhenius plot of the rate of production of the positive peak. The reciprocal of the peak time of the positive peak was taken as a measure of the rate of production of the positive peak to cast the experimental points in the form of an Arrhenius plot. The peak times are expressed in msec

does change slightly with temperature, the effect is small, the temperature coefficient ( $Q_{10}$ ) being only about 1.3. If this correction is applied, we would obtain for the temperature coefficient of the positive peak a value of about 2.4, corresponding to an activation energy of about 13 kcal/mole.

The latency of the initial rise toward the positive peak is obscured by the artefacts but has been estimated to be less than 50  $\mu$ sec at 0° C. An approximate estimate of the latency at -20° C is still only about 100  $\mu$ sec.

The results of our work exclude almost conclusively the transformation of metarhodopsin I to II, the hydrolysis of metarhodopsin II, or the photo-conversion of rhodopsin to pre-lumirhodopsin as a possible mechanism for the generation of the positive peak.

Let us first consider the time-sequence of the bleaching of metarhodopsin. Probably the shortest decay time of metarhodopsin reported is due to Hagins<sup>12</sup>. In his experiments with excised rabbit eyes he estimated the half-time of decay of metarhodopsin at the physiological temperature to be about 120  $\mu$ sec. In the rat both the positive and negative peaks of the early receptor potential begin to appear long before 120  $\mu$ sec at the physiological temperature. The latency of the positive peak is less than 50  $\mu$ sec at 0° C (ref. 3) and only about 100  $\mu$ sec at -20° C. If Hagins's values for the half-time of decay of metarhodopsin at 12° and 26° C were to be extrapolated to 0° and -20° C, one would obtain about 300 msec and 70 sec, respectively. Clearly the positive peak of the early receptor potential must occur long before the decay of metarhodopsin observed by Hagins.

Furthermore, in solutions cattle metarhodopsin I goes over to metarhodopsin II with a half-time of about 1 min at 1° C and about 100 min at -9° C (ref. 6). Again this is much too slow a process to account for the production of the positive peak.

The observed temperature dependence of the rate of production of the positive peak also indicates that the process which converts metarhodopsin I to II is not responsible for the generation of the positive peak. In their investigation with cattle rhodopsin in solutions

Matthews *et al.*<sup>8</sup> obtained a temperature coefficient ( $Q_{10}$ ) of about 100 for the step going from metarhodopsin I to II. The decay of metarhodopsin Hagins examined in the intact rabbit eyes also shows a considerable temperature dependence. From his results the temperature coefficient ( $Q_{10}$ ) can be calculated to be about 8. These values are much too large to be consistent with the value,  $Q_{10} \approx 3$  we obtain for the positive peak.

Perhaps the most crucial piece of evidence we can present for rejecting the possible involvement of the decay or hydrolysis of metarhodopsin in the production of the positive peak is that the positive peak can be observed in the rat at -35° C, at which temperature both metarhodopsin I and II are known to be stable. Cattle metarhodopsin I in solutions is stable up to about -20° to -15° C (refs. 5 and 6), and metarhodopsin II remains stable up to somewhat higher temperatures<sup>6,7</sup>. Therefore, the production of the positive peak, in all probability, precedes both the decay and the hydrolysis of metarhodopsin. If the positive peak is, indeed, produced by one of the early steps of visual excitation, the fact that the production of the positive peak precedes the decay or hydrolysis of metarhodopsin would suggest that the visual excitation itself precedes these steps.

Finally, our investigation of the dependence on temperature of the peak time of the positive peak shows that the positive peak probably is not produced directly by the initial photo-isomerization of the 11-*cis* retinal to all-*trans*. The rate of formation of the positive peak, as indicated by the reciprocal of the peak time, changes by a factor of about 3 in a temperature variation of 10° C. Since the initial photo-excitation of rhodopsin requires photons of several eV, a temperature variation of 10° C (about 10<sup>-3</sup> eV) should have only a negligible effect on the rate of photo-isomerization. The fact that the rate of formation of the positive peak is sensitive to a small variation in temperature indicates that a temperature sensitive process intervenes between the action of the light on the visual pigment and the generation of the positive peak. Since the photo-isomerization of the 11-*cis* retinal to all-*trans* is thought to be represented by the step going from rhodopsin to pre-lumirhodopsin, this step is not likely to be the rate-determining step in the production of the positive peak.

We therefore conclude that the generation of the positive peak, in all likelihood, occurs after the photo-isomerization of the retinal has already taken place but before either metarhodopsin I can decay into metarhodopsin II or metarhodopsin II can hydrolyse into retinal and opsin. Thus the generation of the positive peak probably takes place at some step intermediate between pre-lumirhodopsin and metarhodopsin I.

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## LETTERS TO THE EDITOR

## RADIO ASTRONOMY

## A New Class of Radio Source Spectra

THE flux densities of 35 non-thermal radio sources were measured at 8,000 Mc/s ( $\lambda$  3.75 cm) with the University of Michigan's 85-ft. reflector. Comparison of these measurements with the spectra of the sources at frequencies less than 3,200 Mc/s (ref. 1) showed that most spectra were either straight (constant spectral index) or curved downward (steepening spectrum) at the high-frequency end of the spectrum. However, the radio source 3C 84 which has been identified with the Seyfert galaxy NGC 1275 (refs. 2 and 3) was found to have a flux density at 8,000 Mc/s which was four times greater than that expected from a straight line extrapolation of the spectrum observed at frequencies less than 3,200 Mc/s. This is the first reported instance of a radio spectrum having such a pronounced upward turn in its spectrum with increasing frequency. (The novel spectrum of NGC 1275 was first reported by us at the International Scientific Radio Union General Assembly in Tokyo, September 9-20, 1963.) Thus NGC 1275 may represent a new class of radio source spectra. The radio source 3C 279 was also investigated because a pronounced upward turn of its spectrum was predicted by J. A. Roberts from measurements up to 2,650 Mc/s; we found its spectrum to be similar to NGC 1275. A third source 3C 273 has a spectral flattening at the high frequency end, but does not have a positive spectral index as do the foregoing two sources.

In order to confirm the upward (positive) curvature in the spectra of these sources at centimetre wave-lengths, flux density measurements were also made at 16,500 Mc/s ( $\lambda$  1.82 cm). Table 1 lists the measured flux densities and their standard deviations of NGC 1275, 3C 279, and 3C 273 at 8,000 Mc/s and 16,500 Mc/s along with the flux density measurements of Virgo A, Taurus A, and Cassiopeia A which are given for reference. All these sources were found to be within 1 min of arc of their expected positions.

In these measurements account has been taken of the finite angular size of the source, the variation of the antenna gain with zenith angle, a small daily antenna gain variation, atmospheric extinction, and the measured linear polarization of the sources<sup>4</sup>. The flux density calibration was made relative to an absolute flux density measurement of Taurus A at 9,400 Mc/s (ref. 5), assuming a spectral index of  $-0.26$ . Fig. 1 shows the spectra of NGC 1275, 3C 279, 3C 273, and Virgo A extended to 8,000 Mc/s and 16,500 Mc/s from measurements made at frequencies less than 3,200 Mc/s (refs. 1 and 6). The ordinate of each spectrum has been arbitrarily displaced to give a compact presentation.

The low-frequency spectrum of NGC 1275 has been discussed by several authors<sup>7-9</sup>. Baars, Mezger and Wendker<sup>10</sup> have recently made flux density measurements of NGC 1275 at five frequencies including 5,000 Mc/s and 14,500 Mc/s. Their observations confirm the upward turn in the spectrum of NGC 1275 reported here.

Table 1. MEASURED FLUX DENSITIES AT 8,000 Mc/s AND 16,500 Mc/s

Source designation	Flux density and standard deviation in units of $10^{-26}$ W m <sup>-2</sup> (c/s) <sup>-1</sup> (Epoch 1964-C)	
	8,000 Mc/s	16,500 Mc/s
NGC 1275 or 3C 84	$18.8 \pm 1.0$	$23.6 \pm 2.5$
3C 279	$15.6 \pm 0.8$	$15.4 \pm 1.5$
3C 273	$26.9 \pm 1.3$	$23.3 \pm 2.0$
Virgo A	$45.0 \pm 2.7$	$26.7 \pm 4.2$
Taurus A	$570 \pm 28$	$477 \pm 44$
Cassiopeia A	$580 \pm 29$	$332 \pm 27$

A flux density of NGC 1275 at 8,000 Mc/s was published by Heesch<sup>11</sup>, but the pronounced upward curvature in the spectrum was not found due to the inaccuracy of his measurement. We find an abrupt change in the spectral index from  $-0.67$  below 3,200 Mc/s to  $+0.84$  between 3,200 Mc/s and 8,000 Mc/s. Such a large positive spectral index cannot be produced by synchrotron radiation from any energy distribution of relativistic electrons when absorption processes are not present.

If the low-frequency spectrum of NGC 1275 is linearly extrapolated to high frequencies and subtracted from the observed composite spectrum in Fig. 1, an isolated spectrum of a high-frequency component of the source is presumably obtained (Fig. 2). This spectrum has a spectral index of about  $+2$  at frequencies less than 8,000 Mc/s and is nearly flat at frequencies greater than 8,000 Mc/s. Although models can be made involving absorption of a synchrotron spectrum, this spectrum could be produced by thermal radiation of free-free transitions in a region of ionized hydrogen that becomes opaque near 8,000 Mc/s. The angular diameter of the high-frequency spectral component of NGC 1275 has not been measured. An upper limit of  $90''$  of arc can be inferred from our measurements. If we assume that the diameter of the high-frequency component is  $10''$  of arc, the same as the low-frequency spectral component<sup>12</sup>, and that the high-frequency component is a thermal source with some unknown ionization process, then the electron temperature,  $T_e$ , of the gas is  $4,500^\circ$  K. If the source, at a

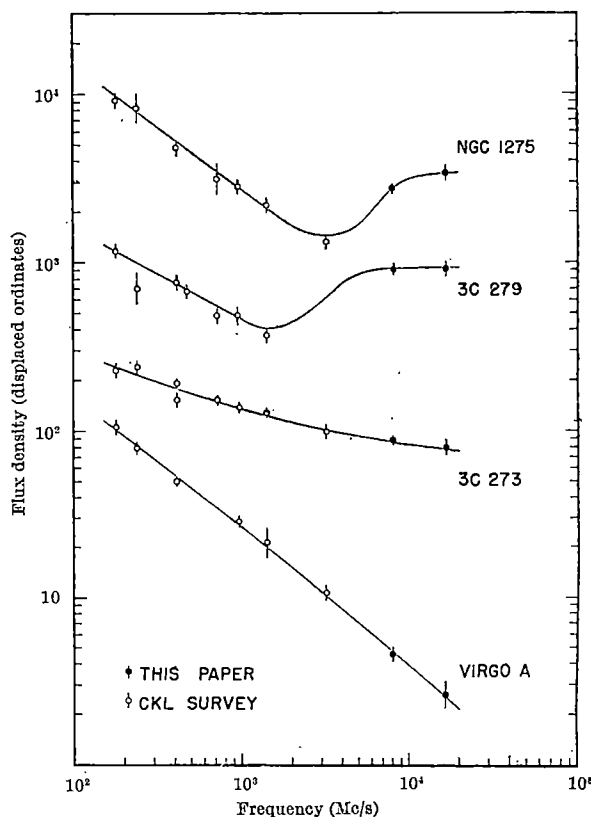


Fig. 1. The high-frequency radio spectra of four sources showing the novel spectral features at 8,000 Mc/s and 16,500 Mc/s of NGC 1275 and 3C 279 in contrast to the normal non-thermal spectrum of Virgo A. The ordinate of each spectrum has been arbitrarily displaced

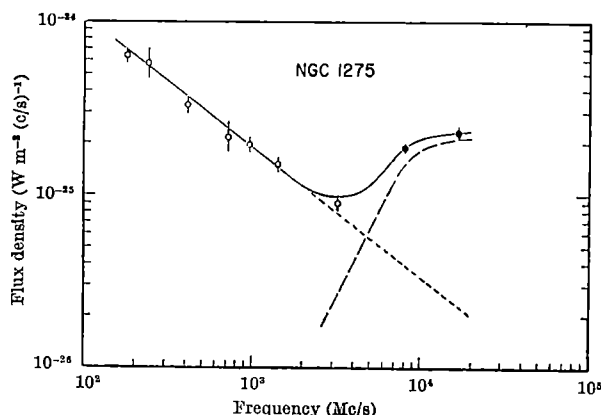


Fig. 2. The observed spectrum of NGC 1275 showing a possible separation into high- and low-frequency spectral components

distance of 54 Mpc (ref. 13), consists of a uniform spherical distribution of thermal electrons, then the electron density is  $170 \text{ cm}^{-3}$  and the total mass,  $M$ , is  $4 \times 10^{10} M_{\odot}$  (about one-tenth the mass of the galaxy). The source is 2,600 parsecs in diameter, presumably located at the centre of the 30 kpc diameter galaxy. It should be noted that the values of the above parameters are sensitive to the adopted angular diameter,  $\phi$ , and the distance,  $R$ , of the source:  $T_e \propto \phi^{-2}$  and  $M \propto R^{-2.5} \phi$ .

The fact that the high-frequency component of NGC 1275 appears to be partially polarized at 8,000 Mc/s (ref. 4) is not evidence against the thermal model since the degree of polarization obtained,  $0.5 \pm 0.4$  per cent, is at the threshold of detection and, in any event, may be attributed to the low-frequency component of the source.

The predicted free-free and Paschen continua at optical frequencies from the above thermal model is not inconsistent with the optical magnitude of the galaxy. However, on the basis of a photographic estimate by Seyfert<sup>14</sup> the integrated flux density of the  $H_{\beta}$  line is a few orders of magnitude less than the  $4 \times 10^{-12} \text{ W m}^{-2}$  predicted for the hydrogen recombination spectrum of the foregoing model. The ratio  $(\lambda 5007 + \lambda 4959)/\lambda 4363$  of [OIII] (ref. 14) is 8.8 in NGC 1275 compared with about 120 in planetary nebulae. An electron density of  $10^7 \text{ cm}^{-3}$  assuming  $T_e \sim 10^4 \text{ }^{\circ}\text{K}$  is required to produce such a ratio by collisional excitation.

The foregoing observed value of the [OIII] line ratio can be made consistent with a thermal interpretation of the radio spectrum on NGC 1275 if  $T_e$  is  $1.5 \times 10^6 \text{ }^{\circ}\text{K}$  and  $N_e$  equals  $6 \times 10^4 \text{ cm}^{-3}$ . To fit the opaque portion of the thermal radio spectrum the diameter of the radio source must be  $0.54''$  or 136 parsecs. The corresponding total mass of ionized hydrogen is  $2 \times 10^9 M_{\odot}$ , which is substantially lower than obtained in the first model. The predicted  $H_{\beta}$  flux in the second model is about  $10^{-13} \text{ W m}^{-2}$  and is within about an order of magnitude of existing optical data<sup>14</sup>. We feel that the value of  $1.5 \times 10^6 \text{ }^{\circ}\text{K}$  for the electron temperature may not be unreasonably high in view of the unusual optical spectrum and fragmented appearance of the galaxy.

Photoelectric measurements of the  $H_{\beta}$  flux of the nucleus of NGC 1275 and future angular diameter measurements of the radio source at frequencies greater than 8,000 Mc/s will be able to test the above two models.

It is also possible that the high-frequency component of NGC 1275 is produced by a relatively flat synchrotron radiation spectrum similar to that of 3C 273B but with a low-frequency spectral cut-off near 8,000 Mc/s due to self-absorption. In this case the spectral index below this frequency would be  $+2.5$ , and this is not inconsistent with the observations. However, synchrotron self-absorption at this high frequency requires exceptionally large magnetic fields; greater than 1,000 gauss if the radio source is larger than 1 parsec in diameter.

Little is known about the radio source 3C 279 since it has not been optically identified. If the high and low

frequency spectral components are separated as was done for NGC 1275, then the spectrum of the high-frequency component is also found to have a thermal-type radio spectrum. A  $10''$  of arc angular diameter for this component of 3C 279 would be required if the electron temperature is  $10^4 \text{ }^{\circ}\text{K}$  and the source is a thermally emitting ionized gas. Angular diameter measurements have been reported for the low-frequency component only:  $20''$  at 158 Mc/s (ref. 15) and less than  $36''$  at 960 Mc/s (ref. 16).

The radio source 3C 273 has been shown<sup>17</sup> to consist of two radio components separated by  $20''$  of arc. Component A, identified optically with a jet of emission<sup>18</sup>, has a steep non-thermal spectrum similar to Virgo A. Component B, identified with a quasi-stellar radio source<sup>18</sup>, has a nearly flat spectrum. The ratios of the flux densities of each component are such that the steep spectrum of component A dominates the observed composite spectrum at frequencies below 1,000 Mc/s while the nearly flat spectrum of component B contributes principally to the total flux density at frequencies greater than 1,000 Mc/s. This difference in the spectral index of each component can explain the positive curvature of the composite spectrum over the frequency range of observations, 178 Mc/s to 16,500 Mc/s.

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## A Radio Investigation of NGC 1265 and NGC 1275

Dent and Haddock<sup>1</sup> have investigated the high-frequency part of the NGC 1275 spectrum and found that the flux density increases between 3 and 10 GHz by about a factor of two.

Earlier low-frequency flux density measurements of NGC 1275 are obscured by the fact that another radio source, NGC 1265, is spaced only half a degree from NGC 1275. Lynds and Sobieski<sup>2</sup> took a mean value of the flux density ratio of NGC 1275 and NGC 1265, measured by Leslie and Elmore<sup>3</sup> at 178 MHz, and their own ratio at 3 GHz to separate the flux densities at intermediate frequencies measured with telescopes of low angular resolution.

Present-day instrumental facilities allow the removal of uncertainties in this method by direct measurement. In order to separate the flux densities of NGC 1265 and NGC 1275 below 3 GHz and to check the feature found by Dent and Haddock<sup>1</sup> in the high-frequency region, we present here a set of new flux-density measurements of both sources. At the frequencies 750 MHz and 1410

MHz, the National Radio Astronomy Observatory (NRAO) 300-ft. telescope was used; at 2.7 GHz, 5 GHz and 14.5 GHz we used the NRAO 85-ft. telescope. The angular resolution of the telescopes is in each case high enough to allow a clear separation of the flux densities of both sources. The flux densities were measured relative to either Cassiopeia A, Taurus A or Virgo A, whichever was more convenient for a particular observation. Some earlier measurements at high frequencies<sup>1,2,4</sup> and the interferometer measurement by Leslie and Elsmore<sup>3</sup> were combined with our own measurements. All these observations also give a clear separation between NGC 1265 and NGC 1275. Our recently analysed high-frequency spectra of the strongest non-thermal radio sources<sup>5</sup> were used to convert the relative flux density measurements into flux densities. The flux densities used for the spectra of NGC 1265 and NGC 1275 are given in Table 1.

Frequency (MHz)	NGC 1275	NGC 1265	Ref.
178	41.0 ± 10% (m.e.)	15.5 ± 12% (m.e.)	3
750	22.9 ± 7.5%	11.5 ± 10%	—
1,400	13.0 ± 12%	6.3 ± 15%	—
2,700	8.7 ± 5%	—	—
3,000	8.7 ± 5.3%	3.7 ± 11%	2
3,000	8.9 ± 5.8%	—	4
5,000	10.7 ± 10%	< 2.5	1
8,000	18.8 ± 5%	—	—
14,500	18.2 ± 12%	—	—
16,500	23.6 ± 9%	—	1

The values are also shown in Fig. 1. The mean errors of the individual measurements are indicated by error bars. The spectrum of both sources steepens between 178 MHz and 3 GHz. But whereas the flux density of NGC 1265 is below our detection limit at 5 GHz, the spectrum of NGC 1275 shows the already mentioned increasing flux density above 3 GHz. This behaviour of the high-frequency part of the NGC 1275 spectrum has been discussed extensively by Dent and Haddock<sup>1</sup>.

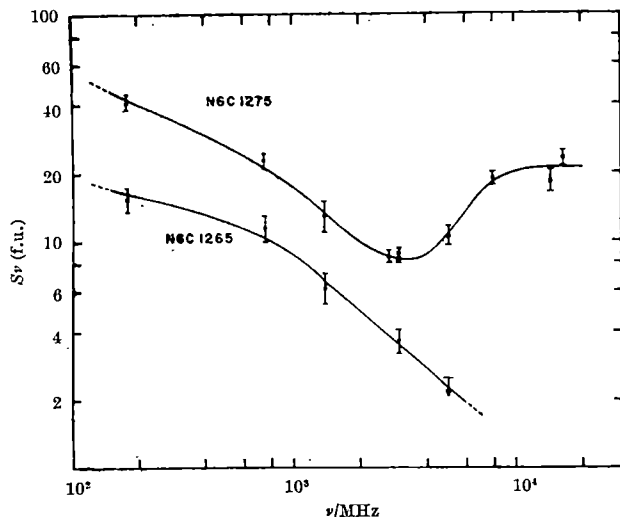


Fig. 1. Spectra of NGC 1275 and NGC 1265

As NGC 1275 is classified as a Seyfert galaxy, we have looked at the other Seyfert galaxies at 5 GHz and 14.5 GHz. In all cases the results were negative. Our detection limit was 5 f.u. and 20 f.u., respectively.

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## PHYSICS

### The Linear Temperature Scale

THE Kelvin temperature scale used at present is linear, forming an ordered sequence of numbers:

$$+ 0^\circ \text{K} \dots 273.16^\circ \text{K} \dots + \infty^\circ \text{K} \quad (1)$$

for all normal thermodynamic states. If the so-called 'abnormal' thermodynamic states are admitted, the foregoing thermodynamic scale can be extended by adding a sequence of negative temperatures<sup>1,2</sup>. Alternatively, if the following relation is introduced:

$$T = e^\psi \quad (2)$$

where  $\psi = \int g(\theta) d\theta$  and depends on the thermal properties ( $\theta$ ) of the system (refs. 3, 4, 5);  $T$ , absolute temperature ( $^\circ\text{K}$ ); it is possible to construct another temperature scale, the  $\psi$ -scale, or the logarithmic scale. Fig. 1 shows the relation between both these temperature scales.

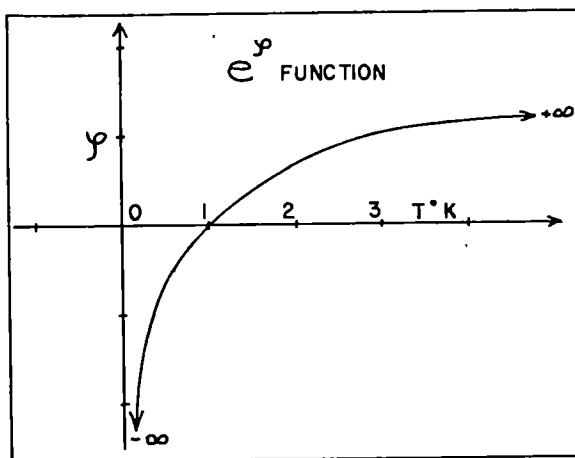


Fig. 1. Relation between temperature scales

The logarithmic nature of the  $\psi$ -function assures that the zero value on the linear absolute scale ( $T$ -scale) will be reached asymptotically, that is,  $T = 0^\circ \text{K}$ , when  $\psi = -\infty$ .

Comparing both these scales (the linear scale and its explicit<sup>4,5</sup> functional form, the  $\psi$ -scale), the inaccessibility of the absolute zero<sup>6,7</sup> follows generically, being not imaginable from the linear  $T$ -scale alone.

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## METEOROLOGY

### An Experimental Determination of the Atmospheric Temperature Profile by Indirect Means

AN article by Kaplan<sup>1</sup> suggested a method of obtaining indirectly the temperature profile of the atmosphere by measuring from a satellite the radiances at several wavelengths in the 15- $\mu$  band of carbon dioxide. To test the practical application of this proposal the U.S. Weather Bureau has developed a grating spectrometer<sup>2</sup> with fixed exit slits and detectors to measure simultaneously the



radiances in 5  $\text{cm}^{-1}$  intervals centred at 669, 677.5, 691, 697, 703 and 709  $\text{cm}^{-1}$  and a 7  $\text{cm}^{-1}$  interval centred at 899  $\text{cm}^{-1}$ .

A 3.2 million cubic ft. balloon ('Dacron-mylar' scrim) carrying the spectrometer was flown by the National Scientific Balloon Flight Station of N.C.A.R. at Palestine, Texas, during the daylight hours of September 11, 1964. The instrument, which has a viewing angle of  $12^\circ$ , was pointed vertically downward, and measured the differences between the atmospheric radiances and those from a 77° K black body.

The balloon reached a maximum altitude of 103,000 ft. and remained above 100,000 ft. for 7.5 h. At the beginning of the flight and at its termination 250 miles to the west, the sky was cloudless in the field of view. Through most of the day there were cumulus clouds, and at one time the balloon passed over a squall area which had clouds extending to about 35,000 ft. and which completely filled the field of view of the spectrometer. The digitized measurements were transmitted by a telemetry-command system furnished by the N.C.A.R. We believe that the flight data were accurate to better than 0.5  $\text{erg/cm}^2 \text{ sec strdn cm}^{-1}$  (about 1 per cent of the signals).

Fundamental to the determination of the temperature profile is an accurate knowledge of the atmospheric transmittance by carbon dioxide in each of the spectral intervals. These were calculated from theory and from parameters determined experimentally in our laboratory, and from the well-supported assumption<sup>3</sup> that carbon dioxide is uniformly mixed in the atmosphere at about 0.031 per cent by volume. It is also vital that the layers of the atmosphere contributing in the various spectral intervals overlap to a considerable extent; but it is likewise important to the stability of the solution that a significant degree of independence remains.

The set of simultaneous equations expressing the measurements in terms of the integral form of the radiative transfer equation may be linearized. Using a minimization technique developed by our group this set may be solved to give the vertical temperature profile.

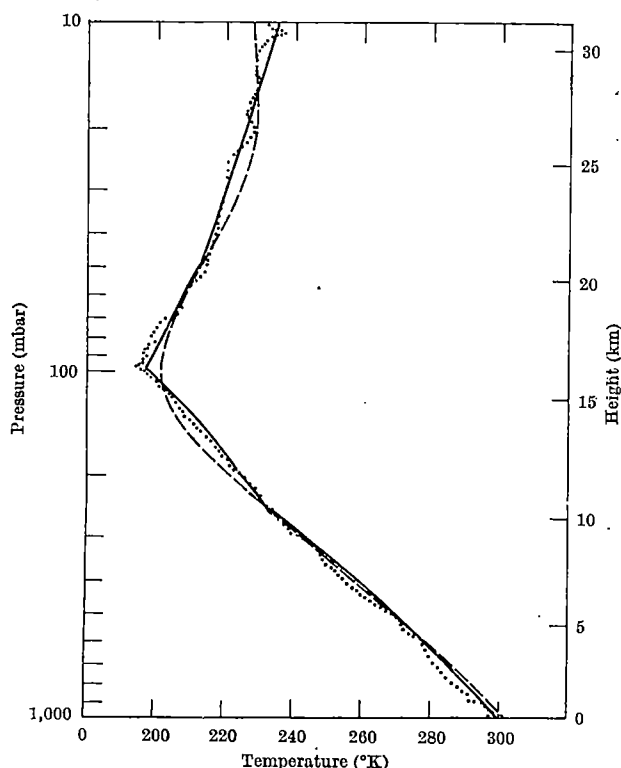


Fig. 1. Temperature profile from radiosondes (dotted line), connected line segment series solution (solid line), trigonometric series solution (dashed line) from data taken with no clouds in the spectrometer's field of view.

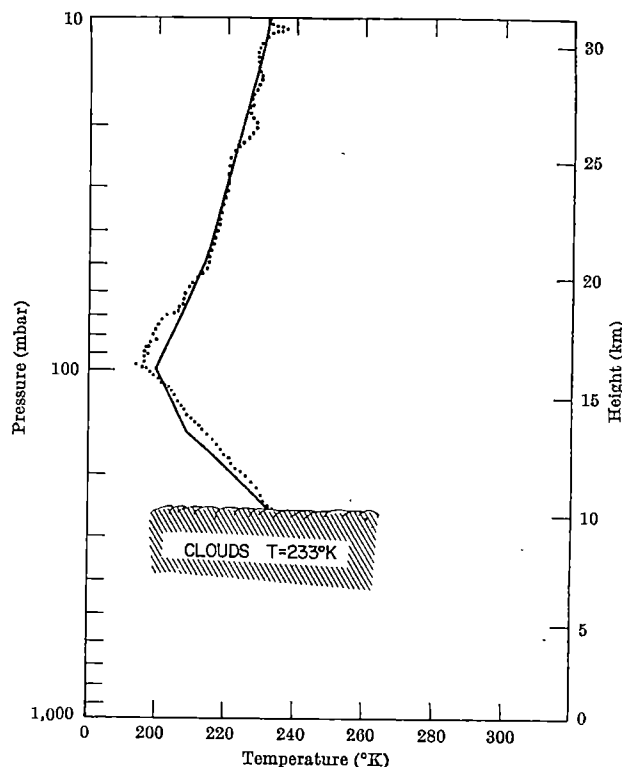


Fig. 2. Temperature profile from radiosondes later in the day (dotted line), connected line segment series solution (solid line) from data taken with clouds filling the instrument's field.

Because of an uncertainty concerning the calibration of the 669  $\text{cm}^{-1}$  flight data they were not used in the calculations; we would expect the inversion of the integral equation to result in a more stable and accurate solution if all six spectral intervals had been used. Results of our computations using only five intervals are shown in Figs. 1 and 2.

Fig. 1 shows the temperature profile determined from radiosonde data (dotted line) early in the flight, when the sky was clear and the entire atmosphere below the instrument contributed to the radiances. Two solutions are given: a series of connected line segments (solid line); and a trigonometric series (dashed line). Each of these two solutions has advantages and disadvantages. The trigonometric series is free to choose levels where changes of slope occur; but it cannot follow sharp changes in slope. The line segments can follow slope discontinuities, but the end points cannot be explicitly determined. In this case, the end points were deliberately selected to show how closely it is possible to approximate the profile with line segments.

Fig. 2 shows the temperature profile (dotted line) above the squall line. In this case the clouds were opaque and the temperature at the top of the cloud was found from the 899  $\text{cm}^{-1}$  results to be about 233° K. The solution in this case requires that the contributions by the cloud to the radiances be subtracted; that is, the contribution by the atmosphere alone must be determined. It is therefore a matter of successive approximations to determine the height of the cloud by having the solution coincide with the cloud temperature, which is already known. This process converges rapidly, inasmuch as there is already, even from climatology, a fair approximation to the height. Also in this solution we have determined the height of the tropopause from the trigonometric series solution, and then performed a line segment solution, spacing the other points so that the information content of the results was equally distributed according to the transmittance functions.

We conclude from our measurements that Kaplan's original suggestion is a practical proposition, and that

with satellite observations of comparable accuracy it should be possible to obtain meteorologically usable atmospheric temperature profiles. The U.S. Weather Bureau on the basis of these results has undertaken continued development of the instrument for a satellite experiment.

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## GEOLOGY

### Age Determinations on Three African Carbonatites

CARBONATITES have been a normal part of the magmatic history of east, central and southern Africa over a long period of geological time. They range in age from those associated with Tertiary volcanoes of the Elgon group to the pre-Karoo carbonatites of Spitzkop and Loolekop in the north-eastern Transvaal. The salient features of most of the known African carbonatites have been summarized by Campbell Smith<sup>1</sup>.

Table 1. POTASSIUM-ARGON AGE DETERMINATIONS

Nature and location of sample	K (%)	Radiogenic <sup>40</sup> Ar	Age (m.yr.)
Mbeya	6.51	0.054 p.p.m.	113 ± 6
Kangankunde	7.59	0.069 p.p.m.	123 ± 6
Nkumbwa Hill	7.94	0.460 p.p.m.	680 ± 25

Decay constants:  $\lambda\beta = 4.72 \times 10^{-10} \text{ yr.}^{-1}$ ,  $\lambda\alpha = 0.584 \times 10^{-10} \text{ yr.}^{-1}$ .  
Isotopic abundance of <sup>40</sup>K = 0.0119 atom per cent.

Table 2. URANIUM-LEAD AGE DETERMINATIONS, MBEYA CARBONATITE

Sample	%U	%Th	%Pb	<sup>204</sup> Pb	<sup>206</sup> Pb	<sup>207</sup> Pb	<sup>208</sup> Pb	<sup>206</sup> Pb/ <sup>238</sup> U	<sup>207</sup> Pb/ <sup>235</sup> U	<sup>206</sup> Pb/ <sup>232</sup> Th
Pyrochlore, Museum Zone	0.022	1.60	0.081	1.17	21.63	18.32	58.68	} 73 m.y.	147 m.y.	185 m.y.
Pyrochlore, Chloritic Zone	0.311	0.71	0.090	1.27	26.47	20.17	52.09			

Decay constants: <sup>238</sup>U =  $1.569 \times 10^{-10} \text{ yr.}^{-1}$ ; <sup>235</sup>U =  $9.7216 \times 10^{-10} \text{ yr.}^{-1}$ ; <sup>232</sup>Th =  $4.8813 \times 10^{-11} \text{ yr.}^{-1}$ ; <sup>238</sup>U/<sup>235</sup>U = 137.7.

This communication gives the results of three K-Ar age determinations on micas from the carbonatites of Panda Hill (Mbeya, Tanganyika), Kangankunde (Chilwa Series, Nyasaland), and Nkumbwa Hill (Northern Rhodesia).

Mica concentrates were prepared using magnetic methods of separation so far as possible. Potassium was determined with an Eel flame photometer; argon was determined by isotope dilution using a 4½ in. radius, 60° Reynolds-type glass mass spectrometer. The argon was extracted by fusion of the mica sample in a glass vacuum system attached directly to the mass spectrometer. The calculated ages are believed to have an uncertainty of no more than ± 4 per cent (95 per cent confidence level).

*The Mbeya carbonatite.* Originally called the Panda Hill pyrochlore deposit, this carbonatite is 13 miles W.S.W. of Mbeya, near the south-west border of Tanganyika. It has been described in detail by Fawley and James<sup>2</sup>. The complex consists of a central plug and steeply inward-dipping outer ring of carbonatite and includes tuffs and agglomerates and an explosion-vent breccia; it also has a pyrochlore-rich zone with the form of an incomplete ring dyke. Much of the structure has been complicated by metasomatic and tectonic activity.

The carbonatite is overlain by Cretaceous sediments, and in the nearby Songwe River valley Karroo sediments are brecciated by minor explosive vents thought to be of the same age as the Panda Hill volcanicity. Thus, geo-

logical evidence indicated a Jurassic or Cretaceous age. The K-Ar age determination gives an age of 113 million years, which in terms of present estimates of the geological time scale corresponds to the Cenomanian stage at the base of the Upper Cretaceous (Table 1).

Age determinations have also been made by the uranium-lead method on two samples of pyrochlore from this carbonatite complex by Schurmann *et al.*<sup>3</sup>. The ages as calculated are discordant and range from 68 to 273 m.yr. However, it is impossible to tell from Schurmann's results whether the discordance reflects chemical alteration or is due to the use of the wrong isotope ratios when correcting for common lead contamination. Fortunately, since two samples have been analysed which can safely be assumed to be of the same age, and were in all probability contaminated by common lead having the same isotopic composition, it is possible to calculate ages independent of any assumptions regarding the isotopic composition of the common lead component. These ages are listed in Table 2 and are still discordant. Thus, it must be assumed that one or both of these pyrochlores have suffered chemical alteration and no other geological significance can be attached to these results.

*The Kangankunde carbonatite.* Kangankunde Hill, formed by an erosion-resistant carbonatite complex, is 50 miles west of Lake Chilwa in the Lower Shire area of southern Nyasaland. This ankeritic carbonatite complex is a member of the Chilwa Series (Dixey *et al.*<sup>4</sup>). The Chilwa Series comprises a group of vents, pipe-like in form, infilled by carbonatite and/or feldspathic breccia; syenite and nepheline-syenite intrusions; and a varied suite of minor intrusions, mainly in the form of dykes. The largest of these vents, namely, Muambe, intersects Karroo sediments and is in part overlain by the Lupata sediments and volcanics of Cretaceous age. The K-Ar age as determined on a phlogopite corresponds to the Albian stage of the Lower Cretaceous.

*The Nkumbwa Hill carbonatite.* This complex forms a hill of limestone rising more than 1,000 ft. above the

surrounding country in the Isoka district of Northern Rhodesia (Reeve and Deans<sup>5</sup>). The carbonatite cuts biotite-gneisses of unknown age. The K-Ar age determination on phlogopite from adjacent metasomatized country rocks indicates a late Precambrian time of emplacement.

*Conclusions.* Age determinations on the Mbeya and Kangankunde carbonatites give results indicating emplacement during the Cretaceous. Such geological evidence as is available indicates an Upper Jurassic or Lower Cretaceous age. Bearing in mind the experimental errors and the possibility that one or both of the analysed micas may have suffered slight argon loss, it is reasonable to suggest that these complexes are actually of the same age. In contrast, the Nkumbwa Hill carbonatite is much older.

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<sup>1</sup> Campbell Smith, W., *Quart. J. Geol. Soc. Lond.*, **112**, 189 (1956).

<sup>2</sup> Fawley, A. P., and James, T. C., *Econ. Geol.*, **50**, 571 (1955).

<sup>3</sup> Schurmann, H. M. E., Aten, A. H. W., Boerboom, A. J. H., Bot, A. C. W. C., Conwenberg, G., Dance, D. F., Hurley, P. M., Ledent, D., Stauffer, H., Steensma, J. J. S., and Suringa, R., *Geol. en Mijnbouw*, **22**, 93 (1960).

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## CHEMISTRY

## Phototropic Enzyme-dye Complexes

THE term 'phototropy' has been applied to the phenomenon of a reversible change of colour induced by coloured lights, as in fulgides, and the change in or loss of colour of dye-stuffs in light of specific wave-length. The term has been used by chemists, botanists, biologists, and biochemists for many years to explain this phenomenon of change in colour of solids and solutions. Several reviews on the subject have been published<sup>1-6</sup>. A number of investigators have attempted to interpret the cause of phototropy in solutions<sup>7,8</sup>. The dyes most commonly used to explain the mechanism of this phenomenon have been the leucocyanides. The phototropy of malachite green leucocyanide has been investigated in dry alcohol and also in mixtures of alcohol and water by a number of authors<sup>9-15</sup>.

The phototropic triphenylmethane dyes are capable of reactions with quantum yields approaching unity in some solvents. Very high extinction coefficients and broad spectral coverage are characteristic properties. However, these dyes exhibit slow reversal time from coloured to colourless state and fatigue during cyclic exposure.

An investigation was conducted to determine the effect of enzymes on the phototropic behaviour of triphenylmethane dyes. It was found that triphenylmethane dyes and certain enzymes form phototropic systems which demonstrate unique properties in changing from a colourless to a highly coloured state under the influence of ultra-violet light. The unique properties demonstrated by this system are resistance to cyclic

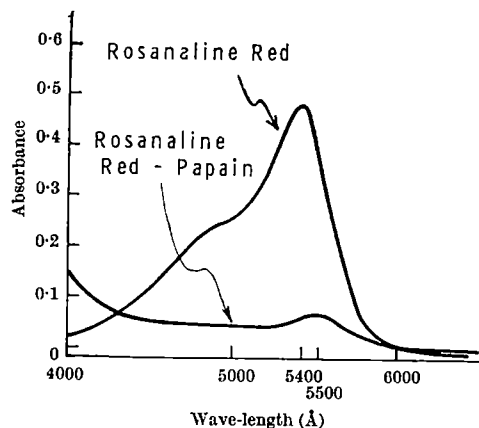


Fig. 1

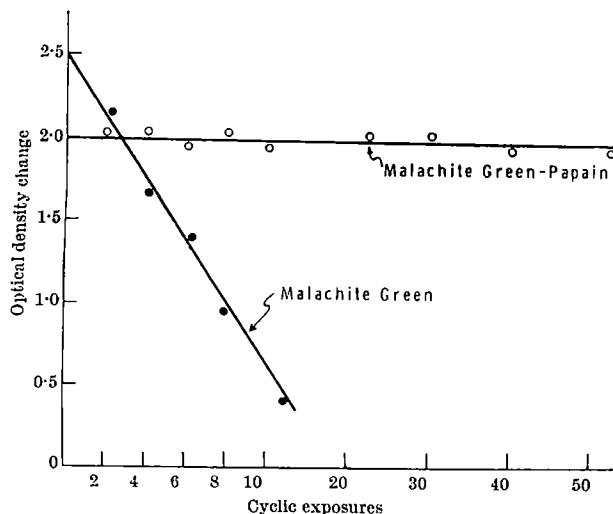


Fig. 2

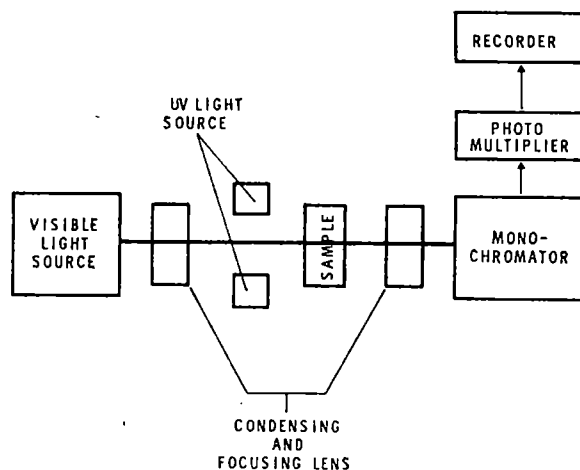


Fig. 3. Flash photolysis unit

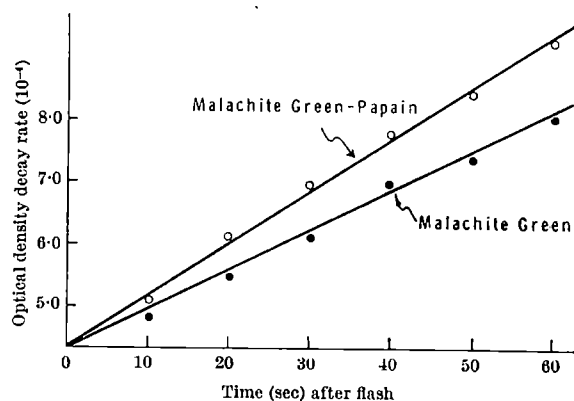


Fig. 4

fatigue, ultra-fast reaction to ultra-violet light stimulus, and reversibility of colour when the source of light is removed. This system is effective as a filter for high-intensity, ultra-violet activated light sources.

Solutions of rosaniline red and malachite green were prepared as follows (in g): dye, 0.02; papain, 0.20;  $\text{NaHSO}_3$ , 0.40; water, 100.

Rosaniline red shows a 100 Å shift (from 5400 Å to 5500 Å) in peak absorption when combined with papain, when measured on a Cary model 14 spectrophotometer, indicating the possible formation of a complex (Fig. 1). Malachite green-papain solutions exhibit a peak intensity at 6180 Å, but little perceptible shift.

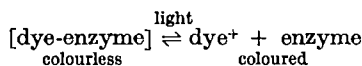
To determine the effect of papain on cyclic fatigue resistance, solutions of malachite green with and without papain were exposed to steady-state illumination of a 275-W ultra-violet lamp for 10 sec periodically, with optical densities measured before and after each exposure in a Bausch and Lomb photometer. Fig. 2 shows that the malachite green-papain solution resists cyclic fatigue after 50 cycles whereas malachite green alone shows a steady deterioration in the ability to resist cyclic fatigue.

Response time to achieve maximum optical density of the malachite green-papain solution was measured on a special flash photolysis unit (constructed by the National Cash Register Company, Dayton, Ohio; Fig. 3). The 200-W-sec ultra-violet flash source attains peak intensity in 25  $\mu\text{sec}$ , with a 50 per cent fall-off from peak intensity in 65  $\mu\text{sec}$  from zero time. Maximum optical density of the malachite green-papain solution was attained in 25  $\mu\text{sec}$ . This response time is independent of either the dye or enzyme concentration. However, the degree of optical density is dependent on light intensity and dye concentration.



Reverse reaction rates (that is, colour decay) were found to be influenced by the pH of the solution, bisulphite concentration and the presence of papain. Another experiment conducted with a malachite green-papain solution at a pH of 7.1 showed definite increase of colour decay rate in the presence of papain as shown in Fig. 4.

It is postulated that an enzyme-dye complex is formed as a first step and one or more additional steps would take place on irradiation until the dye cations were produced, as represented as follows:



The presence of radiant energy in the process may, of course, alter the role of the enzyme as a catalyst, although it should be noted that peak absorption wave-length of papain lies in the ultra-violet range which is beyond the 3000 Å–4000 Å peaks intensity of the light source used in the work recorded here. If the enzyme were acting in some capacity other than as a catalyst, it might be retaining the cation and anion after photo-disassociation in close proximity to each other, thus acting as a cage. Needless to say, these postulations are greatly simplified and may not represent the true mechanism of the process.

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## A New Physical Constant for Solutes

A RELATIONSHIP was established earlier between the dispersion ratio (a measure of the property of gradation of density of the solute in a frozen and thawed column of solution) and the specific gravity of the solute<sup>1</sup>. At the same time it was found that there was no correlation between the dispersion ratio and the molecular weight of the solute, and attention was directed to the significance of this.

Further investigations have now revealed a neat relationship between the mole fraction of the solute and its dispersion ratio. The relevant data obtained in the experiment on a mixture of solutes given in the table in the last communication<sup>1</sup>, plotted on this basis, are shown in the curve in Fig. 1 (a).

The relationship, further, becomes practically linear when the two factors are correlated, as in the graph in Fig. 1 (b), in terms of their logarithms.

This finding has been confirmed by an experiment on the same single solute. Potassium permanganate solutions, in a rising series of initial concentrations, were frozen and thawed in 250 ml. cylinders, and the dispersion ratio determined as before. The direct variation of the logarithm of the dispersion ratio with that of the mole fraction of the solute in the series is brought out in Fig. 2.

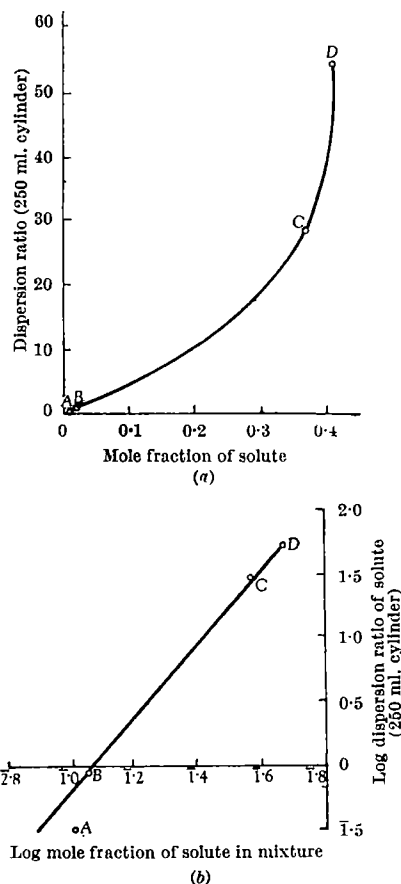


Fig. 1. A,  $K_2SO_4$ ; B,  $Al_2(SO_4)_3$ ; C,  $CuSO_4$ ; D,  $FeSO_4$ .

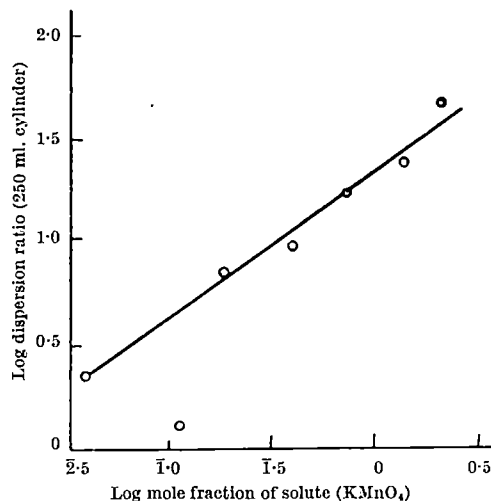


Fig. 2

The rise of the dispersion ratio with the initial concentration of the solute, for the series of concentrations tried in the experiment, fits into the simple equation:

$$Y = Mx$$

where  $Y$  is the logarithm of dispersion ratio;  $x$ , the logarithm of mole fraction of solute;  $M$ , the constant of proportionality.

For potassium permanganate, the constant of proportionality, which may be termed the 'cryoscopic effect constant' (as distinct from the cryoscopic constant<sup>2</sup>), was found to be about 0.71 in this experiment. With the standardization and refinement of the method of determination of the dispersion ratio, the value of the constant will become more exact.

These findings lead to the inference that the 'cryoscopic effect constant', like the dispersion ratio, can be expected to be characteristic of the solute. A comparative investigation of the constant for various categories of solutes and for various solvents, as well as their correlation with other constants of the solute, will perhaps throw more light on the properties of matter.

I wish to thank Mrs. Parvathy Rangappa and Mr. R. N. Sood for assistance in these experiments.

K. S. RANGAPPA

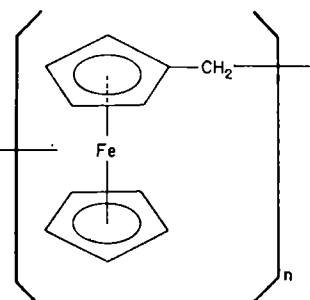
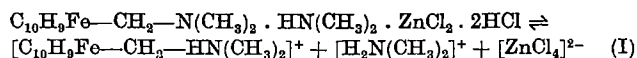
Central Water and Power Commission,  
New Delhi, India.

<sup>1</sup> Rangappa, K. S., *Nature*, **198**, 682 (1963).

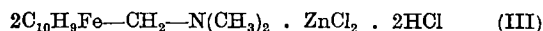
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### Ferrocene-containing Polymers: Intermediary Complex Formation in the Polycondensation of *N,N*-Dimethylaminomethylferrocene

In an earlier paper<sup>1</sup> discussing the  $\text{ZnCl}_2$ -HCl-catalysed polycondensation of *N,N*-dimethylaminomethylferrocene I to give polymer II, the formation of intermediates III and IV was postulated on the basis of the stoichiometry of the starting materials required and the composition of by-product V ( $\text{C}_{10}\text{H}_9\text{Fe}$  = ferrocenyl). While III could indeed be isolated, no interception of IV was possible.



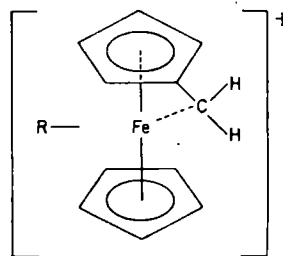
(II)



The isolation of IV has now been accomplished from polycondensation reactions interrupted prior to completion. For example, from experiments (20–40 min, 160°) conducted by the conventional procedure<sup>1</sup>, using I,  $\text{ZnCl}_2$  and HCl in a 2 : 1 : 2 ratio, IV was separated in 5–9 per cent yield besides III and V by fractional crystallization from the isopropanol extract of the reaction mixture. Similarly, equimolar amounts of I,  $\text{ZnCl}_2$  and  $\text{H}_2\text{O}$  (HCl generated by hydrolysis) at 160° (4 h) gave IV in 8.7 per cent yield. The water-soluble complex forms yellow plates, m.p. 140°–142° (calc.: C, 36.22; H, 5.27; Cl, 28.51; Fe, 11.23; N, 5.63; found: C, 36.56; H, 5.35; Cl, 28.31; Fe, 11.52; N, 5.94); in aqueous or alcoholic solution it dissociates (further dissociation of the tetrachlorozincate ion in the equilibrium  $\text{ZnCl}_4^{2-} \rightleftharpoons \text{Zn}^{2+} + 4\text{Cl}^-$  may be neglected here) according to equation I, as indicated by chemical reactions and molecular weight determinations<sup>1</sup>.

The 3.30  $\mu$  band ( $\text{CH}_3$  on  $\text{N}^+$ ) in the infra-red spectrum (potassium bromide pellet) of IV was almost as strong as in V, whereas the stretching band near 2.85  $\mu$ , strong in III, showed considerable attenuation. Decreased intensity was

also observed with the  $\text{NH}_2^+$  bands near 4.2, 6.4 and 11.35  $\mu$  (stretching, bending, rocking). No  $\text{NH}^+$  absorption in the 3.8–4.1  $\mu$  region (as exhibited by the hydrochloride of I) was noticed. These findings suggest protonation of the dimethylamine nitrogen, while the Mannich base nitrogen appears to exist essentially in a non-protonated form as analogously suggested<sup>1</sup> for III, and, hence, may involve co-ordinate covalent  $\text{N} \rightarrow \text{Zn}$  bonding.



VIa,  $R = \text{H}$

VIb,  $R =$  residual polymer chain

The isolation of IV in addition to III during intermediate stages of the polycondensation of I corroborates the reaction course proposed earlier. (IV was also directly obtained by fusing a 1 : 1 mixture of III and V. This reaction, well in accord with the evidenced double-salt character of the compound, is expected and not contradictory to the reaction path postulated.) The primary complex III is formed from I,  $\text{ZnCl}_2$  and HCl (equation (3a), *loc. cit.*<sup>1</sup>). Aided by  $\text{N} \rightarrow \text{Zn}$  co-ordination, III dissociates at one of the two  $\text{C}_a-\text{N}$  bonds, and the resulting IV undergoes further ionization at the remaining  $\text{C}_a-\text{N}$  bond, thus leading to V. The metallocarbocations VIa produced in these two ionization steps (and, similarly, VIb arising from polymeric analogues of III and IV in advanced stages of propagation) may now undergo the earlier proposed propagation and termination steps leading to II.

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### Stereochemistry of Copper in *Bis*(*N*-*t*-butylsalicylaldiminato)copper(II)

It has been established that copper(II) will adopt an approximately tetrahedral environment under certain circumstances but evidence on the exact geometry of this configuration is still meagre. That which is available, with two possible exceptions<sup>1,2</sup>, shows a stereochemistry which is more nearly planar than tetrahedral<sup>3</sup>. For instance, in 2,2'-biphenylbis-(2-iminomethylenephenoate) copper(II), where copper is complexed by a tetradentate molecule with donor atoms which are naturally tetrahedrally disposed, the ligand is considerably distorted and the expected 90° angle between the two planes containing the salicylaldimine groups is reduced to 43°.

A recent investigation of *bis*(*N*-isopropylsalicylaldiminato)copper(II) (ref. 1) has shown the crystals to be nearly, but not perfectly, isomorphous with those of the analogous nickel complex, which in its turn has been shown by X-ray analysis<sup>4</sup> to adopt a configuration which is only slightly distorted from tetrahedral. The angle between the salicylaldimine planes is 81° and the six bond angles about the nickel atom range from 94° to 125°. It is deduced that the copper atom has a similar configuration. Steric arguments, together with spectral data and dipole moment

evidence<sup>1</sup>, suggest that *bis*(*N*-*t*-butylsalicylaldiminato) copper(II) is even more likely to be tetrahedral, and in order to provide some precise information we have made a three-dimensional X-ray analysis of this compound.

The crystals are orthorhombic with  $a = 9.11$ ,  $b = 11.17$ ,  $c = 21.35$  Å and the space group is  $P2_12_12_1$ . The copper atoms were located from the Patterson syntheses  $P_{uu}$  and  $P_{vv}$ , and a three-dimensional heavy-atom electron-density synthesis, computed with weighted coefficients<sup>2</sup>, then showed all the light atom positions. The distinction between carbon, nitrogen and oxygen was made on chemical grounds. One 'difference' cycle, to confirm the co-ordinates, and three least squares refinement cycles have reduced the reliability index to 0.099 for the 2,209 observed reflexions. Separate isotropic temperature factors were applied to all atoms.

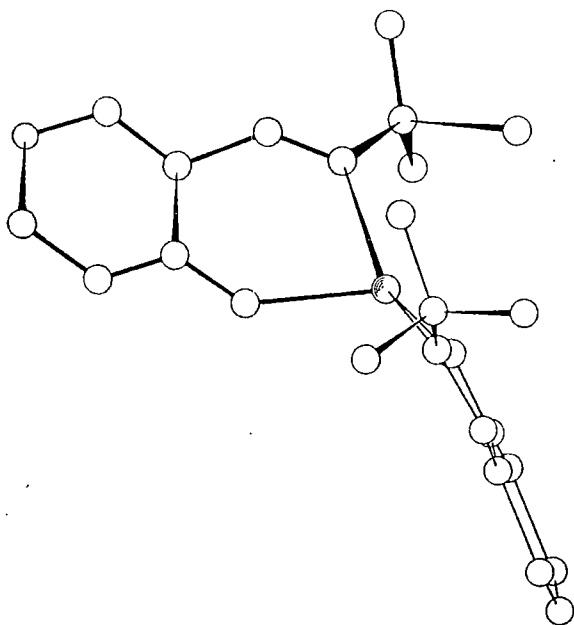


Fig. 1

The atomic arrangement in the molecule is shown in Fig. 1. Most bond lengths and angles are normal but the stereochemistry about the copper atom is far from regular. The angle between the mean planes containing the salicylaldimine residues is  $80^\circ$ , suggesting a basically tetrahedral environment, but the bond angles about the copper atom show this to be more flattened than in the above nickel compound. The most striking feature is the distortion of one of the salicylaldimine groups, but not the other, from co-planarity with the copper atom. In one-half of the molecule the plane of the benzene ring contains, within small deviations, all atoms of the chelate ring, including the copper. In the other half the deviations from the plane defined by the benzene ring are, for the atoms copper, nitrogen, aldehyde carbon and oxygen,  $+0.67$ ,  $-0.01$ ,  $-0.12$  and  $+0.04$  Å respectively. The tertiary carbon atom of the *t*-butyl group is  $-0.27$  Å from this plane. (The sense of the signs is apparent from Fig. 1.)

There is no obvious explanation for this quite unusual distortion. The chelate group has been twisted away from the position it would naturally occupy if the stereochemistry were flattened tetrahedral but not in any way so as to restore some planarity to the co-ordination arrangement. Space models built with planar salicylaldimine groups and with the measured bond angles about the copper atom show satisfactory approach distances to the neighbouring *t*-butyl group and give no indication of intramolecular steric strain. There are no intermolecular

contacts of less than  $3.6$  Å in the observed structure but the packing of the molecules is indeed compact and it may be that with unwieldy molecules, such as these, crystal packing forces have more influence on the shape of the molecule than might otherwise be supposed. On the other hand, it is possible that the copper atom itself induces the deformation. Certainly the observation that the dipole moment of this compound in benzene solution is only two-thirds that of its cobalt(II) analogue<sup>1</sup> suggests it has some structural influence.

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## New Types of Aromaticity

RECENTLY it has been shown<sup>1</sup> that the two ideas of aromatic character and the aromatic sextet, which are generally associated in current theory, are not necessarily connected and that in addition to the well-known  $p_\pi-p_\pi$  bonding of the cyclic polyenes and aromatic hydrocarbons there is a second type,  $d_\pi-p_\pi$  bonding, which is exemplified notably by the phosphonitrilic halides. The two types have been termed<sup>2</sup> 'homomorphic' and 'heteromorphic', respectively, and it has been demonstrated<sup>1,2</sup> that, in terms of the site-symmetry of the atomic orbitals combined to form molecular  $\pi$ -orbitals, there are only these two forms of cyclic  $\pi$ -bonding.

Each of the two forms may be subdivided into two topological classes, however, giving four types of cyclic  $\pi$ -bonding which are distinguished by a particular relationship between the total  $\pi$ -electron energy and the number of conjugated atoms of the cyclic system. The first of the four types to be identified was that of the Hückel cyclic polyenes<sup>3</sup>. In the approximation where each of the  $n$  conjugated carbon atoms has the same coulomb integral  $\alpha$ , and each of the carbon-carbon bonds the same resonance integral  $\beta$ , the energies of the molecular orbitals are:

$$E_j = \alpha + 2\beta \cos(2\pi j/n) \quad (1)$$

where the quantum number  $j = 0, \pm 1, \pm 2 \dots (n/2)$ . The Hückel cyclic polyenes have a closed-shell ground-state configuration if  $n/2$  is odd and a total  $\pi$ -electron energy given by:

$$E_\pi = n\alpha + 4\beta \operatorname{cosec}(\pi/n) \quad (2)$$

whereas the rings with  $n/2$  even have an open-shell ground-state configuration and a total  $\pi$ -electron energy of:

$$E_\pi = n\alpha + 4\beta \cotan(\pi/n) \quad (3)$$

The second type of cyclic  $\pi$ -bonding, exemplified by the phosphonitrilic halides, consists<sup>1,2</sup> of alternating  $p_\pi$  and  $d_\pi$  orbitals, the latter being tangential to the ring. The resonance integrals  $\beta$  of the  $\pi$ -bonds have the same magnitude, but those of adjacent bonds now have opposed signs in the basis model exhibiting the cyclic equivalence of each of the two kinds of atomic orbital. If it is assumed for the purpose of illustration that all of the  $n$  conjugated atoms have the same coulomb integral  $\alpha$  the energies of the molecular  $\pi$ -orbitals are:

$$E_j = \alpha \pm 2\beta \sin(2\pi j/n) \quad (4)$$

where the quantum number  $j = 0, \pm 1, \pm 2 \dots (n/4)$  or  $(n-2)/4$  for the cases of  $n/2$  even or odd, respectively. In



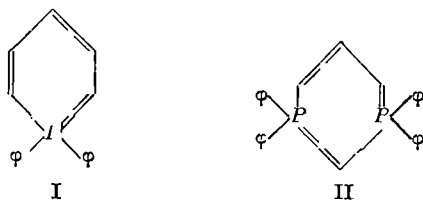
this approximation the rings with  $n/2$  either odd or even have an open-shell configuration in the ground state and the lower  $\pi$ -energy per electron given by equation (3). The introduction of different coulomb integrals for the two sets of atoms, the nitrogen and phosphorus atoms of the phosphonitrilic halides, gives the system a closed-shell ground state, but it does not affect the conclusion<sup>1,2</sup> that the  $\pi$ -energy per electron is a smooth function of  $n$  and not an oscillatory function, depending on the even or odd character of  $n/2$ , as in the case of the Hückel cyclic polyenes.

The formalism of the third type of cyclic  $\pi$ -bonding may be derived<sup>4</sup> from a model constructed schematically by cutting any one bond of a Hückel cyclic polyene and rejoining the ends after twisting them mutually by  $180^\circ$  so that the  $\pi$ -electron system consists of a Möbius strip of conjugated  $p_\pi$  orbitals. The resonance integrals  $\beta$  of the Möbius cyclic polyenes have the same magnitude, but one of them is of opposite sign to the others, and for such a system with  $n$  conjugated atoms, each having the same coulomb integral  $\alpha$ , the energies of the molecular  $\pi$ -orbitals are:

$$E_j = \alpha + 2\beta \cos(\pi j/n) \quad (5)$$

where the quantum number  $j = \pm 1, \pm 3, \pm 5, \pm 7 \dots \pm (n-1)$ . The Möbius cyclic polyenes are anti-Hückel<sup>4</sup> in that those with  $n/2$  odd have ground states with open-shell configurations and the lower  $\pi$ -energy per electron given by equation (3), whereas the Möbius rings in which  $n/2$  is even have closed-shell ground-state configurations and the higher  $\pi$ -energy per electron given by equation (2).

The Möbius ring formalism applies to any cyclic  $\pi$ -bonded system in which one or an odd number of  $\pi$ -bond resonance integrals have the same magnitude but an opposite sign to the others, for example a Hückel cyclic polyene in which one  $p_\pi$  orbital has been removed and replaced by a tangential  $d_\pi$  orbital. The phosphorus analogue (I) of benzene, recently prepared<sup>5</sup>, is of this type. The molecule (I) has a closed-shell ground-state configuration, owing to the different coulomb integrals of phosphorus and carbon atoms, and it is probable that the resonance integrals  $\beta_{PC}$  and  $\beta_{CC}$  have different magnitudes, but the general formalism of the Möbius rings suggests that the corresponding phosphorus analogues of cyclobutadiene and cyclo-octatetraene, as yet unknown, would have a larger  $\pi$ -energy per electron than (I).



The fourth type of cyclic  $\pi$ -electron system consists of a Möbius strip of alternating  $p_\pi$  and tangential  $d_\pi$  atomic orbitals or, what is formally equivalent, a ring of the second type in which one of the  $d_\pi$  orbitals has been replaced by a  $p_\pi$  atomic orbital, or one of the  $p_\pi$  by a  $d_\pi$  orbital. If all of the  $n$  atoms of the ring have the same coulomb integral  $\alpha$  and all of the  $\pi$ -bonds a resonance integral  $\beta$  with the same magnitude the energies of the molecular  $\pi$ -orbitals are:

$$E_j = \alpha \pm 2\beta \sin(\pi j/n) \quad (6)$$

where the quantum number  $j = 1, 3, 5, 7 \dots (n-1)$ . Rings of the fourth type with  $n/2$  either even or odd have a closed-shell ground-state configuration and the higher  $\pi$ -energy per electron given by equation (2). Another recently prepared<sup>5</sup> phosphorus analogue (II) of benzene

exemplifies the fourth type of cyclic  $\pi$ -bonded system, although in any detailed treatment of molecule (II) it would be necessary to take account of the different magnitudes of  $\alpha_P$  and  $\alpha_C$  and of  $\beta_{PC}$  and  $\beta_{CC}$ .

The cyclic  $\pi$ -bonded systems of the first and the third types are fundamental topologically in that their characteristics derive, respectively, from the even or the odd number of resonance integrals with a given sign in the ring. Thus in the second type of ring system there is an even number of resonance integrals with the same sign if  $n/2$  is even (type 1 behaviour), but an odd number if  $n/2$  is odd (type 3 behaviour), so that all rings of the second type have the smaller  $\pi$ -energy per electron (equation (3)) and an open-shell ground-state configuration in the approximation where it is assumed that the coulomb integrals of the two sets of atoms are equal. Conversely, for the fourth type of ring system there is an odd number of resonance integrals with a given sign if  $n/2$  is even (type 3 behaviour) and an even number if  $n/2$  is odd (type 1 behaviour), giving all rings of the fourth type the larger  $\pi$ -energy per electron—(equation (2)).

I thank Prof. E. Heilbronner and Prof. D. P. Craig for their advice.

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## BIOPHYSICS

### A Resemblance between the Clotting of Blood Plasma and the Breakdown of Cytoplasm

THE urge to regard vertebrate blood plasma as a descendant of protozoan cytoplasm is an old one. It is supported by discovery of various similarities between the clotting of plasma when shed, and that of cytoplasm when extruded. Both need calcium ions, yield similar (thromboplastin) enzymes, are accelerated by thrombin and in some cases inhibited by heparin<sup>1</sup>.

Some physical changes occurring in the cytoplasm of damaged cells appear transient. They were investigated by Kopac<sup>2</sup> with a technique based on the principle that an oil drop introduced into a cell will adsorb a protein film at the oil/cytoplasm interface; if afterwards one reduces the interfacial area, one will compress such a film until visible wrinkling (Devaux effect) occurs. This wrinkling is provoked by a minimum of compression or even spontaneously if the oil drop had been introduced shortly after the cell had been damaged by other means. Apparently, at that time cytoplasmic lipophilic proteins become available which are in part adsorbed at the oil interface where they expand (perhaps by becoming more helical); those left unadsorbed will become restabilized by mutual attachment at available apolar groups, since, in an aqueous medium, exposure of hydrophobic amino-acid groups will be unstable<sup>3</sup>. The newly formed protein/protein complexes may be of such strength that an apolar interface introduced at this time can no longer compete for their masked apolar sites; hence no spontaneous Devaux effect can be provoked from then on.

During the clotting of mammalian blood, similar interactions may take place among various proteins. These may be arranged (Fig. 1) according to the availability of their apolar sites, as judged by wettability<sup>4</sup>, activity<sup>5,6</sup> and optical thickness<sup>7</sup> of films adsorbed at hydrophilic and hydrophobic interfaces. Factors XI, V, VIII, thrombin and fibrinogen are preferentially adsorbed

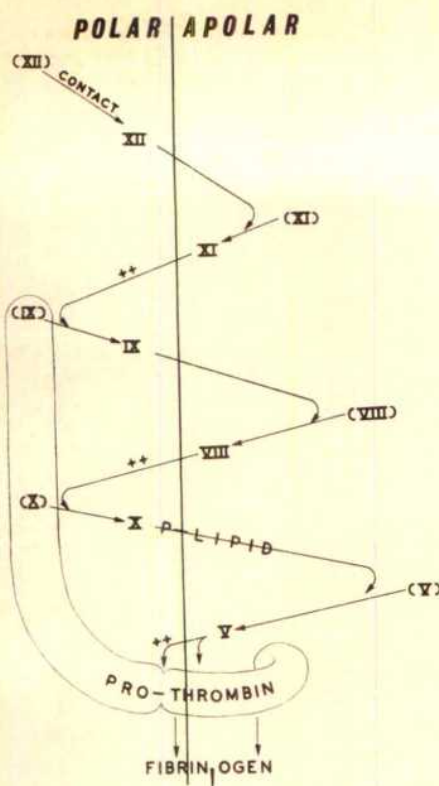


Fig. 1. Diagram of coagulation reactions. Outline around factors IX, X and prothrombin is intended to suggest that these factors may be part of one molecule. Presumed degree of effective apolarity among factors shown as increasing toward the right. ++ indicates reaction demands calcium ions

on to hydrophobic surfaces such as barium stearate and aluminium octoate powders; experimental and other evidence was reported in detail elsewhere<sup>8</sup>. *In vivo*, and before surface activation, those clotting factors with apolar sites most available may circulate as dimers, or as complexes with each other and with lipids. From the chain of clotting reactions proposed by most current theories (arrows, Fig. 1), the following can be selected to suggest involvement of hydrophobic bonds. At the onset of clotting, factor XII is electrostatically adsorbed on to a surface (such as glass), so that its more apolar sites become exposed to the medium and will in turn adsorb factor XI by hydrophobic bonding. Factor XI, changed in shape with its hydrophobic bonds now fixed by those of factor XII, activates factor IX in an electrostatic interaction which demands a bridge of calcium ions. Hydrophobic sites in factor IX then become attached to those readily available in factor VIII, and allow interaction of factor VIII with polar factor X. The resulting complex absorbs a negatively charged phospholipid micelle and opens it to expose its apolar fatty acid tails which can now adsorb and activate factor V, a protein we presume to be rich in available hydrophobic bonds. The final product enzymatically disrupts prothrombin molecules, exposing the hydrophobic bonds of thrombin which in turn will serve as point of attachment to similar bonds in fibrinogen, thus aligning enzyme and substrate. It is equally possible that factors IX, X and thrombin are parts of one molecule<sup>4</sup> (prothrombin) which demands disruption of increasing numbers of hydrophobic bonds, and thus increasingly hydrophobic enzymes, as its thrombin core is reached (Fig. 1). After coagulation, the activity of most 'apolar' factors disappears, or their apolar sites become permanently unavailable.

Both coagulation of mammalian blood plasma, and the breakdown of cytoplasm in damaged cells, may therefore involve the passage of transient hydrophobic bond

exposure from protein to protein, and techniques so far used for the examination of the one phenomenon may be applied to the other.

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## BIOCHEMISTRY

### Cross-over Electrophoresis of Acid Mucopolysaccharides

WE wish to direct attention to a simple modification of an electrophoretic technique for acid mucopolysaccharides (AMP) which makes definition of zones clear. The equipment used is the Beckman 'Microzone' electrophoresis unit which permits the placing of constant volume samples (0.25  $\mu$ l.) on buffer soaked strips of cellulose acetate.

In the commonly used method the separation proceeds with a constant voltage of 250 V and an initial 3 m.amp for approximately 25 min with either a pH 8.6 veronal or pH 2.5 acetic acid buffer. After the separation the strip is dried and spray-stained for AMP with toluidine blue, alcian blue or acridine orange solution<sup>1,2</sup>.

In the proposed modification the dye is deposited in a continuous line at the positive end of the electrophoretic strip 5 min after the current is turned on. A narrow strip of blotting paper saturated with concentrated dye in the electrophoresis buffer gives the best results. The dye migrates toward the AMP and when the dye and AMP fronts meet they co-precipitate. The slower, or diffused, part of each zone of AMP is allowed to catch up and precipitate at the front of each zone. The dye, present in excess, crosses the zone of precipitation and migrates toward the next zone of polysaccharide. The run is continued until the dye migrates past the AMP origin. Unreacted dye is rinsed away with distilled water. The dye most suited to this process, 'cross-over electrophoresis', is acridine orange. It should be recrystallized from concentrated ethanol solution in the presence of the buffer salts used in the electrophoresis and should be stored in light-proof containers<sup>3</sup>. The ionic strength of the buffer should be no higher than 0.10 because the dye complex with non-sulphated AMP dissociates above this ionic strength.

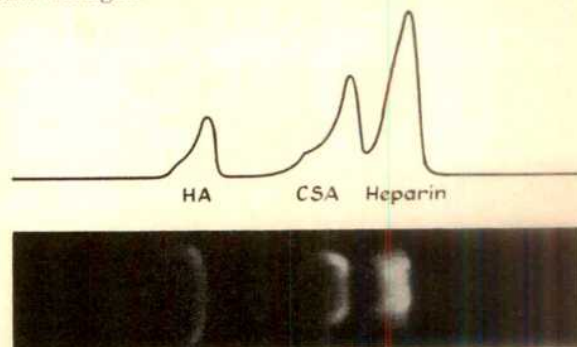


Fig. 1



A buffer made by mixing 4 volumes of formic acid with one volume of sodium acetate, each at 0.10 M, gives a pH of 2.0. This seems most useful for separating AMP of different degrees of sulphation. With veronal buffer at pH 8.6 the technique can be used for the separation of non-sulphated glycoproteins and AMP.

The AMP present in a 1 cm square 10 $\mu$  section of cartilage are easily detected and separated. As little as 0.05  $\mu$ g of standard AMP is easily detected and 0.3  $\mu$ g can be measured by scanning cleared strips in the Beckman 'Microzone Analytrol'.

Fig. 1 illustrates the separation of hyaluronic acid (HA), chondroitin sulphuric acid (CSA) and heparin from a mixture of standards. The scan is made directly from the cleared strip which was also used as the 'negative' for the enlarged print of the separated zones.

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### Enzymatic Action of Lipoglycoprotein Preparations from Sperm-acrosomes on Rabbit Ova

THE cumulus oophorus of rabbit eggs can be dispersed *in vitro* by suspensions of rabbit spermatozoa<sup>1,2</sup> or by hyaluronidase<sup>3</sup>, but the corona radiata remains intact<sup>4</sup>. Both the corona and the zona pellucida can be dissolved by proteolytic enzymes such as ficin, papain, 'Pronase' (a bacterial proteinase) and trypsin<sup>5,6</sup>, but the mechanism by which dissolution is brought about *in vivo* remains obscure<sup>6</sup>. However, it is known that when spermatozoa penetrate the layers of cumulus and corona cells of rabbit eggs the acrosomes become detached<sup>6</sup>, and it has been suggested that this leads to liberation of a 'zona lysin', a hypothetical enzyme which is localized between the sperm head and the acrosome<sup>7</sup>.

Acrosomes can be dislodged from ram, bull and rabbit spermatozoa by means of anionic detergents and obtained as suspensions which are almost free from any other parts of the sperm cell<sup>8,9</sup>. The separated acrosomes become solubilized, in the form of a lipoglycoprotein complex, following treatment with ethanol<sup>9</sup>.

When freshly ovulated rabbit eggs were incubated at pH 7 and 37° C with lipoglycoprotein complex from ram, bull or rabbit spermatozoa the eggs became denuded, that is, cumulus and corona cells were dispersed. In some cases dissolution of the zona pellucida was also observed. When the eggs were stationary in the lipoglycoprotein solution, up to 24 h was necessary for full effects to be observed, but with occasional gentle shaking the process of denudation occurred more rapidly. Control experiments without lipoglycoprotein complex showed no denudation. The denuding activity of lipoglycoprotein complex was much lower after it had been heated to 100° C. Activity was also decreased by iodoacetate and by the polyanionic hyaluronidase inhibitor 53DK<sup>10</sup>, although with both inhibitors the extent of inhibition varied widely from one preparation to another. The lipoglycoprotein complex preparations contained some hyaluronidase; but there was no parallelism between denuding activities and hyaluronidase contents. The preparations also showed proteolytic activity, reflected in the liberation of amino groups, when incubated alone at pH ~7. In presence of

air this proteolytic activity was low (0.2–0.6  $\mu$ equiv. amino-acids/h/mg lipoglycoprotein complex) but rates in absence of oxygen were 2–3 times greater.

The experiments briefly reported here provide the first demonstrations of a denuding effect on ova, and a dissolution of the zona pellucida, by a cell-free enzyme preparation with proteolytic activity, obtained from mammalian spermatozoa.

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### Relationship between Concentrations of Ruminal Nucleic Acids and Excretion of Purine Derivatives by Sheep

PURINES can be synthesized in the animal body from glycine, serine, aspartic acid, glutamine, formate and respiratory carbon dioxide, but they are also derived from dietary and cellular nucleoprotein. Recent work with cattle<sup>1</sup> and sheep<sup>2</sup> has shown that purine derivatives (uric acid and allantoin in urine) are quantitatively important end-products of these ruminants when they are fed on diets with a high-energy, low-protein content and when the animals are in either N equilibrium or slight positive balance. With such diets protein is utilized very efficiently. It is well known that efficiency of protein utilization in the ruminant depends on the catabolic and synthetic activities of the rumen micro-organisms. For high efficiency, the breakdown products of dietary protein and other nitrogenous compounds must be rapidly synthesized into microbial protein in the rumen<sup>3</sup>. This evidence suggests that most of the allantoin and uric acid excreted by ruminants may be derived from nucleic acids of rumen micro-organisms. Some support for this suggestion has been reported by Blaxter and Martin<sup>4</sup>, who showed that after ruminal infusion of casein (which is free of purine and pyrimidine bases) slightly more allantoin was excreted than after abomasal infusion of the same quantity of casein.

In trials briefly described in this communication, the relationship was examined between ruminal concentrations of nucleic acids and the urinary excretion of allantoin and uric acid by sheep which were fitted with rumen cannulae and fed on a variety of low-protein diets.

Sixteen diets, ranging in crude protein content from approximately 2.6 to 10 per cent and varying in digestible energy content (four levels) were offered *ad lib.* to four groups of four fistulated wether sheep for periods of 28 days (the detailed composition of the diets and the experimental plan have been described previously<sup>5</sup>). In the last week of each 28-day period the sheep were housed in metabolism crates and total urinary collections were made. Samples of rumen fluid, approximately 50 ml. in volume, were taken at 7 a.m., 8 a.m., 9 a.m., 11 a.m. and 4 p.m. during one day of this collection period. The 7 a.m. and 4 p.m. samples were withdrawn immediately before feeding at these times. The rumen fluid was



aspirated from a constant depth (6 in.) into a  $\frac{3}{8}$ -in.-diameter glass tube and strained through four layers of muslin to remove coarse food particles. Four ml. of strained fluid were mixed with 10 ml. of 10 per cent (w/v) trichloroacetic acid, and centrifuged. The precipitate was extracted with 10 per cent trichloroacetic acid, ethanol and a mixture of ethanol and ether, and then hydrolysed using the method of Schneider<sup>6</sup>. The hydrolysed extract was diluted to 50 ml. with 5 per cent (w/v) trichloroacetic acid and its absorption of ultra-violet light measured on a Unicam spectrophotometer SP500 at 263 m $\mu$ . Compensation for absorption of ultra-violet light by the solvent was made by measuring the extracts against a blank of 5 per cent trichloroacetic acid. In the absence of a more suitable standard, yeast nucleic acid (supplied by B.D.H.) was hydrolysed by the same procedure and was used as a reference material. It was found that the maximum absorption of hydrolysates of ruminal extracts varied between 260 and 268 m $\mu$  but all measurements were made at 263 m $\mu$ . Allantoin content of the urine samples was determined by the method of Young and Conway<sup>7</sup>, and uric acid by the procedure of Benedict and Franke<sup>8</sup>.

Table 1. MEAN VALUES FOR URINARY LOSS OF URIC ACID PLUS ALLANTOIN, AND CONCENTRATIONS OF RUMINAL NUCLEIC ACIDS, FOR GROUPS OF FOUR SHEEP GIVEN FOUR DIFFERENT TYPES OF DIET

Diet	Urinary loss of uric acid plus allantoin nitrogen (mg/24 h)	Concentration of nucleic acid in rumen fluid ( $\mu$ g/ml.)
High energy	544 $\pm$ 260	187 $\pm$ 139
High roughage	312 $\pm$ 93	76 $\pm$ 39
All roughage	307 $\pm$ 120	91 $\pm$ 35
Dried veldt grass	236 $\pm$ 109	78 $\pm$ 50

The 16 different diets used in the trial comprised four high-energy diets (equal parts of concentrate and roughage), four high-roughage diets (6 parts of roughage with 1 part of concentrate), four diets composed entirely of roughage and four diets of dried veldt grass. Mean values of ruminal nucleic acid levels and uric acid plus allantoin excretion by sheep fed on the four groups of diets are shown in Table 1. Both the excretion of purine derivatives by sheep and the level of nucleic acids in the rumen were highest when they were given the high-energy diets. These diets, which were rich in readily fermentable carbohydrate, would encourage multiplication of micro-organisms in the rumen, which in turn would tend to cause high levels of ruminal nucleic acids. This association between nucleic acids and amount of synthesis in the rumen is supported by the observation that the concentration of nucleic acids in the rumen liquor during the day was generally highest in the 4 p.m. sample, that is, in 46 of the 64 samplings, while second highest concentration occurred most frequently at 7 a.m. During the time between feeding and these observed peaks there is probably a steady increase in the amount of microbial protein synthesized from the food ingested.

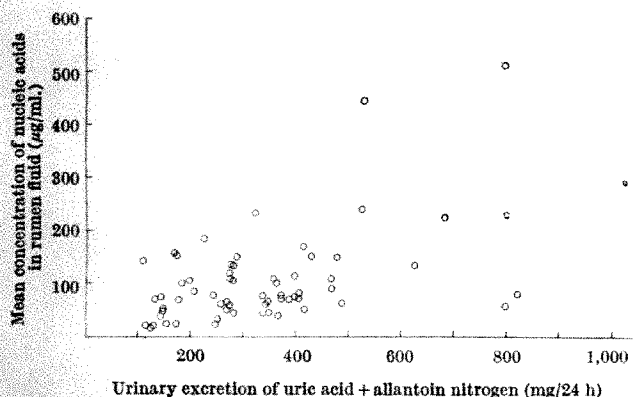


Fig. 1. Relationship between concentration of ruminal nucleic acids and excretion of purine derivatives by sheep

In Fig. 1 the mean daily excretion of nitrogen, in the form of uric acid plus allantoin, by the 16 sheep each given four different diets, is plotted against the weighted mean concentration of nucleic acid in the sheep's rumen during a day when the sheep was given the particular diet (a total of 64 observations). The weighted mean concentrations of nucleic acids were obtained by assuming linear changes between consecutive observations during the day. There was a highly significant correlation between the concentration of ruminal nucleic acids and the amount of uric acid plus allantoin nitrogen excreted ( $r = 0.537$ ;  $P < 0.001$ ).

Unfortunately there is, as yet, no convenient method of assessing the total daily production of substances in the rumen. The concentration observed at any time is dependent on the rate of synthesis of the substance and its rate of disappearance from the rumen. The latter is controlled by the rates of absorption, decomposition, and propulsion along the digestive tract. However, studies with metabolites such as volatile fatty acids<sup>9</sup> have shown that there is a close relationship between their concentration in the rumen and their rate of production. The highly significant correlation obtained in the trial now described, between ruminal concentrations of nucleic acids and the amount of purine derivatives excreted in urine by sheep, strongly indicates that nucleic acids produced in the rumen are the main source of purines from which uric acid and allantoin are derived by partial oxidation. It is possible that a more precise correlation would have been obtained between levels of uric acid plus allantoin excreted and ruminal nucleic acids had it been feasible to measure the daily rate of production of the latter with sufficient accuracy.

If ruminal nucleic acids are the main source of allantoin and uric acid found in the urine of ruminants, this does explain why the percentage of urinary nitrogen present as allantoin was found to be a useful index of efficiency of protein utilization<sup>1</sup>. For similar reasons, levels of nucleic acids in the rumen may be another useful characteristic for assessing the efficiency of protein utilization by ruminants.

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### Histochemical Localization of $\beta$ -Glucuronidase in the Rat Brain

THERE are earlier reports of the biochemical demonstration of  $\beta$ -glucuronidase in brain tissue<sup>1,2</sup>. Strong  $\beta$ -glucuronidase activity in the choroid plexus and in the Purkinje cells was established by Pearse<sup>3</sup>. We have, however, been unable to find a more detailed investigation of the distribution of this enzyme in the brain.

Our histochemical investigations showed that  $\beta$ -glucuronidase activity is widely distributed in the rat brain. A pronounced and precisely localized reaction was found



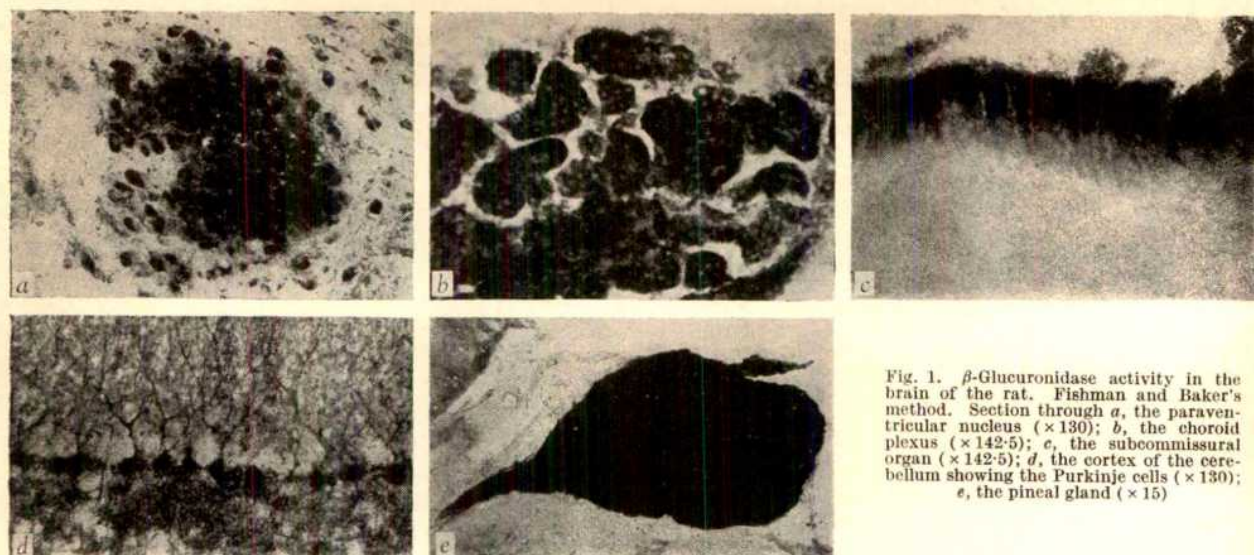


Fig. 1.  $\beta$ -Glucuronidase activity in the brain of the rat. Fishman and Baker's method. Section through *a*, the paraventricular nucleus ( $\times 130$ ); *b*, the choroid plexus ( $\times 142.5$ ); *c*, the subcommissural organ ( $\times 142.5$ ); *d*, the cortex of the cerebellum showing the Purkinje cells ( $\times 130$ ); *e*, the pineal gland ( $\times 15$ ).

particularly in the hypothalamic magnocellular neurosecretory nuclei, in the subcommissural organ, in the choroid plexus, in the Purkinje cells, and in the pineal gland. In the other parts of the brain, for example in the cortex of the cerebrum and cerebellum, and in some basal nuclei there was a rather diffuse reaction, and it was considerably weaker than that in the areas mentioned here.

The brains of 20 albino rats of both sexes were investigated by Fishman and Baker's ferric hydroxyquinoline method<sup>3</sup>, with slight modifications. Frozen sections 15–30  $\mu$  were cut with the Pearse freezing microtome, mounted on cover glasses, and fixed in chloral-formalin at 8°C, or at room temperature for 10 min to 2 h, incubated in substrate solution for 17 h at 8°C and then for 3–7 h at 37°C, placed in oxalate buffer for 15 min and finally in acid ferrocyanide solution. The specificity of localization was controlled by treating sections with 0.001 M saccharic acid.

The sites of the most intense reaction are shown in Fig. 1. Activity in the neurosecretory ganglia is cytoplasmic. In the subcommissural organ, strong activity is localized in the cytoplasm of the ependymal cells; the hypendyma is quite negative. The enzymatic activity is similarly localized in the ependyma in the choroid plexus. In this connexion it may be pointed out that the ependyma covering the walls of the cerebral ventricles shows only a very weak reaction or none. Activity in the Purkinje cells is cytoplasmic and it could be demonstrated also in the neurites and dendrites. The pineal gland exhibited a strong  $\beta$ -glucuronidase activity which seemed to be evenly distributed throughout the gland.

These observations suggest that the strongest  $\beta$ -glucuronidase activity is found in those parts of the brain which have, or are presumed to have, a secretory function. The only exception is the Purkinje cells, the physiological significance of which is problematic.

Precise evaluation of the functional role of  $\beta$ -glucuronidase in the brain must await further investigation. One might speculate that it is related to the synthesis of secretion in the 'secretory' regions which are rich in the enzyme. The hypothalamic-hypophyseal 'neurosecretory material' and the 'secretion' of the subcommissural organ are of protein character. It has also been suggested that the pineal gland secretes some factor of a protein nature. However, it is impossible to state on the basis of present knowledge whether  $\beta$ -glucuronidase is in fact involved in protein synthesis. The possible relationship between the activity of  $\beta$ -glucuronidase and liquor secretion in the choroid plexus is obscure.

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### A Light-catalysed Reaction in the Aqueous Humour of the Eye

Kon and Watson<sup>1</sup> found that ascorbic acid in milk was destroyed by light and Hopkins<sup>2</sup> showed that this destruction was caused by the photocatalytic oxidation of ascorbic acid by riboflavin. Oxygen was necessary and peroxide was formed. In connexion with an examination of glutathione peroxidase of lens I became interested in the ascorbic acid of the aqueous humour as a possible source of hydrogen peroxide. In many mammals, including man, the level of ascorbic acid in the aqueous humour is about 1.0 mM, which is far higher than the level in serum<sup>3</sup>. Light freely penetrates through the aqueous humour whenever the eye is open, and Philpot and Pirie<sup>4</sup> found traces of riboflavin and/or flavinadenine dinucleotide in the aqueous humour of cattle eyes. It seemed possible, therefore, that a photocatalysed oxidation of ascorbic acid might take place in the eye *in vivo*, as it does in milk *in vitro*.

At the pH of the aqueous humour of the rabbit, which is near pH 7.6 (ref. 5), ascorbic acid is autoxidizable and small amounts of dehydroascorbic acid are present in both aqueous humour and lens. Traces of  $H_2O_2$  are also present in aqueous humour of cattle and rabbit (Pirie, to be published).

To examine the effect of light and of riboflavin on the oxidation of ascorbic acid in aqueous humour, this was removed from cattle eyes into a syringe, about 15 min after death, and kept in the dark on ice until used. Table 1 shows that exposure to ultra-violet light (max. 360 m $\mu$ ) at 0° or exposure to sunlight at 25° increases the rate of disappearance of ascorbic acid; at 25° the rate is about doubled in sunlight. Table 2 shows that addition of 0.125  $\mu$ g/ml. of riboflavin still further accelerates removal of ascorbic acid from aqueous humour in light but has no effect in the dark. The same is true of riboflavin phosphate



Table 1. EFFECT OF LIGHT ON ASCORBIC ACID OF CATTLE AQUEOUS HUMOUR

1. *Exposure to sunlight:* Cattle aqueous humour contained in a quartz cuvette was exposed to sunlight on an open windowledge. A sample of the same aqueous humour was put in a black-lined light-tight box also on the windowledge. Temp. 25°. Samples of 0.1 ml. were removed at intervals into spectrophotometer cups containing 1.0 ml. 0.05 M potassium phosphate pH 6.6, 1.6 ml. H<sub>2</sub>O + 0.3 ml. 2:6 dichlorophenol indophenol, made up so that 1.0 ml. = 0.1 mg ascorbic acid. The absorption at 600 mμ was measured before and immediately after addition of the sample of aqueous humour. The decrease in reading measures the ascorbic acid. The amount present can be read from a calibration curve made with known amounts of ascorbic acid and dye

Time (min)	Ascorbic acid $\mu\text{g/ml}$ . aqueous humour			
	Dark	Sun	Dark	Sun
0	208	208	110	110
5	204	194	—	—
25	180	152	105	85
75	160	110	—	—
120	—	—	80	40

2. *Exposure to ultra-violet lamp:* Max. emission 360 mμ. Temp. 2°. Aqueous humour in open beaker; depth of fluid 5 mm; distance from lamp 5 cm. Another sample of aqueous humour kept at 2° in dark

Time (min)	Ascorbic acid $\mu\text{g/ml.}$ aqueous humour			
	Dark	Light	Dark	Light
0	120	120	192	197
10	125	117	190	190
30	124	108	190	180
45	125	105	192	175
60	124	97	—	167

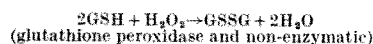
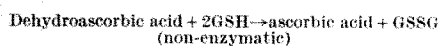
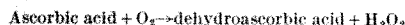
Table 2. EFFECT OF ADDED RIBOFLAVIN (RF), RIBOFLAVIN PHOSPHATE (RFP) AND FLAVIN ADENINE DINUCLEOTIDE (FAD) ON THE ASCORBIC ACID OF AQUEOUS HUMOUR IN SUNLIGHT OR DARKNESS

0.125 μg riboflavin or its compounds were added to 1.0 ml. aqueous humour. Temp. 25°. Conditions and estimation as described in Table 1

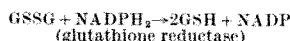
Time (min)	Ascorbic acid $\mu\text{g/ml.}$ aqueous humour						
	Dark	Sun	Dark + RF	Sun + RF	Dark + FAD	Sun + FAD	Sun + RFP
0	142	142	145	142	145	138	—
25	128	113	127	103	128	110	—
80	82	82	87	57	87	68	—
220	30	25	37	0	30	15	—
0	182	182	—	182	—	182	182
10	175	162	—	155	—	157	150
30	157	142	—	125	—	147	135
80	112	90	—	74	—	95	76

and to a lesser extent of flavin adenine dinucleotide. To estimate ascorbic acid, aqueous humour was added directly to 2:6-dichlorophenol indophenol buffered at pH 6.6 in a spectrophotometer cup and the change in absorption at 600 mμ was measured. After this, H<sub>2</sub>O<sub>2</sub> was identified by adding peroxidase. If the method of titration with the dye in metaphosphoric acid had been used, peroxidase would have been inactivated. At pH 6.6, peroxidase and H<sub>2</sub>O<sub>2</sub> re-oxidize 2:6-dichlorophenol indophenol so that there is an increase in absorption at 600 mμ on addition of the enzyme<sup>6</sup>. Hydrogen peroxide was detectable in all samples but it did not accumulate, probably because it reacts with ascorbic acid.

At oxidation of ascorbic acid in the aqueous humour even in the dark is fairly rapid, but the results show that light accelerates it and that it is accelerated still more by addition of small amounts of riboflavin. It is possible that this reaction has physiological importance. Experiments (to be published in detail elsewhere) have shown that glutathione is oxidized during removal of ascorbic acid and that this oxidation is partly enzymatic, being catalysed by glutathione peroxidase of lens.



Oxidized glutathione can be re-reduced by NADPH<sub>2</sub> and glutathione reductase which is present in lens.



Kinoshita and Masurat<sup>7</sup> found that the supply of NADP in lens limited the oxidation of glucose by the pentose phosphate pathway and that, in certain circumstances, addition of oxidized glutathione stimulated oxidation of glucose. It is possible, therefore, that the whole

series of reactions, starting with uptake of oxygen by ascorbic acid in the aqueous humour, is advantageous to the lens. Zeller<sup>8</sup> found that lens contains only traces of catalase so that nearly all H<sub>2</sub>O<sub>2</sub> formed may be available to glutathione peroxidase.

The effect of light on the retina is so fundamental for vision that attention has been focused on this and we have largely overlooked the fact that most tissues and fluids of the eye are bathed in light. Oxidation of ascorbic acid in aqueous humour is perhaps simply one of a group of light-catalysed reactions in the eye.

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### Effect of Solvated Ions on the Activity of Esterases in the Housefly

A NUMBER of electrolytes, which are in common use in the fractionation of proteins, have been studied for their effect on choline- and ali-esterase of the housefly. The substrates were acetylcholine and ethylbutyrate, respectively. Enzyme activity was measured by a pH-titrimetric method using barbitone-HCl (pH 7) as buffer.

The results, illustrated in Fig. 1a and b, show that all salts that were tested activated acetylcholinesterase and inhibited the ali-esterase in more or less the same order. The order of the effect of the salts corresponds to the order of their energy of hydration. Heats of hydration<sup>1</sup> are 490, 410, and 114 kcal/g-ion for Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Na<sup>+</sup>, respectively, while the lyotropic number<sup>2</sup> for SO<sub>4</sub><sup>2-</sup> is 2 and that for Cl<sup>-</sup> is 10, indicating that the former has a higher affinity for water than the latter. However, MgSO<sub>4</sub> may have a lower energy of hydration than MgCl<sub>2</sub> due to its low degree of dissociation<sup>3</sup>.

It is possible, therefore, that the effect of electrolytes on esterases is related to the solvation of ions, the consequences of which can be discussed as follows.

Proteins have a high affinity for water, with which they bind possibly through hydrogen bonds. Water of solvation confers a high degree of stability on protein colloids by blocking the polar groups on the surfaces of the molecules and thus preventing cohesion. Water of solvation must also exist in equilibrium with the free water, the capture of a large part of which due to ion hydration will reduce the thickness of the water shell around the protein molecule, and this intensifies the electrostatic field on the surface of the enzyme leading to cohesion. Thus, with the aliesterase, the attraction between enzyme particles seems greater than between enzyme and the ethylbutyrate molecule. However, in the case of cholinesterase which possesses an anionic site in its active centre, ion hydration should result in increase in activity in the presence of a very polar substrate such as acetylcholine. This is supported by the fact that MgCl<sub>2</sub> at 0.1 M reduced the activity of acetylcholinesterase towards the dimethyl amino analogue of acetylcholine, which is non-polar by 25 per cent.

The inhibition of cholinesterase by large concentrations of salts of high hydration energy can be explained on the basis of polarity, but may throw light on another more important aspect of the water of solvation which could be largely responsible for the inhibition of the aliesterase.



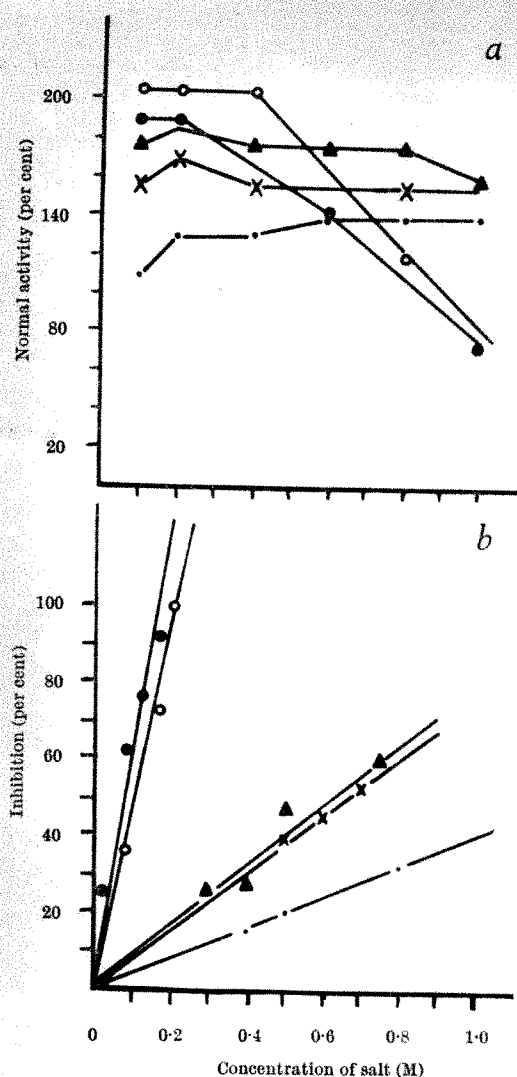


Fig. 1. Effect of electrolytes on the activity of (a) cholinesterase; (b) alkaline phosphatase. ●, CaCl<sub>2</sub>; ○, MgCl<sub>2</sub>; ▲, MgSO<sub>4</sub>; ×, Na<sub>2</sub>SO<sub>4</sub>; — · —, NaCl

Esterase activity, like that of many enzymes, is influenced by the concentration of the substrates, which are the ester and water. Relatively little attention has been given to the state in which water exists, in view of the fact that enzyme reactions take place in aqueous solutions. However, ion hydration will lead to the existence of large quantities of water in an unavailable ion-bound form and the concentration of enzyme-bound and free water will be lowered. Since enzyme reactions are adsorption reactions, it is not unnatural to regard the water of solvation which is already adsorbed on the surface of the molecule as the one directly used for hydrolysis, the partial removal of which will affect enzyme activity. This suggests that during hydrolysis the enzyme particle is involved in a continuous process of dehydration and solvation.

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## Reaction of Pyridine Nucleotides with 2,4-Dinitrophenylhydrazine

WHILE attempting to isolate and identify pyridine nucleotide-dependent and enzymatically-formed aldehydes and  $\alpha$ -keto acids as their 2,4-dinitrophenylhydrazone derivatives, it was noted that derivatives were formed which, when chromatographed on paper, were unlike known standards and gave blue colours when sprayed with alcoholic KOH. The reaction mixture in which this phenomenon was first observed was a control tube that contained: *tris*-HCl buffer, pH 8.5, 1,500  $\mu$ moles; nicotinamide-adenine-dinucleotide (NAD), 10.0  $\mu$ moles; and crude cell-free extract containing 1.2 mg protein in a total volume of 3.8 ml. The incubation was terminated by the addition of an equal volume of saturated 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl. The reaction mixture was centrifuged to remove precipitated protein and the supernatant fluid decanted into a clean test-tube and incubated overnight at 37° C. Further investigations revealed that the flocculent precipitate which formed was due to a reaction between NAD and DNPH. This product was purified by the usual extraction procedure including initial extraction with ethyl acetate to remove unreacted DNPH, neutral and acidic hydrazones; the latter were re-extracted into 10 per cent sodium carbonate, leaving unreacted DNPH and neutral hydrazones behind in the organic phase; the carbonate layer was acidified at 4° C and re-extracted with ethyl acetate. The ethyl acetate layer was evaporated and aliquots were used for spotting on to paper chromatograms.

In the light of the results obtained with NAD, the investigation was extended to nicotinamide-adenine-dinucleotide-phosphate (NADP) and the reduced forms, NADH and NADPH. These latter compounds also gave additional products when treated with DNPH. To each of

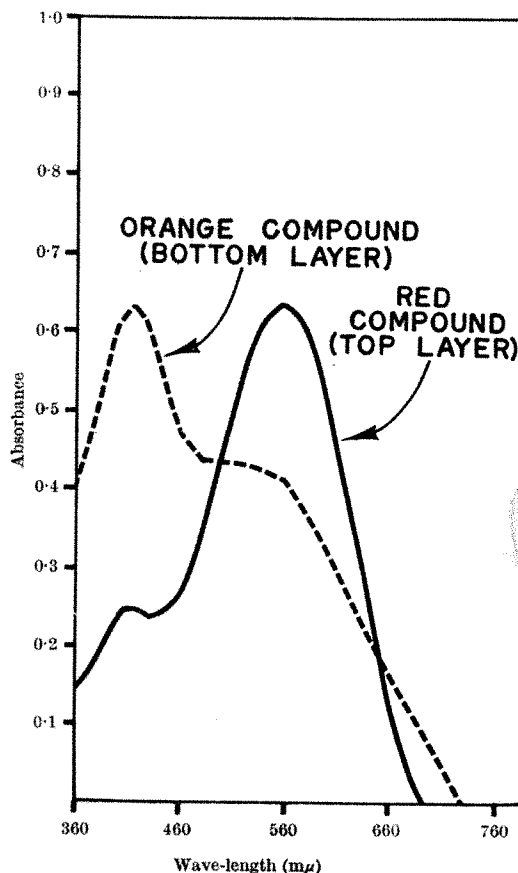


Fig. 1. Absorption spectra of compounds formed between 2,4-dinitrophenylhydrazine and NADH or NADPH. See text for details.

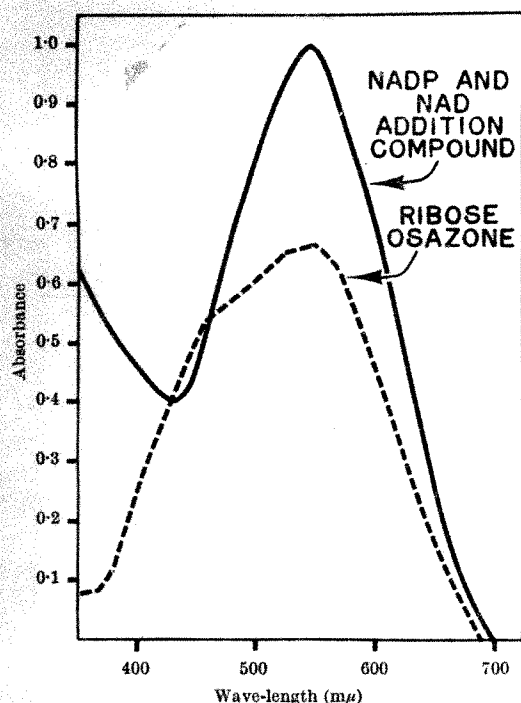


Fig. 2. Absorption spectra of compounds formed between 2,4-dinitrophenylhydrazine and NAD, NADP, and ribose. See text for details

four tubes, containing 300–500  $\mu$ moles of one of the pyridine nucleotides were added 50 ml. of DNPH solution. While tubes containing NADH and NADPH became cloudy almost immediately, those containing the oxidized forms of the pyridine nucleotides formed precipitates slowly. After 16 h at 37° C, large quantities of flocculent material were evident in the tubes containing NADH and NADPH, while the tubes containing the oxidized forms contained considerably less precipitated material. The addition compounds thus formed were only slightly soluble in 1 N HCl but were quite soluble in water. They were also soluble in ethyl acetate, ether, pyridine and acetone.

Samples were analysed spectrophotometrically in a Cary Model 14 recording spectrophotometer by dissolving a small amount of precipitate in alkaline solution (1 part 2 N NaOH and 1.5 parts 10 per cent  $\text{Na}_2\text{CO}_3$ ). Fig. 1 shows that either NADH or NADPH forms two additional compounds with distinct optical properties. These two addition compounds settled in the tube as two distinct layers of precipitate; a denser orange compound below and a less dense, red compound on top of it. Samples were removed for spectral analysis by carefully collecting them from the separate layers with a capillary pipette. Efforts to separate the two additional compounds by centrifugation were unsuccessful. As shown in Fig. 1, the two compounds had maximal absorptions in the 410–420  $\text{m}\mu$  and 550–560  $\text{m}\mu$  regions, respectively. The small peaks and shoulders probably represent contamination of one compound with the other. Absorption spectra were obtained within 10 min after placing the precipitates in alkali, since it was noted that the absorbance of the 550–560  $\text{m}\mu$  peak would decrease, while the 410–420  $\text{m}\mu$  peak would increase with time.

Samples for spectral analysis of NAD and NADP additional compounds were obtained in the way described for their reduced forms. These precipitates, however, appeared homogeneous. The spectra of materials collected from either tops or the bottoms of the precipitate layers were identical, as shown in Fig. 2. Included in Fig. 2 for comparative purposes is the absorption spectrum of authentic ribose osazone. It can be seen that, with one exception, the absorption in the 410–420  $\text{m}\mu$

region, the addition compounds of the pyridine nucleotides resemble the sugar osazone.

Treatment of pyridine nucleotides with strong acid will hydrolyse the pyrophosphate moiety<sup>1</sup>. Complete acid hydrolysis of NAD yields adenine, nicotinamide, ribose and phosphoric acid in the ratio of 1 : 1 : 2 : 2 (refs. 2, 3). The action of strong acid on NADP results in the formation of the same products with an additional phosphate group<sup>4</sup>. Thus, it might be expected that the addition compounds described herein are 'ribose-like'. It is to be noted that the most significant difference is the solubility of these products in water as compared to the ribose osazone, which is insoluble.

Reduced pyridine nucleotides are very unstable in strong acid. Anderson and Berkelhammer<sup>5</sup> showed that the nicotinamide moiety adds water to form a compound that reacts with both phenylhydrazine and dinitrophenylhydrazine. They postulate that these reactions represent an acid-catalysed ring opening at the 6-position to give the amino aldehyde. We suggest that it is the amino aldehyde-ribose-DNPH compound in our mixtures which absorbs maximally in the 410–420  $\text{m}\mu$  range.

This report is intended to clarify difficulties which may arise from attempts to isolate aldehydes or  $\alpha$ -keto acids as their 2,4-dinitrophenylhydrazone derivatives from mixtures containing pyridine nucleotides. It originated from work supported by grants from the U.S. Public Health Service (A1-03868-03) and the National Science Foundation (GB 2020). One of us (H. C. R.) holds a research career development award (5-K3-A1-6928-02) from the National Institutes of Health.

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## PHYSIOLOGY

### Long-term Preservation of Bone Marrow

PRESERVATION of the functional integrity of cells stored at low temperatures has become routinely successful. Current efforts in cryobiology centre around improvement of the techniques, assessment of the physico-chemical alteration of cells by low temperatures, and evaluation of the length of time for which frozen cells will maintain their functional capacity. It has been reported, for example, that bull spermatozoa can be stored successfully at  $-79^\circ\text{C}$  for at least 7 years<sup>1</sup>, and that some human cell lines remain viable for 33 months<sup>2</sup>. During the course of experiments with various techniques for freezing and storing mouse bone marrow<sup>3</sup>, we froze a number of samples to be tested at long intervals. The present interim report describes the results of these experiments.

The reproductive integrity of mouse bone marrow after preservation at low temperatures was measured by the ability of the infused cells to promote recovery of lethally X-irradiated mice. The procedures used were the same as previously reported<sup>3</sup>, with the exception that the irradiated recipient was the ( $\text{C3H} \times \text{C57BL}$ ) $\text{F}_1$  mouse rather than the ( $\text{C57BL} \times 101$ ) $\text{F}_1$ . The preserved marrow was from the ( $\text{C57BL} \times 101$ ) $\text{F}_1$  hybrid, and it would have been desirable to use the same type hybrid, but these were not readily available. The C3H and 101 strains, however, possess the same major histocompatibility antigens.



To present a complete picture of the data obtained so far, Table 1 includes results of some experiments (section A) previously reported<sup>3</sup>. From section A of Table 1, it is seen that marrow stored at  $-30^{\circ}\text{C}$  for 25 weeks was ineffective in promoting survival of lethally irradiated mice. By ineffective we mean that in the injected inoculum of  $10^6$  nucleated cells there were not enough viable cells to prevent death of any of the recipients.

Table 1. LONG-TERM STORAGE OF FROZEN MOUSE BONE MARROW. X-RAY EXPOSURE WAS 900 OR 950 R. EACH MOUSE RECEIVED  $10^6$  MARROW CELLS INTRAVENOUSLY IN 0.5 ML. SECTION A FROM DATA OF BENDER, TRAN AND SMITH (REF. 3)

	No. mice	Storage temp. ( $^{\circ}\text{C}$ )	Storage time	Per cent 30-day survival
A	25	-30	1 hour	83
	25	-30	4 weeks	57
	25	-30	25 weeks	0
	25	-70	1 hour	80
	25	-70	22 weeks	80
	25	-196	1 hour	68
	25	-196	4 weeks	80
	25	-196	25 weeks	72
	292			0 (X-ray control)
B	29	-196	29 months	79
	13			0 (X-ray control)
	16	-196	57 months	88
	10			0 (X-ray control)

In a solid  $\text{CO}_2$  refrigerator ( $-70^{\circ}\text{C}$ ), effective cells were present after 22 weeks' storage. It was not possible to test cells stored at  $-70^{\circ}\text{C}$  beyond 22 weeks because of accidental thawing of the refrigerator. At liquid nitrogen temperature ( $-196^{\circ}\text{C}$ ), effective cells were present after 25 weeks' storage. Section B of Table 1 shows the results of the most recent tests of marrow stored at  $-196^{\circ}\text{C}$  for 29 and 57 months. It is apparent that some cells retained their reproductive capacity for almost 5 years at  $-196^{\circ}\text{C}$ . On thawing, the 57-month preparation contained a slight amount of white fibrous material, and the cell concentration was reduced by about 50 per cent. In part, this reduction could have resulted from a moderate degree of cell clumping that was observed microscopically. Eosin uptake of this preparation was 58 per cent. Biennial testing of the stored marrow is proposed.

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### Antigenic Components in Human Pituitary Follicle-stimulating Hormone Preparations of Various Purities

THE production of an antiserum to a preparation of human pituitary follicle-stimulating hormone (HP-FSH) which had been fractionated by electrophoresis on starch gel (SG4) has been described previously<sup>1,2</sup>. Immuno-electrophoresis of various HP-FSH preparations with this antiserum showed the presence of two precipitin lines. These two lines obtained with antigens of 1 mg/ml. solutions (w/v) were now investigated by comparing three HP-FSH preparations of different purity, CM 1 (ref. 3), CP 1 (ref. 3) and SG 4 (Fig. 1, A, B, C), and by absorbing the antiserum with various proteins.

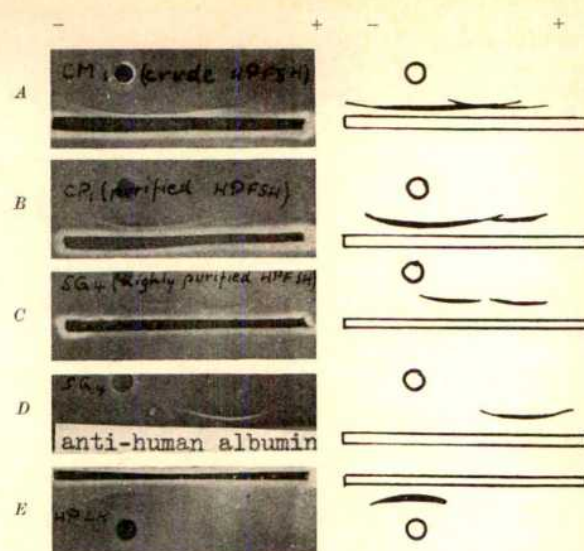


Fig. 1. Immuno-electrophoretic pattern of: A, CM 1 (crude human pituitary FSH); B, CP 1 (purified human pituitary FSH); C and D, SG 4 (highly purified human pituitary FSH); E, human pituitary LH. A, B, C and E developed by antiserum to SG 4. D developed by antiserum to human serum albumin. The antigens were applied in 1 mg/ml. solutions. Left—contact prints; right—schematic drawings

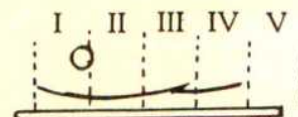


Fig. 2. Schematic pattern of human pituitary FSH showing the agar sections I-V which were eluted after electrophoresis for injecting into mice

The anodic precipitin line was probably due to the presence of albumin or a closely related protein in the HP-FSH preparations. It appeared with all preparations in the position of human serum albumin after electrophoresis and was not seen after the antiserum had been absorbed with normal human serum or human serum albumin. Moreover, an antiserum to human albumin gave a precipitin line in the same position when it was used instead of the anti-HP-FSH serum (Fig. 1D). When the HP-FSH antigens were used in higher dilutions (500  $\mu\text{g}/\text{ml}$ .) the albumin line was removed while the second line remained. The appearance of this second precipitin line varied with the purity of the HP-FSH preparations. The line of the crude material (CM 1) and of the purified material (CP 1) showed a cathodic tail which was not present when highly purified HP-FSH (SG 4) was applied. This cathodic end of the line occurred in the  $\gamma$ -globulin position and was removed after the antiserum had been absorbed by a preparation of human pituitary luteinizing hormone (HP-LH) which had been fractionated by chromatography on DEAE-cellulose<sup>4</sup>. HP-LH, on the other hand, gave a precipitin line in the  $\gamma$ -globulin position (Fig. 1E).

Evidence in favour of the anodic part of this line (in  $\alpha$ - $\beta$ -globulin position) being due to HP-FSH was obtained by eluting sections of the agar gel marked I-V in Fig. 2 after electrophoresis of purified HP-FSH (CP 1). Most activity was detected by the ovarian augmentation assay for FSH in the region of this part of the band (Table 1).

It appears, therefore, that all three HP-FSH preparations probably contained a contaminating protein related

Table 1. RESULTS OF BIO-ASSAYS WITH ELUATES OF AGAR SECTIONS

No. animals	Section	Mean ovarian wt. (mg) with 95% confidence limits
6	I	8.4 (5.7-12.8)
6	II	16.5 (11.3-24.1)
6	III	8.9 (6.1-13.0)
6	IV	4.2 (2.9-6.1)
6	V	3.6 (2.5-5.3)



to human serum albumin and that the less-purified preparations contained another contaminant closely related to HP-LH. The HP-LH is largely removed in the highly purified HP-FSH as has previously been shown by bioassays<sup>1</sup>. The immuno-electrophoretic pattern can thus be used as a rough indication of the purity of a HP-FSH preparation.

The presence of contaminants would probably interfere with the haemagglutination assay of FSH but it is possible to improve the specificity of the method by suitable absorption.

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## PHARMACOLOGY

### Effects of Noradrenaline and Isoprenaline on the Permeability of Depolarized Intestinal Smooth Muscle to Inorganic Ions

It has recently been shown<sup>1,2</sup> that stimulation of the intrinsic inhibitory nerves of the guinea-pig taenia coli can cause a transient increase in the membrane potential of the smooth muscle cells. The magnitude of the hyperpolarization was found to vary with the external potassium concentration in a way which suggested that the transmitter, almost certainly largely noradrenaline, acts by increasing the permeability of the membrane to potassium ions. According to another hypothesis<sup>3,4</sup>, adrenaline, and presumably noradrenaline, can hyperpolarize the membrane by stimulating electrogenic extrusion of sodium from the cells.

The effect of noradrenaline on the permeability of the smooth muscle membrane to sodium and potassium ions has been further investigated in the present work. We have also attempted to identify the receptors involved in the permeability change since little is known of the relative functions in intestinal muscle of the two types of catecholamine receptor (usually termed  $\alpha$  and  $\beta$  following Ahlquist<sup>5</sup>) which have been demonstrated in many tissues. A comparison was therefore made of the actions of noradrenaline and isoprenaline, which were selected as representative catecholamines having predominantly  $\alpha$ - and  $\beta$ -activating properties, respectively.

The techniques used were essentially similar to those developed during an earlier investigation of the actions of carbachol on permeability<sup>6</sup>. As before, strips of taenia coli mounted on stainless-steel holders were bathed throughout in fluid containing sufficient potassium sulphate to depolarize the cells. Radioisotopes could then be used to examine the exchange of ions between tissue and bathing fluid without the complication of changes in ion movement secondary to alterations in membrane potential.

Fig. 1 illustrates the effect of  $3 \times 10^{-7}$  g/ml. *l*-noradrenaline bitartrate on the efflux of tracer potassium from muscle equilibrated at 20° C in a bathing fluid containing 235 mM K<sup>+</sup> and 6.5 mM Na<sup>+</sup>. An increase in the rate of loss was consistently observed, whereas the same concentration of *dl*-isoprenaline sulphate was without effect. Other experiments in this series showed that this concentration of noradrenaline also enhanced inward movement of tracer potassium, isoprenaline again having little action. These results confirm that noradrenaline

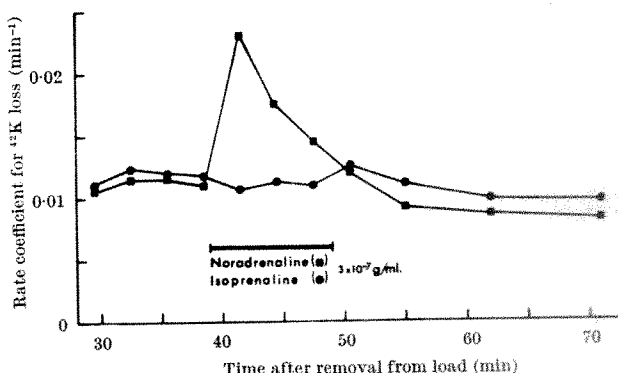


Fig. 1. Effect of *l*-noradrenaline bitartrate ( $3 \times 10^{-7}$  g/ml.) and *dl*-isoprenaline sulphate ( $3 \times 10^{-7}$  g/ml.) on the rate of loss of tracer potassium from taenia coli bathed in potassium-rich fluid. ■, Noradrenaline,  $3 \times 10^{-7}$  g/ml.; ●, isoprenaline,  $3 \times 10^{-7}$  g/ml.

can increase the permeability of the membrane to potassium ions.

Experiments of a more preliminary nature have also been made to find whether the permeability increase extends to sodium ions. These measurements were made at 10° C in a fluid containing 190 mM K<sup>+</sup> and 52 mM Na<sup>+</sup>. The lower temperature and higher sodium concentration were adopted in an attempt to overcome earlier difficulties in the analysis of tracer sodium flux at 20° C (ref. 6). It was thought necessary first to demonstrate that a substance considered to increase sodium permeability did, in fact, alter sodium uptake under these conditions. Carbachol was chosen for this purpose, and a comparison was made of the effects on sodium exchange of concentrations of carbachol and noradrenaline ( $5 \times 10^{-7}$  and  $3 \times 10^{-7}$  g/ml., respectively) shown to cause a similar increase in potassium uptake. It was found that whereas carbachol did produce a significant increase in sodium uptake, noradrenaline was without effect. The actions on sodium efflux have not yet been determined.

Since noradrenaline produced a change in permeability to potassium ions, whereas isoprenaline was ineffective, it seems likely that this effect is mediated through the  $\alpha$ -receptors. This conclusion was supported by experiments with adrenergic blocking agents (Fig. 2). The  $\alpha$ -blocking agent phentolamine hydrochloride ( $10^{-7}$  g/ml.) was found to greatly reduce the effect of noradrenaline ( $3 \times 10^{-7}$  g/ml.) on potassium exchange. Moreover, the  $\beta$ -blocking agent pronethalol ( $10^{-7}$  g/ml.) was without effect.

It is interesting in relation to these findings that catecholamines can reduce the tension that is developed

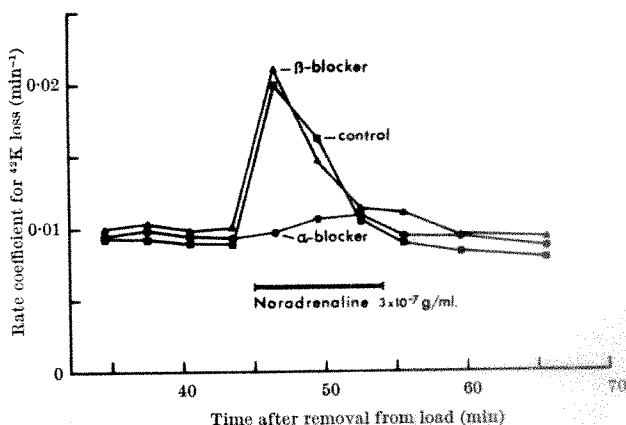


Fig. 2. Comparison of the effects of an  $\alpha$ -antagonist (phentolamine hydrochloride,  $10^{-7}$  g/ml.) and a  $\beta$ -antagonist (pronethalol,  $10^{-7}$  g/ml.) on the increase in the rate of loss of tracer potassium due to noradrenaline ( $3 \times 10^{-7}$  g/ml.). The antagonists were present in the bathing fluid from 5 min after removal of muscles from the load solution.



by depolarized smooth muscle in the presence of calcium ions<sup>7</sup>. We have found that, in the depolarized taenia, isoprenaline was much more active in this respect than noradrenaline, indicating that the effect was mediated through  $\beta$ -receptors. This was confirmed by the observation that pronethalol, at the same concentration as in the experiment illustrated in Fig. 2, strongly antagonized this action of noradrenaline.

Summarizing, our results suggest that noradrenaline can act through the  $\alpha$ -receptors to increase the permeability of the membrane to potassium, but not to sodium, ions. Since under physiological conditions the membrane potential is most probably below the potassium equilibrium potential, an increase in potassium permeability would tend to hyperpolarize the membrane and reduce the frequency of spontaneous action potentials. These  $\alpha$ -mediated effects would, in themselves, serve to account for many features of the relaxation of intestinal smooth muscle in response to catecholamines. However, under conditions where the membrane potential had been abolished, activation of the  $\beta$ -receptors was also found to cause relaxation. There is, as yet, little to suggest what mechanism underlies this response, although it seems possible that a change in the availability of metabolic energy (cf. refs. 8 and 9) might be involved. If this mechanism operates under physiological conditions, then both  $\alpha$ - and  $\beta$ -receptors would contribute to the relaxation induced by catecholamines; this conclusion is supported by other evidence<sup>10,11</sup>.

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### Neuropathological Effects of Dosing Dogs with Isonicotinic Hydrazide and with its Methanesulphonate Derivative

THE neuropathological effects of dosing dogs over a prolonged period with some monoamine oxidase inhibitors have already been described by us<sup>1</sup>. Two of the drugs, phenyl-iso-propyl hydrazine ('JB 516' or 'Catron') and tetra-hydronaphthyl hydrazine, produced strikingly focal bilateral degeneration of the inferior olives. Phenelzine ('Nardil') and indanyl carbethoxy hydrazine resulted in oedema and disintegration of sub-cortical myelin in the cerebrum, accompanied by microglial proliferation. It was suggested that perhaps a contributory cause of both types of lesion might be ascribed to elevated levels of brain serotonin.

Opportunity has since arisen to examine the effects of dosing dogs over a prolonged period with two hydrazides, which are not monoamine oxidase inhibitors, and this has thrown light on the pathogenesis of the cerebral oedema seen in the previous experiments with phenelzine and indanyl carbethoxy hydrazine. The two compounds in question are isonicotinic hydrazide (isoniazid) and its methanesulphonate derivative. Oral administration of these drugs was carried out for periods of up to 6 months



Fig. 1. Section of sub-cortical white matter from dog (No. 285) dosed with 'Isoniazid' (15 mg/kg/day) showing clefts and spaces, disintegration of myelin, glial activation and capillary hyperplasia. Tissue fixed in formal saline, embedded in paraffin and section stained with haematoxylin and eosin



Fig. 2. Section of sub-cortical myelin from control dog. Fixation, embedding, staining and magnification the same as for Fig. 1

using the method described by us. After preliminary trials, the dose-levels finally used were: isoniazid 15 and 5 mg/kg/day and for its methanesulphonate derivative 20, 10 and 6 mg/kg/day.

The neuropathological effects of the two compounds appear to be similar. In both, at the higher dose-levels, there is separation of fibres in certain areas of the sub-cortical myelin. Clefts or spaces occur, accompanied by activation of microglia and hypertrophy of astrocytes (Figs. 1 and 2). There is pallor of affected regions, with variable staining of myelin and occasional ballooning of the sheath suggesting its disintegration. Frozen sections show the presence of only a few lipid-filled phagocytes around vessels. The axons remain intact, and the majority of cortical neurones appear healthy although there is microcavitation of the ground substance.

The neuropathological effects of dosing dogs with 15 mg/kg/day of isoniazid are about the same as those of dosing with 20 mg/kg/day of its methanesulphonate derivative, which on this evidence would appear to be slightly less toxic than isoniazid. In two dogs dosed with the methanesulphonate at 10 mg/kg/day there was, however, severe laminar necrosis of the cerebral cortex, a change not seen with isoniazid.



The effect of isoniazid and its methanosulphonate derivative on the brain of the dog appears to be the production of oedema in the cerebral white matter, leading to loss of myelin, and accompanied by glial proliferation. These changes are very similar both morphologically and in distribution to those produced previously with phenelzine and indanyl carbethoxy hydrazine, but as isoniazid and its methanosulphonate derivative are not monoamine oxidase inhibitors, it would suggest that the changes in the myelin produced by all four drugs are not due to monoamine oxidase inhibition. The histological changes are reminiscent of those induced in rats by administration of triethyl tin compounds by Magee *et al.*<sup>2</sup>, in which there is widespread oedema of myelin, with spaces between the fibres, but axons and myelin are intact; later work has shown that the oedema accumulates in large clefts within myelin sheaths<sup>3</sup>.

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## HAEMATOLOGY

### Relationship between Activity of Pyruvate Kinase and Age of the Normal Human Erythrocyte

MATURE erythrocytes of human beings derive energy chiefly through anaerobic glycolysis. Pyruvate kinase catalyses a key<sup>1,2</sup>, adenosine triphosphate-generating glycolytic reaction. We have examined the relationship between the activity of pyruvate kinase and the age of the normal human red cell.

Freshly drawn venous blood, anticoagulated with heparin, was obtained from each of six normal adult males. A modification<sup>3</sup> of a method of serial centrifugation<sup>4</sup> was used to separate erythrocytes into fractions containing relatively young and relatively old packed red cells. Reticulocyte, erythrocyte, leucocyte, and platelet counts of these fractions were determined. Haemolysates were prepared by freezing and thawing 1 ml. of each fraction of red cells three times and by subsequent addition of distilled water. Activities of both pyruvate kinase and glucose-6-phosphate dehydrogenase (G-6-PD) were measured. Available evidence indicates that the activity of G-6-PD in the normal human red cell decreases as the cell ages *in vivo*<sup>5,6</sup>.

Results are shown in Table 1. Reticulocyte counts and activities of G-6-PD provide evidence that centrifugation effected significant separation of young and old cells. The mean activity of pyruvate kinase in haemolysates of relatively old red cells proved 45 per cent less than that in haemolysates of relatively young red cells. The mean platelet count of fractions of relatively young red cells (78,000/mm<sup>3</sup>) was less than that of fractions of relatively old cells (103,000/mm<sup>3</sup>); contamination by platelets, therefore, does not account for the difference detected between the mean activities of pyruvate kinase in haemolysates of young and old red cells. The mean leucocyte count of fractions containing relatively young red cells was 840/mm<sup>3</sup> and that of fractions containing relatively old cells was 340/mm<sup>3</sup>. We investigated the activity of pyruvate kinase in lysates of leucocytes purified by the method of Fallon *et al.*<sup>11</sup>. These investigations indicated that less than 6 per cent of the activity of pyruvate kinase in any of the haemolysates examined (Table 1) was

attributable to contamination by leucocytes. Contamination by leucocytes accounted for approximately 3-4 per cent of the mean activity of pyruvate kinase in haemolysates of relatively young red cells and for approximately 2 per cent of the mean activity of this enzyme in haemolysates of relatively old red cells. The results indicate that the activity of pyruvate kinase in the normal human red cell decreases markedly as the cell ages *in vivo*. These findings coincide with the results of investigations reported by Tanaka, Valentine and Miwa<sup>9</sup>. The results suggest that a decrease in activity of pyruvate kinase sustained during ageing of the normal human red cell may be important in senescence of the cell.

Table 1. NORMAL HUMAN RED CELLS SEPARATED BY SERIAL CENTRIFUGATION INTO FRACTIONS CONTAINING RELATIVELY YOUNG AND RELATIVELY OLD CELLS

Subject	Reticulocytes (%)		Activity of G-6-PD*		Activity of pyruvate kinase†	
	Young cells	Old cells	Young cells	Old cells	Young cells	Old cells
1	1.5	0.2	362	256	17.0	9.2
2	2.1	0.4	267	190	15.9	10.8
3	2.9	0.4	286	199	17.4	10.1
4	2.2	0.5	277	183	17.1	10.1
5	2.8	0.3	275	149	13.7	6.0
6	5.7	0.1	319	187	19.4	10.1
Mean	2.9	0.3	298	194	16.8	9.3

Significance‡ 0.001 < P < 0.01

P < 0.001

P < 0.001

\* Activity of glucose-6-phosphate dehydrogenase, assayed by a modification of the method of Glock and McLean (ref. 7) similar to that used by Zinkham and Lenhard (ref. 8), expressed as  $\mu$ moles nicotinamide-adenine dinucleotide phosphate reduced/g haemoglobin/h.

† Activity of pyruvate kinase, assayed by a modification of the method described by Tanaka, Valentine and Miwa (ref. 9) (modifications consisted of doubling the concentration of adenosine diphosphate and trebling the concentration of phosphoenolpyruvate in the assay mixture), expressed as  $\mu$ moles nicotinamide-adenine dinucleotide formed/g haemoglobin/min.

‡ Evaluated by Student's *t*-test as outlined by Fisher (ref. 10).

It has not been established, however, that activity of pyruvate kinase falls below a rate-limiting level during the life span of the normal red cell. Hexokinase has been considered a pacemaker of glycolysis in the human red cell and evidence has been presented indicating that activity of hexokinase decreases substantially as normal red cells age *in vivo*<sup>3</sup>. Many other age-related changes in the human erythrocyte have been reported<sup>12</sup>. Considerable additional information will be required for clear delineation of the factors which limit the survival of the normal human red cell.

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## PATHOLOGY

## Tumour Transplantation: a Simple Technique

NUMEROUS techniques of tumour transplantation have been used since Hanau<sup>1</sup> established the transplantability of animal tumours in 1889. One of us reported a technique using a curved surgical needle in 1963 (refs. 2 and 3).

We have now developed an even simpler method of tumour transplantation which makes use of a modified shoemaker's sewing machine needle with the tip ground down almost to the eye (Fig. 1). A 1-3 cm tumour is suitable for transplanting purposes. The donor animal is anaesthetized and immobilized on a cork board. The shoemaker's needle is plunged once into the unexposed tumour, removed and inserted horizontally into the unprepared thigh of the unanaesthetized recipient. As the needle is withdrawn a finger is pressed firmly against the thigh. The five or ten thousand tumour cells that were initially caught in the eye of the needle remain in the recipient. One donor tumour can be used for twenty or more recipients.

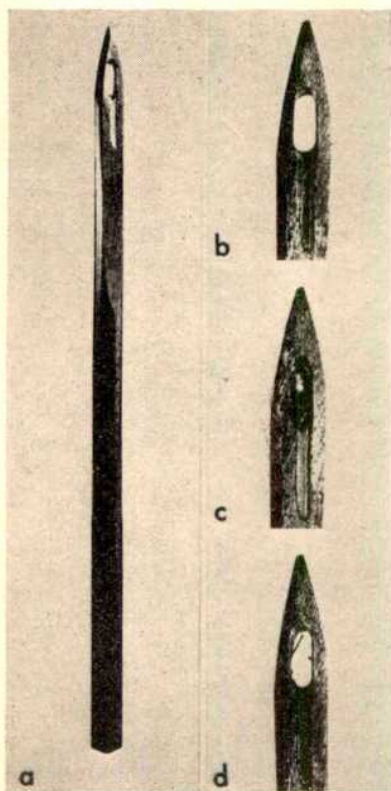


Fig. 1. *a*, Shoemaker's needle, ground down. *b*, Tip of needle empty ready for plunging into donor tumour. *c*, Tip of needle full after being plunged into donor tumour. *d*, Tip of needle relatively empty after withdrawal from recipient thigh.

A *C<sub>3</sub>H* rhabdomyosarcoma transplanted using this technique becomes evident in two or three weeks in almost 100 per cent of the recipients.

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## IMMUNOLOGY

## Failure of Spleen Cells from Immunologically Tolerant Mice to form Antibody Plaques to Sheep Erythrocytes in Agar Gel

DURING the past decade there have been numerous investigations concerned with establishment of specific immunological tolerance or unresponsiveness to either viable tissues or to a variety of non-living antigens, such as soluble serum proteins, bacterial products, and mammalian or avian erythrocytes<sup>1,2</sup>. Most investigators believe that tolerance is due to a central inhibition of antibody formation and not to masking or binding of antibody by excess antigen<sup>3</sup>.

Acquired tolerance to non-living antigens is often demonstrated by lack of an expected immune response, such as failure to demonstrate circulating antibodies following immunization, failure to induce anaphylactic shock or an Arthus reaction, or failure to establish delayed or immediate skin reactivity following attempted sensitization. It has not been unequivocally established in such investigations whether or not the lack of demonstrable immunological activity or suppressed antibody titres is caused by absence of antibody-forming cells, or due to formation of low concentrations of antibody by relatively normal numbers of immunologically competent cells.

Fluorescent antibody investigations by Sercarz and Coons<sup>4,5</sup> and cell transfer work by Brooke and Karnowsky<sup>6</sup> have demonstrated an absence of cells forming specific antibody to a soluble serum protein antigen or to pneumococcal polysaccharide in tolerant adult mice. Others have also reported failure to transfer specific antibody-forming activity with lymphoid cells from tolerant donors<sup>7-9</sup>. This communication is concerned with investigations utilizing the 'antibody plaque' technique of Jerne<sup>10</sup> to demonstrate directly the relative absence of haemolysin-forming cells in young mice receiving throughout early life tolerance-inducing injections of sheep red blood cells.

For these experiments, litters of new-born *NIH* albino mice were injected intraperitoneally with 0.05-0.1 ml. of a 50 per cent suspension of washed sheep red blood cells twice a week from birth to shortly before testing. As controls, litter mates were injected either with saline or left untreated. At 6-8 weeks of age, the mice were challenged intravenously with 0.1 ml. of a 20 per cent suspension of sheep erythrocytes. The mice were bled from the retro-orbital plexus before and after immunization to obtain serum specimens for determination of serum haemolysins to sheep erythrocytes. At varying intervals after challenge immunization, individual mice were killed, spleens obtained, and cell suspensions prepared<sup>9</sup>. Viability was determined by trypan blue stain, and cell counts were obtained with a haemocytometer. A 0.1 ml. of a suspension of cells containing  $10 \times 10^6$  to  $5 \times 10^5$  nucleated cells per ml. was used for pouring agar plates essentially as described by Jerne *et al.*<sup>10,11</sup>. In brief, 0.1 ml. of the test spleen cell suspension was added to 2.0 ml. of melted 0.7 per cent Noble agar containing 0.1 ml. of a 10 per cent suspension of freshly washed sheep erythrocytes and 1.0 mg DEAE. This mixture was carefully layered over a previously prepared 3-mm thick layer of agar in a 100-mm diameter Petri plate. After the upper cell-agar layer had solidified, the plates were incubated for 1 h at 37° C. The plates were then treated with 5.0 ml. of a 1:10 dilution of fresh guinea-pig complement and incubated for an additional 30 min at 37° C. Following this incubation, zones of haemolysis could be readily seen against the light pink background of unlysed erythrocytes, indicating lymphoid cells which had secreted haemolysins to sheep red blood cells<sup>10,11</sup>.

Table 1 indicates the results observed after plating lymphoid cell suspensions obtained from mice immun-



ized at 6 weeks of age and either previously untreated or injected with sheep erythrocytes at birth and twice weekly thereafter. Whereas approximately 1,200–1,500 antibody plaques were formed per million viable nucleated lymphoid cells from control immunized mice, fewer than 50 plaques were formed with the same number of spleen cells from mice injected with sheep erythrocytes from birth to the fifth week of age. The mean peak haemolysin titres of immune control mice averaged 1:450. Haemolysin titres of mice tolerant to sheep red blood cells averaged 1:60.

Table 1. SERUM HAEMOLYSIN TITRES AND NUMBER OF HAEMOLYTIC PLAQUES FORMED WITH SPLEEN CELL SUSPENSIONS FROM CONTROL AND TOLERANT MICE BEFORE AND AFTER IMMUNIZATION WITH SHEEP ERYTHROCYTES

Group	Day following immunization†	No. of mice tested	Mean haemolysin titres	No. of mice with No. of haemolytic plaques per 10 <sup>6</sup> lymphoid cells plated in range of:			Mean plaque count/10 <sup>6</sup> cells plated
				Less than 50	51–500	500 or more	
Tolerant*	0	12	<1:10	12	0	0	0.2
	+2	10	<1:10	10	0	0	2.9
	+4	10	1:32	9	1	0	32.6
	+6	8	1:58	8	0	0	23.9
	+10	11	1:40	10	1	0	17.5
Normal	0	10	<1:10	10	0	0	0.2
	+2	8	1:62	1	1	0	38.9
	+4	12	1:364	2	5	5	1,243.5
	+6	10	1:404	2	3	5	1,140.0
	+10	10	1:160	3	6	1	245.5

\* Injected intraperitoneally at birth and twice weekly thereafter for 5 weeks with 0.1 ml. of 20 per cent suspension washed sheep erythrocytes.

† Injected intravenously at 6 weeks of age with 0.1 ml. sheep erythrocytes.

Table 2 indicates mean serum titres and number of haemolytic plaques formed per million spleen cells from control and tolerant animals immunized at several age intervals. All mice injected with sheep erythrocytes at birth and periodically thereafter exhibited markedly depressed antibody titres and plaque counts in comparison to controls. Maximum depression of plaque counts was observed with mice challenged with sheep red blood cells 1–2 weeks following cessation of the tolerance-inducing injections of sheep erythrocytes. An interval of several weeks between the last tolerance-maintaining red blood cell injection and the intravenous immunization resulted in increased plaque counts and higher haemolysin titres (Table 2).

Table 2. SERUM HAEMOLYSIN TITRES AND ANTIBODY PLAQUE FORMATION BY SPLEEN CELL SUSPENSIONS FROM TOLERANT AND CONTROL MICE IMMUNIZED WITH SHEEP ERYTHROCYTES AT VARYING AGES

Mouse group	Age at challenge immunization† (weeks)	No. of mice	Mean peak haemolysin titres	Haemolytic plaques per 10 <sup>6</sup> spleen cells plated†	
				Average	Range
Tolerant*	6	10	1:39	36	1–94
	8	10	1:78	43	12–265
	10	10	1:85	87	36–765
	12	8	1:214	689	38–1,148
Normal	6	9	1:438	1,128	648–2,543
	8	10	1:390	1,520	548–2,945
	12	10	1:412	1,438	675–2,780

\* Injected intraperitoneally with sheep erythrocytes at birth and twice weekly thereafter for 5 weeks.

† Injected intravenously with sheep erythrocytes at age indicated.

‡ Spleens obtained for antibody plaque assay four days after challenge immunization.

Although there was a marked difference in the number of plaque-forming cells in suspensions of spleen cells from normal and mice tolerant to sheep red blood cells following immunization, the relative sizes of individual plaques were similar. There was no indication of reduced haemolysin secretion activity by individual cells from the tolerant mice. The only discernible difference between control and tolerant mice was in the number of plaques formed per million viable nucleated spleen cells. In addition, mice rendered tolerant to *Shigella* micro-organisms at birth responded normally with haemolysin formation and antibody plaque production when immunized with sheep red blood cells<sup>13</sup>.

These results demonstrate that injection of mice from birth with relatively large quantities of foreign erythrocytes markedly suppresses not only their capability of forming circulating haemolysins, but also suppresses the absolute number of lymphoid cells capable of forming localized zones of haemolysis in agar. Such marked inhibition of plaque-forming ability persisted at least 2–3 weeks following cessation of red blood cell injection. In further experiments<sup>13</sup>, it has been observed that tissue culture suspensions of spleen cells from tolerant and from normal mice also differ markedly in ability to form antibody plaques. Suspensions of lymphoid cells from normal animals immunized with sheep erythrocytes readily form plaques following incubation in tissue culture medium for a period of 1–10 days. Similar suspensions from tolerant animals are much less capable of forming plaques either initially or following incubation in tissue culture medium for a period up to 10–15 days<sup>12</sup>.

The results of these experiments support the belief that specific antibody-forming cells are absent or in low numbers in lymphoid tissue of animals exhibiting a specific acquired tolerance to non-living antigens. It seems unlikely that low post-challenge haemagglutinin titres in mice rendered tolerant to sheep erythrocytes are due to suppressed antibody formation by many cells or due to rapid elimination of normal levels of antibody bound to 'excess' antigen. Similarity in size of individual plaques from tolerant and control mice suggests that individual cells competent to make haemolysins in tolerant animals form antibody similar in quantity to amounts formed by 'normal' cells in control animals.

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### Immunoglobulin-levels in Mice undergoing Graft-versus-host Reaction

It is generally admitted that mice undergoing graft-versus-host reaction reveal diminished immunological competence. In particular, the antibody production following antigenic challenge is known to be depressed<sup>1–3</sup>, although there is little conclusive evidence concerning the behaviour of immunoglobulins<sup>4,5</sup>. This particular aspect of the graft-versus-host reaction has been examined by us.

Adult (C57BL × CBA)<sub>F</sub><sub>1</sub> hybrid mice of both sexes were injected intravenously with 30 × 10<sup>6</sup> spleen cells from C57BL donors. Donors and recipients were matched according to sex. Control animals received (C57BL × CBA)<sub>F</sub><sub>1</sub> hybrid cells, using the same technical procedure. A semi-quantitative dosage of immunoglobulins was performed by the micro-modification of the Ouchterlony technique<sup>6</sup>, using specific antisera prepared against purified immunoglobulin fractions of mice<sup>7</sup>. Thus, the three immunoglobulins (IgG, IgA and IgM) could be

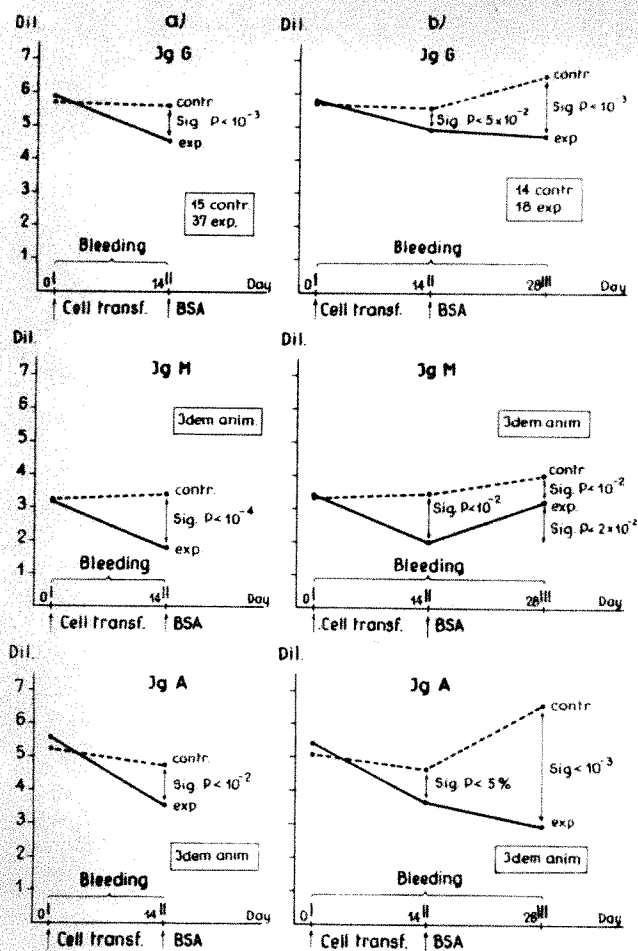


Fig. 1. Immunoglobulin-levels in experimental and control animals

evaluated separately. Total protein values were determined as well, using Folin's method<sup>8</sup>.

Anti-bovine serum albumin antibodies were titrated by passive haemagglutination<sup>9</sup>.

The first signs of the graft-versus-host reaction could be observed at the end of the first week after cell transfer. At the end of the second week the syndrome was well established as judged from the decrease in body-weight (10–20 per cent) and the drop of both total leucocyte and lymphocyte counts (50–70 per cent) with respect to initial values. Simultaneously the levels of all the three immunoglobulins were significantly reduced as compared with initial values and simultaneous control values of animals treated with hybrid cells (Fig. 1). At day 14 after the cell transfer, the mice were stimulated with 50γ of bovine serum albumin, in complete Freund's adjuvant, injected into both hind pads. At the end of the third week the mortality rates were 61 per cent; all control animals were in a perfectly healthy condition. The surviving experimental animals checked at day 28 presented a progressive decrease of the IgG and the IgA globulins, but a significant increase of the IgM fraction was recorded. Control animals displayed a significant rise in all the three Ig fractions (Fig. 1b). Although a drop in total protein values was recorded during the 4 weeks following cell transfer, this drop affected both experimental and control animals in a similar manner and could be attributed to successive bleedings. Anti-bovine serum albumin antibodies, as examined by the passive haemagglutination method 14 days after challenge, were greatly depressed in the experimental group (Table 1). It was further observed that animals undergoing the graft-versus-host reaction, as compared with controls, developed a significantly

lesser local inflammatory reaction at the site of bovine serum albumin plus Freund's adjuvant injection. The reading of reactions was scaled from 0 to +++; results are given in Table 2.

Table 1. PASSIVE HAEMAGGLUTINATION STUDY FOR PRESENCE OF ANTI-BSA ANTIBODIES AT DAY 14 AFTER IMMUNIZATION WITH BOVINE SERUM ALBUMIN

Dil.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Exp.	4	2	1			1					1					9
Contr.		1					1	2	3	5	4	2	2		1	21
Pos. contr.																2
Neg. contr.	1															1

Titres are given in reciprocals of serial doubling dilutions numbered as 1–14, corresponding to dilutions 10<sup>-1</sup>–81–920<sup>-1</sup>.

Table 2. LOCAL INFLAMMATORY REACTIONS AFTER THE INJECTION OF BOVINE SERUM ALBUMIN WITH FREUND'S ADJUVANT

Intensity	Exp.	Contr.	Total
0	8	0	8
+	7	3	10
++	5	17	22
+++	1	15	16
Total	21	35	56

$\chi^2 = 26.55$ ,  $P < 10^{-3}$ .

(1) In the course of the acute phase of the graft-versus-host reaction in adult non-irradiated mice a drop in the levels of all three immunoglobulins was observed. The decrease of IgG and IgA values continues in mice surviving the acute phase of the reaction despite antigenic stimulation, although the IgM level tends to rise again and reaches almost initial values.

(2) The antibody response against bovine serum albumin was significantly depressed in animals undergoing the graft-versus-host reaction.

(3) Animals undergoing the graft-versus-host reaction are less susceptible than controls to local inflammatory reactions to a protein antigen incorporated in Freund's adjuvant.

(4) Our observations are in agreement with the poor immunological reactivity generally observed in animals undergoing graft-versus-host reactions.

It seems likely that the reduction of immunoglobulin-levels, of antibody production and of the local inflammatory reactions plays an important part in the establishment of the clinical image of the graft-versus-host reaction.

We thank Prof. P. Grabar, Dr. G. A. Voisin and Dr. P. Burtin for their advice and Mrs. Jeanine Schaffner and Mr. Claude Bernard for their assistance.

**Addendum.** We have examined three immunoglobulins without having considered a recent work of Fahey *et al.*<sup>10,11</sup> showing the existence in mice of a fourth immunoglobulin, which is assimilated by these authors to a  $\beta_2$  A globulin. The protein which we have called IgA corresponds to Fahey's  $\gamma_1$  7 S globulin; the one called by us IgG should correspond to the  $\gamma_2$  7 S globulins.

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## RADIOBIOLOGY

Effect of X-ray Dose on the Growth Rate and Delay of the First Post-irradiation Division in a Strain of *Paramecium aurelia*

*Paramecium* is particularly useful in the investigation of the effects of radiation on growth and division. Its size and moderately long generation-time permit accurate accounting of growth. Kimball, Geckler and Gaither<sup>1</sup> have reported an extensive investigation of division effects induced by mustards, ultra-violet light and X-rays on *Paramecium aurelia* and *Paramecium caudatum*. The action of ultra-violet light they observed is rather complex and is in general accord with the action of this agent on a number of protozoa<sup>2-4</sup> (including my investigations<sup>5,6</sup>). However, a series of X-ray experiments has shown some differences from the type of division effects observed by Kimball *et al.* in X-irradiated *Paramecium aurelia*, perhaps because a different strain was used.

This strain of *Paramecium aurelia* was isolated in Texas in 1960 and has been grown as a stock culture since then. Test animals were always grown as isolates in depression slides. Growth was in bacterized lettuce medium prepared in the manner suggested by Sonneborn<sup>7</sup> with *E. coli* as the food bacteria; the strain of *E. coli* was isolated locally. All growth was at room temperature,  $24.5^\circ \pm 1.5^\circ \text{C}$ .

Log phase animals were irradiated with X-rays from a Picker Vangard 250 X-ray machine at 43 krad/min (half value layer, 0.5 mm Al). Dosimetry was carried out with a ferrous sulphate chemical dosimeter using the parameters suggested by Shalek, Sinclair and Calkins<sup>8</sup>. Animals were concentrated by centrifugation to approximately 500–2,000/ml. and a 2-ml. sample was irradiated. No specific radical scavenger such as broth was added to the medium before irradiation; the addition of broth to cultures leads to complications because it promotes an excessive bacterial growth harmful to the protozoa. The animals were irradiated to a desired dose-level, the machine was stopped, an amount of unirradiated medium equal to the sample was added and, after thorough mixing using a transfer pipette, a sample was removed. The X-ray machine was then started and additional doses were given. The samples were diluted with a small amount

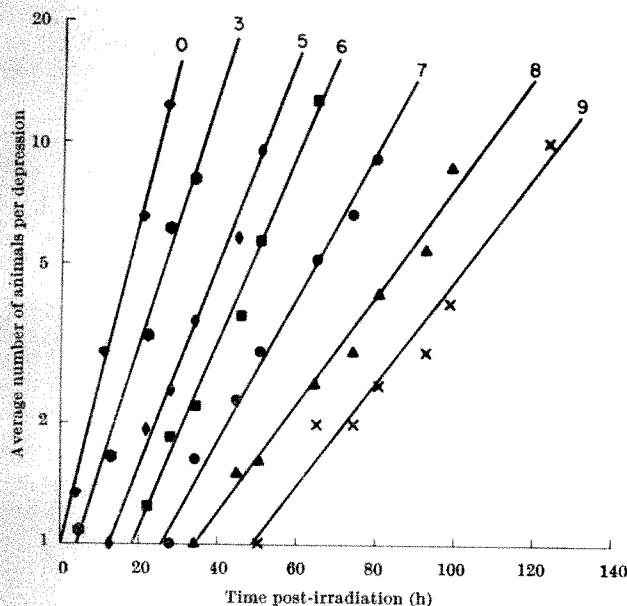


Fig. 1. Log-linear plot of increase in average numbers of X-irradiated paramecia as a function of post-irradiation time. Lines are fitted through the points 'by eye'; numbers at the top of various lines indicate X-ray doses in min at 43 krad/min. Actual numbers (not logarithms) are indicated on the vertical axis

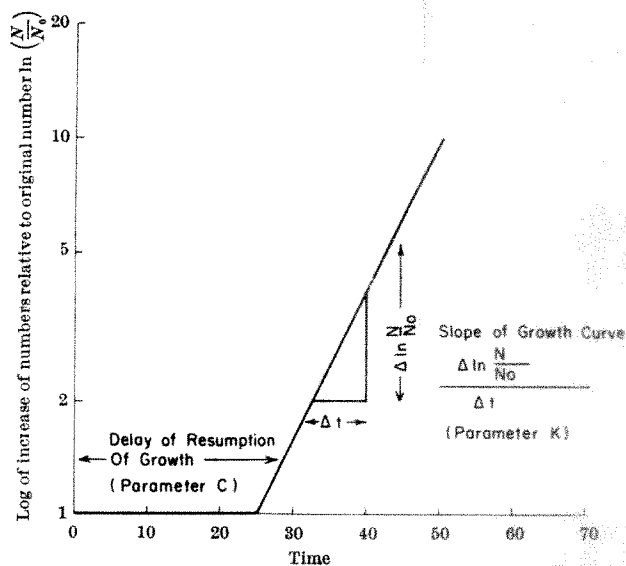


Fig. 2. Idealized plot of growth following irradiation. The two parameters (*C* and *K*) described in the text are indicated. Actual numbers (not logarithms) are indicated on the vertical axis

of fresh medium and within 1 h of irradiation single animals were isolated from the irradiated samples into depressions containing approximately 4 drops of medium.

The isolated animals were counted at intervals suitable for the determination of growth rate. Counts on control animals, which continued to grow with negligible delay on isolation, were made at 4- to 10-h intervals until the depressions held a large population. The maximum population a depression will support depends on the food supply, and with the amount of medium used populations ordinarily reached 32–100 animals before divisions completely stopped. Growth to 16 animals was used as the criterion of survival and counts were not necessary after a line descending from an isolated animal reached this number. X-irradiated animals which resumed division continued, with only rare exceptions, to grow to the limit imposed by the food available.

Fig. 1 is a plot of the average number of animals in a depression as a function of time following various X-ray doses. This figure is taken from one particular experiment but it is typical of a number of such experiments. Certain aspects of the response to X-irradiation are clearly illustrated in this figure. The X-rays have produced a progressive delay of the first division following the exposure. When growth does resume, and for the first four divisions after the resumption, the rate of growth of the population is constant within the resolution of the method of growth rate measurement used. It can also be seen in Fig. 1 that the growth rate is progressively reduced with increasing X-ray dose.

The growth of each surviving line descending from an isolated animal has been analysed in terms of a model using two parameters illustrated in Fig. 2. According to the model, when growth is resumed after irradiation it will be at a constant rate for the isolated animals and all their progeny. This type of growth would, in fact, be stepwise because if all progeny grew at the same rate a perfectly synchronized culture would descend from a single animal. The growth rate (measured as 1/doubling time) could be measured by determining the time between a point on one step of the growth curve and the same point on the next step, for example, the time between separation of an animal into two daughters and the separation of those daughters into four animals. The reference time in the cycle could be taken anywhere during the cycle, but division of animals is the only point which can conveniently be recognized, without using procedures which stop further observation.

The growth rate could be determined as indicated here by observations of the exact interval between divisions of isolated animals; however, the number of observations required for growth rate determinations in this manner would impose a severe limitation on the scope of the investigation. Instead, the growth rate of isolated lines has been determined by fitting values of  $K$  and  $C$  to the observed data on increase of numbers. The equation

$$\ln_2 \frac{N}{N_0} = K(t - C)$$

is assumed to be the mathematical relation of increase in numbers with time. In the equation  $N$  is the observed number of animals,  $\ln_2$  is log to the base 2,  $K$  is the growth rate,  $t$  is the elapsed time from the starting point (where  $N = N_0$ ). This equation would accurately represent the growth of a large random phase culture, but deviations are to be expected for small numbers of animals which increase in numbers in a discontinuous manner. The parameter  $C$  will, in small populations, depend on the average amount of the growth cycle completed when the animals are isolated; the effects of radiation on the resumption of growth, as shown by Fig. 1, can also be manifest by the constant  $C$ .

If observations of growth of isolated animals were made many times during each growth cycle (which was not the case) then the best fit of the data, using the model equation, would pass through the centre of the growth steps. Observations were made without any relation to the growth cycle and are observed to be at random locations on the steps. Thus large errors in growth rate determinations for single animals can occur but, assuming random errors, by combining data on a large number of isolated animals an accurate average growth rate can be determined.

Since high-radiation doses can produce quite long delays before growth resumes, and the inclusion of a large number of observations of a single animal (before growth resumed) would tend to reduce the apparent growth rate, counts before the first post-irradiation division were not used for growth rate or delay determinations. It was also observed that, under the conditions of the experiment, growth began to slow after 16 animals were present in a depression (the growth rate of the animals began to be limited by the available food), so the data on growth were not used after this. With these limitations in mind it is obvious that the data presented concern only the first four post-irradiation divisions; effects on growth rate after extended periods of growth have not been investigated.

Parameters  $C$  and  $K$  were determined which best fit the counts of each surviving line descending from a single animal. All animals in an experiment receiving the same treatment were then analysed together and the standard errors of the parameters  $C$  and  $K$  were calculated. The actual computations were done on an IBM 1410 computer using a special programme prepared by Dr. Alan Ross and Mr. Stuart Riley of this Institution.

Fig. 3 shows the relation of radiation dose to growth rate  $K$ . Results from several experiments are plotted on this figure. The standard errors for the points are indicated by the bars. Data from two experiments which seem to show the trend of response most clearly are plotted. Data from a number of other experiments are consistent with the effects shown in the various figures.

Fig. 4 is a plot of the delay of resumption of growth induced by various radiation doses, that is, values of  $C$  for the irradiated animals minus the  $C$  value for the controls, plotted against the square of the dose. The two experiments are again shown by different symbols and the bars indicate standard errors.

Fig. 3 shows that, in *Paramecium aurelia*, recovery from some aspects of X-ray damage is not complete by the time of first division. The radiation-induced depression of growth rate continues for a number of early divisions. The persistence of the effect suggests it is not

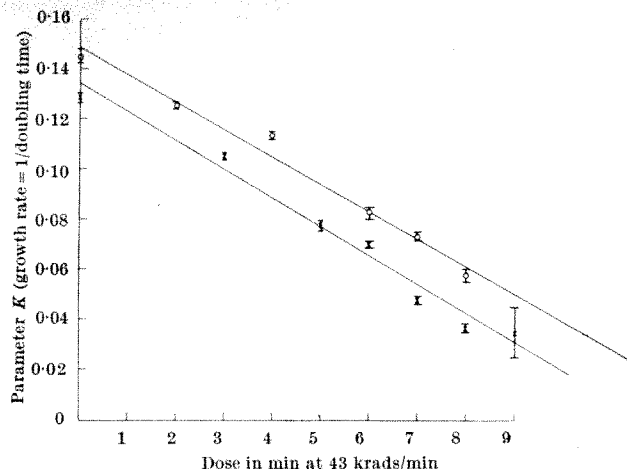


Fig. 3. A plot of growth rate (Parameter  $K$ ) as a function of X-ray dose. Two different experiments are shown by the different symbols. Bars indicate standard errors and the lines are drawn through the points 'by eye'.

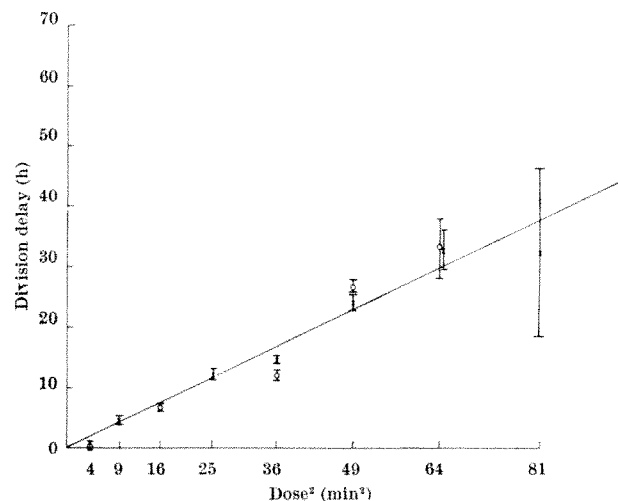


Fig. 4. A plot of the radiation-induced delay of division (Parameter  $C_{\text{irradiated}}$  minus  $C_{\text{control}}$ ) as a function of the dose squared. Symbols indicate results from the same two experiments as in Fig. 3. Bars indicate standard errors and the line is drawn 'by eye'.

merely destruction of some metabolite but probably a depression of the synthetic capacity of the irradiated animals inherited for a few generations at least. The linear relation between depression of growth and dose is amenable to many hypotheses regarding the nature of the X-ray-induced lesion. One interpretation which seems to fit the data is that the growth is depressed by lesions which are equivalent to mutations in the macronucleus. Information from these damaged DNA molecules would not function properly, but because of the redundant nature of the information in the macronucleus the undamaged duplicate information would permit growth at a reduced rate. Since division of the macronucleus is not perfectly regular, individuals with fewer mutations could occur and show an increased growth rate, representing partial recovery.

The dependence of division delay on the square of the dose is highly indicative of a situation where two lesions interact. Experiments with fractionated doses, to be reported elsewhere, should give an indication of the stability of such lesions if this is the correct interpretation. An interpretation suggested by the dose-delay relation is that a pair of X-ray-induced lesions in the macronucleus interact to cause cross-linkage of DNA molecules; the number of these cross-linkages would depend on the square of the dose. A mechanism for removing cross-linkages would be required before normal growth and

division could be resumed. The time to restore the DNA to its original condition might then be expected to depend on the extent of cross-linkage and thus on the dose squared.

The experiments reported here are not sufficiently definitive to rule out many alternative explanations to those proposed, but the relations between dose and response do suggest hypotheses susceptible to test by experiment.

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### A Method for reacting Submicrogram Quantities of Radioactive Substances; Observations on the Preparation of [<sup>131</sup>I]-Iodotyrosines of Very High Specific Activities

We described recently a method of preparation of [<sup>131</sup>I]-iodotyrosines of very high specific activity<sup>1,2</sup>. Further work has shown a considerable difference in the specific activities of these substances obtained in a number of iodinations though the experimental conditions were kept as nearly identical as possible.

Eighty-nine iodinations were carried out with thirty-six different batches of [<sup>131</sup>I]-iodine (Radiochemical Centre, Amersham, Code No. IBS.3) by the method described in our paper<sup>1</sup>. The addition of the various solutions into the reaction flask (1.00-ml. 'E-MIL' volumetric flask) was done with Hamilton syringes and micropipettes. However, even using the same batch of <sup>131</sup>I in a number of successive experiments performed in the course of an hour, the specific activities of the reaction products varied considerably. For example, eleven successive iodinations using 0.09 µg of L-tyrosine from the same standard solution gave monoiodotyrosines with specific activities varying between 0.3 and 1.7 c./µmole, while the specific activities of the di-iodotyrosines varied between 0.6-3.4 c./µmole. In another series of four iodinations, using 0.05 µg of L-tyrosine, the specific activities of di-iodotyrosines varied between 1.3 and 2.5 c./µmole.

We noticed that it was very difficult to discharge the small volumes (10-25 µl.) of successive reactants so near each other that they mixed thoroughly with sufficient speed, especially as the reactions with these small amounts of substance are completed in a few seconds. To obviate these difficulties we proceeded as follows: a Hamilton syringe (total capacity 100 µl.) was linked through a specially adjusted connector (Tully Anaesthetic Co.; Luke's female Luerlock gland connexion No. 2) to a 250-mm length of polytetrafluorethylene tubing (A.E.I. Plastics, Ltd., size 0.023 in. internal diameter, wall thickness 0.010 in.; type TWT 24). The connector carried on one end a soldered-in Luer fitting for connecting the syringe; on the other end of the connector the polytetrafluorethylene tubing was threaded through a tight-fitting hole in a piece of rubber which was then compressed by the movement of a screw of the connector.

Moving the syringe, 25 µl. of the chloramine-T solution were first sucked into the tubing, followed by 5 µl. of air;

Table 1

	µg L-Tyrosine	<sup>131</sup> I-MIT (c./µmole)	<sup>131</sup> I-DIT (c./µmole)
First set	0.04	1.80	3.54
	0.20	1.61	3.20
	1.00	0.29	0.58
Second set	0.04	1.99	3.97
	0.18	1.69	3.38
	0.91	0.29	0.58
Third set	0.04	2.26	4.51
	0.18	1.74	3.48
	0.91	0.33	0.65

then 25 µl. of the L-tyrosine solution were sucked into the tubing followed by 5 µl. of air; and 20-25 µl. of radioactive iodine solution (100 µl. = approx. 20 mc.) were sucked into the same tube, again followed by 5 µl. of air. In this way all the reactants were contained in the polytetrafluorethylene tube, separated from each other by long air bubbles, and the length of tubing was such that the solutions were far enough from the syringe to prevent contamination.

To carry out the reaction, the tubing was firmly introduced to the bottom of the reaction vessel, and the reactants expelled at a steady rate. In this way they were not only discharged into the same place within the reaction vessel, but the succeeding air bubbles mixed the solutions thoroughly. To terminate the reaction the metabisulphite solution (100 µl.) was added through another length of tubing. The relatively inexpensive polytetrafluorethylene tubing was discarded after use.

Three sets of iodinations performed in three succeeding weeks, each set using a different batch of iodine-131, gave the results shown in Table 1.

These results form part of an investigation into the occurrence of iodotyrosines in human plasma. We thank the Medical Research Council for a grant in support of this work.

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## BIOLOGY

### Anatomical Adaptations for Olfaction in the Snow Petrel

THE accompanying photographs show the exceptional development of the olfactory apparatus of the snow petrel, *Pagodroma nivea*. As we have previously pointed out<sup>1</sup>, all Procellariiformes (tube-nosed birds, or tubinares) have great olfactory equipment. The snow petrel, however, is the peer of them all. In four other tube-nosed species, Dr. Stanley Cobb<sup>2</sup> has found that the ratio of the diameter of the olfactory bulb to the diameter of the hemispheres is 29-30 per cent. He measured the snow petrel shown in Fig. 2 and found the corresponding ratio to be 34 per cent. Not only is the receptor surface area of tubinares much enlarged by an additional concha which arises from the septum and interdigitates with the olfactory concha proper (Fig. 2), but the whole nasal fossa is apparently engineered for optimum olfactory reception. Using the snow petrel as a model, I would like to point out two structures in the entry area of the tubinarial fossa which I believe are directly concerned with olfaction, yet the function of which in this respect has been obscured by having other functions attributed to them in the literature. These are the anterior concha and the anterior valve.

The tubinarial anterior concha is rather small, but its position and shape render it important in distributing inspired air and segregating the out-flowing lateral nasal gland fluids from the airstreams. Its position at two



vertical levels is shown in Fig. 3. A twist in its course, not demonstrable in this plane, directs a medio-dorsal air-stream on to the olfactory surfaces and a ventral stream over the respiratory area and into the pharynx. The concha fulfils all the criteria of the anterior concha of the Aves, yet has been described as a structure peculiar to tubinares, and has been variously called a 'nasal flange', a 'paraseptal process'<sup>3</sup> and a 'lamina vestibuli'<sup>4</sup>.

The anterior valve is, I believe, entirely concerned with keeping water off the olfactory organ of pelagic diving birds. It is as prominent a structure in other pelagic diving species as it is in the tubinares. Two quite different functions have been attributed to it. Portmann<sup>5</sup>, proposing that birds smell via the choanae, says: "In general the plan of the nasal fossae prevents the direct entrance of the respiratory (air) current into the olfactory region. In the tubinares this entrance is even closed by a special membrane, a sort of valve which permits only the passage of the expiratory current". Mangold<sup>6</sup> suggests that the anterior valve in 'dynamic gliders', such as the tube-nosed birds, might be an air-velocity sense organ or air-pressure indicator, and illustrates the idea with clear

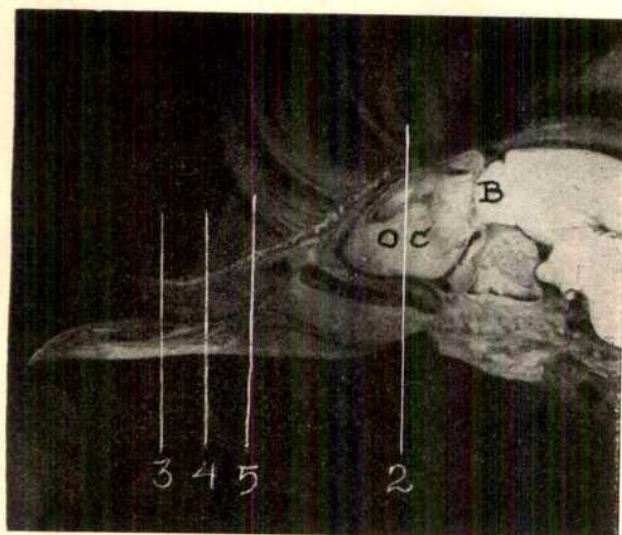


Fig. 1. Sagittal section of snow petrel nasal fossa showing relative size of medial surface of main olfactory concha. Note size of stem of concha. B, olfactory bulb; OC, olfactory concha. Numbered lines indicate level of vertical sections in Figs. 2-4

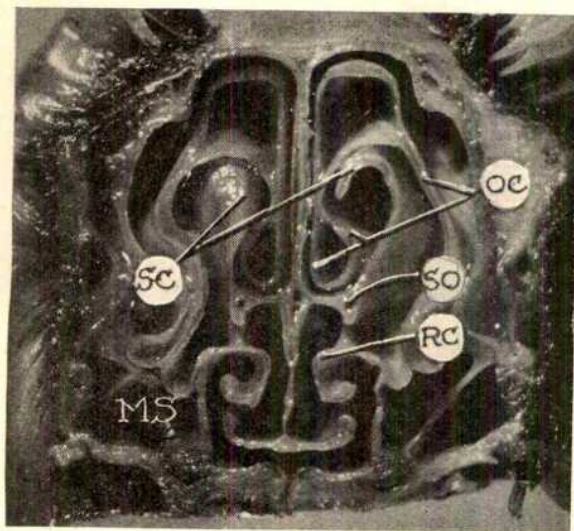


Fig. 2. Vertical section through mid-olfactory area. MS, maxillary sinus; OC, main olfactory concha; RC, respiratory concha; SC, septal olfactory concha; SO, sinus ostium

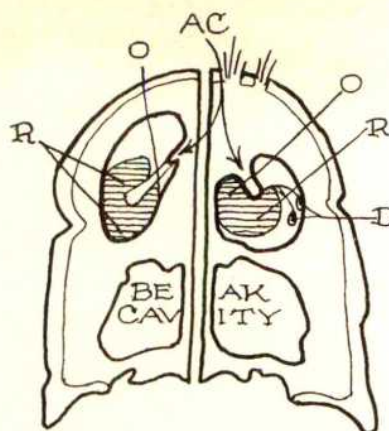


Fig. 3. Left: vertical section through right half of tube nostril showing origin of anterior concha on septum. AC, anterior concha; O, olfactory airchannel; R, respiratory airchannel. Right: Section through left half of tube nostril showing base of anterior concha as it blends with lateral wall of vestibule; D, duct mouths

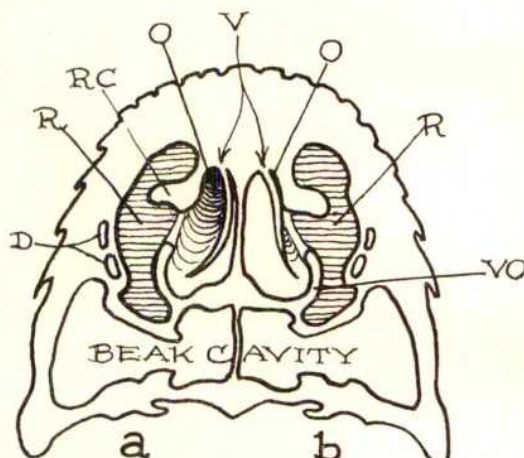


Fig. 4. Section at valve level showing (a) valve open for inhaled odour reception, (b) valve closed to seal off olfactory mucosa from water during dive. Note role of vomer in segregating olfactory and respiratory channels and supporting valve action. D, lateral nasal gland ducts; RC, respiratory concha; V, valve; VO, vomer. Other labels as in Fig. 3

but inaccurate drawings of fulmar valves, which have been widely copied. However, none of the more than sixty large or small gliding, or other, land species in our collection has anterior valves except the diving kingfishers, the well-developed ones of which have apparently been acquired by parallel evolution<sup>7,8</sup>. In all species which have them, the crescent-shaped valves are formed by a single fold of the most anterior mucous epithelium; the free border faces the nostrils and the blind end is firmly anchored to the mucoperiosteum. In freshly killed birds, and in histological sections through the valves, it is apparent that in the 'resting' state the valves lie close against the septum, and that they tend to adhere to the septum by means of the adjacent (valve-septal) mucous blankets. It is suggested that this is the case when the birds are on land or are flying, and that the air passages are then fully open for olfaction and respiration (Fig. 4a). When the birds dive, however, the valves would be opened by the force of the water (Fig. 4b). The olfactory mucosae would thus be protected from the abrasive impact of seawater, while the respiratory epithelium, which carries the trigeminal free nerve endings so important to pelagic animals<sup>9</sup>, remains open to the water. Bits of shale or seaweed are often caught in the valves of pelagic divers, or stuck on the respiratory conchae, but I have never yet found debris of any kind on the olfactory membranes. I did some crude tests with fresh herring gull heads sectioned



just at valve level and found that the valves remained adherent to the septum during successive quick mock airdives, but that they opened instantly if a submerged head was pulled gently upward in a tank, nose first.

Dr. Robert Cushman Murphy informs me that snow petrels feed on fish to a greater extent than do any other antarctic petrels, and that most of their fishing is done at night in the small channels of ice floes. Since in tubinares the olfactory bulb is much larger than in most other birds, since the nasal apparatus apparently ensures optimum olfaction, and since extraordinary feats of food-finding have been described for several species<sup>1</sup>, it is hard to escape the conclusion that in the Procellariiformes olfaction is a dominant sense and that food-finding is one of the functions of that sense. It is worth reiterating, however, that the olfactory epithelium is also strongly developed in species which lack olfactory conchae, or lack nostrils, or both, as well as in species the habits of which suggest olfactory behaviour other than food-finding<sup>2</sup>.

I thank Mr. Robin Woods for the pair of snow petrels used in this investigation, obtained from the waters of Signy Island in the South Orkneys in co-operation with the Falkland Islands Dependency Service, Dr. Cobb for his collaboration and advice, and Dr. Murphy for permission to quote his correspondence.

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<sup>1</sup> Bang, B. G., *Nature*, **188**, 547 (1960).

<sup>2</sup> Cobb, S., *Perspect. in Biol. and Med.*, **3**, 383 (1960).

<sup>3</sup> Murphy, R. C., and Harper, F., *Bull. Amer. Mus. Nat. Hist.*, **44**, 501 (1921).

<sup>4</sup> Wood-Jones, F., *Emu*, **36**, 281 (1937).

<sup>5</sup> Portmann, A., in *Grasse's Zoologie*, **15**, 210 (1950).

<sup>6</sup> Mangold, O., *Die Naturgewiss*, **33**, 19 (1946).

<sup>7</sup> Allen, G. M., *Birds and their Attributes* (Dover, reprint 1962).

<sup>8</sup> Bang, B. G., *PAVO*.

<sup>9</sup> Kellogg, R., *Quart. Rev. Biol.*, **3**, 174 (1928).

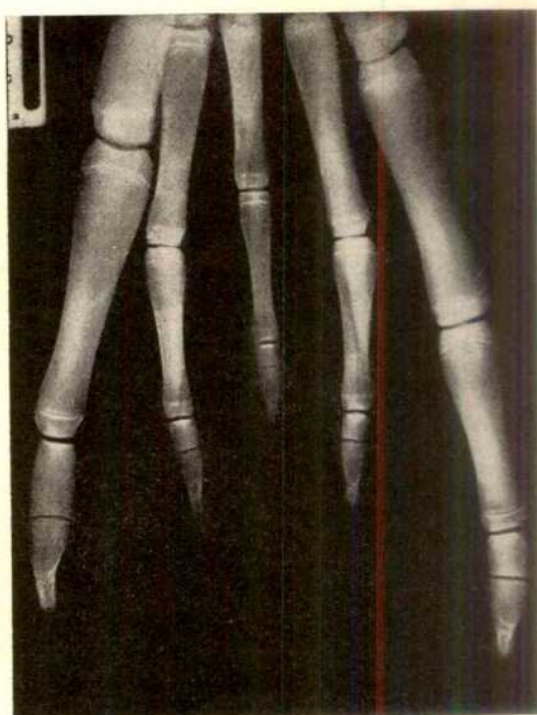
### Giant Epiphyses in a Ross Seal

THE ROSS SEAL (*Ommatophoca rossi*) is a relatively little-known animal and it was not until recently, when the British Museum (Natural History) received two frozen specimens from the British Antarctic Survey, that any detailed investigation of it could be attempted. Several curious characters have been noted in the first of these seals to be dissected, including a peculiarity of the flippers.

The elongated, rather delphinoid shape of the fore flippers is brought about by an increase in length of the first digit and a decrease in length of the fifth. This is mainly achieved by means common to other members of the Lobodontini, and to the Otariidae—the metacarpal and first phalanx of the first digit are elongated, while the second phalanx of the fifth digit is reduced. This tendency to increase the length of the flipper has been carried further in *Ommatophoca* by an unusual means, not previously recorded. An X-ray photograph of the fore flipper of one frozen animal, a female, shows, by the presence of unfused epiphyses, that the seal was not yet physically mature. The epiphyses on all the bones of the manus, except those of the terminal phalanges, are unremarkable and 'normal' in their dimensions. Their approximate lengths vary between 7 and 10 mm, and their proportion to the shaft is about 10–16 per cent. The epiphyses on the terminal phalanges are, however, very noticeable both for their size and shape. Their lengths range between 7 and 24 mm, that on the first digit being the largest, and their proportions to the rest of the bone between 40 and 82 per cent. As can be seen from Fig. 1a,



(a)



(b)

Fig. 1. Radiographs of Ross Seal (a) fore and (b) hind flipper

this epiphysis on the first digit is very nearly as long as the shaft, and its size and its rectangular shape are quite unusual. Those on the other terminal phalanges are also much larger than might be expected. Skeletons of *Ommatophoca* are rare in collections, but skins brought back by some of the early Antarctic expeditions still have the terminal phalanges *in situ*. X-ray photographs have shown that in every specimen so far available these giant epiphyses can be seen.

Tips of seal flippers of appropriately immature animals are not easy to find, but an examination has been made of the fore flippers of as many phocid seals as possible. Only in the Leopard Seal (*Hydrurga leptonyx*) are there comparable epiphyses, although they are relatively not so big as in the Ross Seal.

On the terminal phalanges of the hind flippers of *Ommatophoca* (Fig. 1b) similar large square or rectangular epiphyses are present, that on the first digit being most spectacular with a length of 33 mm, the rest of the phalanx being 44 mm long. Large but less peculiar epiphyses are present in *Hydrurga*. There is some evidence that there are unusually large epiphyses on the terminal phalanx of the first digit of the hind flippers in such northern phocids as *Pagophilus* and *Cystophora*.

The elongation of these epiphyses, particularly that of the terminal phalanx of the first digit, which involves elongation of the digit as a whole, probably has some bearing on the animal's superior powers of aquatic locomotion. It may be significant that the fore flipper of *Ommatophoca*, the most specialized phocid, exhibits a character usually found (and to a much lesser degree of development) only in the hind flippers of less-adapted phocids. Some other characters, to be reported later, have a similar distribution.

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### Early European Description of an Australian Mammal

In the account of an early description of an Australian mammal given by Dr. Wilma George<sup>1</sup> several suggestions were made as to the possible identity of this animal. No definite conclusion was reached and *Thylogale stigmatica*, *Hypsiprymnodon moschatus* and *Uromys caudimaculatus* were all offered as possible species which might satisfy the original description.

One particular group of marsupials which does not appear to have been considered in this problem is the genus *Phalanger* containing the cuscuses. Seven features were tabulated which were derived from the original description by Prado and then each species was scored for closeness of fit. A maximum of 5 points out of a possible 7 was scored by both *Thylogale stigmatica* and *Hypsiprymnodon moschatus*.

If similar scoring is applied to species of *Phalanger*, a better result is obtained.

Cuscuses are widely distributed in south-east New Guinea<sup>2</sup>, they have a strong musky odour from which the name castor could be derived and they have a naked scaly prehensile tail. They feed mainly on leaves and are eaten extensively by the native people of New Guinea<sup>3</sup>. The descriptions of the testicles given by Prado which "hung from a nerve like a thin cord" would fit the cuscus as well as any other marsupial. This feature alone in the description would seem likely to preclude any group of Australian mammals other than the marsupials, which are characterized by a typically pendulous scrotum. The description of "an animal which is in the shape of a dog, smaller than a greyhound" could be distorted to fit many small or medium sized mammals and would be as equally applicable to a cuscus as to any of the other species listed.

Even if this latter feature were scored zero, some species of *Phalanger* would still rate 6 points out of a possible 7.

Some features in the description are of greater diagnostic value than the remainder and could be given greater emphasis without risk of subjective distortion. These include the scaly tail, pendulous testes and distribution in south-east New Guinea. Only the genus *Phalanger* possesses all these characteristics since *Thylogale* does not

have a scaly tail and *Hypsiprymnodon* is confined to Queensland.

It is therefore probable that the animal described by Prado in 1606 was actually a cuscus, *Phalanger* sp.

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<sup>1</sup> George, W., *Nature*, **202**, 1130 (1964).

<sup>2</sup> Tate, G. H. H., "The Marsupial Genus *Phalanger*", *Amer. Mus. Novitates*, **1283**, 1 (1945).

<sup>3</sup> Waterhouse, G. R., *Natural History of the Mammalia*, **1**, Marsupialia, 277 (Baillière, London, 1846).

DR. WILMA GEORGE has recently brought to scientific notice what is apparently the earliest known description of an Australo-Papuan mammal and discusses its possible identity<sup>1</sup>. Briefly, in 1606 Don Diego de Prado y Tovar sailed along the southern coast of New Guinea and through Torres Strait, and in his *Relación* described how on the south-eastern coast of New Guinea his party killed an animal "in the shape of a dog smaller than a greyhound, with a bare and scaly tail like that of the snake, and his testicles hang from a nerve like a thin cord; they say that it was a castor, we ate it and it was like venison, its stomach was full of ginger leaves and for that reason we ate it".

George gives equal weight to all the characters listed by Prado and tests the results against a series of Australian and Papuan mammals, the information about them being obtained from standard works. She concludes that the description best fits the musky rat-kangaroo (*Hypsiprymnodon*), but that a wallaby (*Thylogale*) and a rat (*Uromys*) are strong contenders. None of the works listed, however, has the kind of detail necessary for a good analysis of Prado's description. As George remarks there is no reason to doubt the accuracy of the description, and for that and other reasons I cannot appreciate her view that weighting of the various points would lead to difficulties.

A key character is the description of the testes, which George interprets to mean merely 'prominent' or 'evident'. Anyone familiar with living marsupials must be struck by the aptness of Prado's lay description of the suspended pre-penile scrotum of most male marsupials. This is particularly obvious in the Macropodidae. In adult kangaroos of the larger species, the large roughly spherical scrotum hangs several inches below the body by a thin scrotal pedicle. Movement of the scrotum is apparently under conscious control and it is drawn up against the body during such activities as hopping, fighting, and copulating. In death it hangs freely in the extended position. I believe that this part of Prado's description diagnoses his animal as marsupial and removes any of the eutherian rats, such as *Uromys*, from consideration.

Large marsupials with scaly tails which could have been encountered by Prado on the south-eastern coast of New Guinea are two wallabies, *Dorcopsis veterum* (= *muelleri*) and *Thylogale brunii*, and one or two species of cuscuses (genus *Phalanger*). The last-named genus was not considered by George. The distal inch or so of the tail of *Dorcopsis* is scaly and bare of hair but the rest of the tail is well furred. In *Thylogale brunii* and other species of that genus the greater part of the tail is scaly with sparse hair on the sides and somewhat more hair on the upper and lower surfaces. In some specimens of *Thylogale* the hair on the tail is very sparse indeed, perhaps depending on stage of moult or age. The scaly nature and sparseness of hair on the tail of *Thylogale brunii* is well shown by Alston's illustration of this species (under the name of *Macropus lugens*)<sup>2</sup>. The tails of *Phalanger* spp. are naked for two-thirds or more of their length on the ventral surface and approximately half on the dorsal surface and there are large scales on a considerable part of the ventral surface.



It is reasonable to assume that when Prado described his animal as shaped "like a dog smaller than a greyhound" he meant that it resembled a small example of that breed of dog in shape as well as in size. To Europeans seeing macropodids for the first time the comparison in shape with a greyhound is not at all unreasonable. It will be recalled that Captain Cook, when he saw a kangaroo for the first time at Endeavour River, recorded in his journal that it was "the full size of a grey hound and shaped in every respect like one"<sup>1</sup>. *Dorcopsis veterum* and *Thylogale brunii* are slenderly built and have dog-like muzzles and rhinaria, ears roughly proportional in size to those of greyhounds and dark grey short fur. Cuscuses, on the other hand, are short-legged and do not resemble greyhounds in body conformation or shape of head. Moreover, they have extremely short ears and woolly fur. The male of the widespread *P. maculatus* is strikingly blotched reddish-brown and cream, while the forms of the other widespread species group, *P. orientalis*, are brown or dark grey. One would think that had Prado seen a cuscus he would have compared it to some other breed of dog or a well-known European animal.

George's choice of *Hypsiprymnodon* does not fit well on the score of size as it is a diminutive creature with a head-and-body length of little more than 1 ft. and weighs only about 1 lb. Moreover, there is no recent or fossil evidence that it ever occurred in New Guinea or even the northern part of Cape York Peninsula, or its offshore islands where some authorities claim that Prado touched.

The reason why Prado's animal was called a castor may have been because of a smell it emitted, or the fact that it had gnawing teeth or perhaps even because it had a scaly tail. George apparently does not realize that many male marsupials including some species at least of macropodids and phalangerids have strong characteristic odours. Of the mammals known to Europeans the teeth of macropodid and phalangerid marsupials have a marked superficial resemblance to those of rodents, because of the single pair of procumbent lower incisors, the diastemata between the incisors and cheek teeth, and in most macropodids, the lack of canines. The teeth of diprotodont marsupials were very puzzling to early naturalists, and in some of their classifications these animals were placed in or near the rodents. The unity of the Marsupialia was finally recognized by de Blainville<sup>4</sup>, and it is significant that in his classification he used the name 'Rongeurs' for the subdivision of the marsupials (his "Didelphes Normaux") containing the diprotodonts.

The fact that the stomach of Prado's animal was full of 'ginger leaves' is of no significance and merely indicates a herbivore. Likewise it is of no importance in attempting to determine the nature of the creature that the flesh was eaten and tasted like venison to early seventeenth-century European sailors.

In summary, I believe that Prado's mammal was certainly a marsupial, probably a wallaby and possibly a cuscus; the species that best fits his description is *Thylogale brunii*.

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<sup>1</sup> George, W., *Nature*, **202**, 1130 (1964).

<sup>2</sup> Alston, E. R., *Proc. Zool. Soc. Lond.*, 123 (1877).

<sup>3</sup> Cook, J., *The Journals of Captain James Cook*, edit. by Beaglehole, J. C., 1, 351 (Hakluyt Soc., 1955).

<sup>4</sup> de Blainville, H. M. D., *Bull. Soc. philom.*, Paris, 105 (1816).

Mr. Calaby and Mr. Marlow have added interesting comments towards establishing the identity of Prado's Australasian mammal. I am grateful to them for pointing out the claims of *Phalanger*.

Testing for an association between Prado's description and modern descriptions of likely animals I was particularly concerned not to weight any characteristic more than any other since there is no consistent system for applying such weighting. Inconsistent weighting leads Mr. Calaby and Mr. Marlow to different conclusions. Mr. Calaby gives little weight to the interpretation of the term castor and to the question of edibility but stresses the prominent testicles and the likeness to a small greyhound and the scaly tail. In contrast Mr. Marlow by implication gives little weight to the likeness to a greyhound but stresses the other characteristics.

In spite of being an adherent of the rigorous numerical method of assessing associations of a range of characteristics<sup>1</sup> and, by this method, eliminating the greater part of the Australasian mammal fauna from the discussion, I am prepared to apply at this stage a certain amount of subjective freedom to the final choice. It seems necessary in this case because none of us can examine the specimen we are trying to identify and because we are making a basic assumption that Prado's description is accurate.

If it is accepted that the original method eliminates the outsiders there remain only *Thylogale*, *Hypsiprymnodon* and *Uromys* with the addition of *Phalanger*. A new assessment can be made of these four. One method of weighting is to accept the opinions of those experienced in the study of mammals and of the fauna of the region. I suggest that the opinions of Mr. Calaby, Mr. Marlow and myself are taken into account together with some of the original zoological descriptions<sup>2-6</sup> of the animals and some early explorers' descriptions<sup>7,8</sup>. By doing this, weighting can be applied to each characteristic to a maximum of four points derived from our three opinions and those of either the early describers or the casual explorers.

All are agreed that the prominence of the testicles is a peculiarly marsupial feature and can therefore be weighted more heavily than any of the other qualities. Whether greater weighting should be given to *Thylogale* and *Hypsiprymnodon* than to *Phalanger* in this respect is a difficult decision to take; but, having accepted weighting, I am inclined to weight ruthlessly and score say four for *Thylogale* and *Hypsiprymnodon* and two or three for *Phalanger*, since I believe the thick soft fur makes them less obvious than in the macropodids. *Uromys* does not score.

It is fair to say that all four genera could equally have been eating ginger leaves, and this characteristic can either be eliminated or scored the same for each.

All four genera are presumably edible. Mr. Calaby says it is of no importance, Mr. Marlow that the natives eat cuscuses and it is, therefore, important. There is good evidence for the natives of New Guinea eating cuscuses and a great deal of historical and present-day evidence for Europeans and natives eating and enjoying wallabies and kangaroos. Both Dampier<sup>7</sup> and Cook<sup>8</sup> record that these animals were very good eating, and kangaroo meat is a considerable export to-day. It becomes difficult to know how to score this except by giving two points to *Thylogale* and two to *Phalanger* as being better to eat than the other two, which score only one each.

Their occurrence in south-east New Guinea is an unreliable point since it is not known exactly where Prado was; nevertheless, all except *Hypsiprymnodon* can score one.

I do not agree with Mr. Calaby that the term castor would have been used to describe a type of jaw and dentition which in its general features is common to all rodents. It seems more likely to apply to an odour resembling that of castoreum or castor from the genital glands of the beaver and for which, among other things, the beaver was hunted. Cuscuses and *Hypsiprymnodon* have a strong musky odour, and score two at least on this point. I am aware of Mr. Calaby's point that some

macropodids have strong odours and I attach considerable importance to the possibility of differentiating species of some animals, particularly rodents, by their distinctive odours. However, I cannot find any record of the scents of any of these animals, and would appreciate further information on this point. Failing this factual information no more than one can be scored for *Thylogale*.

We are not agreed on the tail. Mr. Calaby and other authors<sup>2-4</sup> describe the sides of the tail of *Thylogale* as scaly, Mr. Marlow says that *Thylogale* does not have a scaly tail. Reading through the early descriptions of *Thylogale* and *Phalanger* species<sup>2-5</sup>, I conclude that the tails of either of these genera could in some circumstances be called scaly. *Hypsiprymnodon* and *Uromys* certainly have scaly tails so this character can also be eliminated as common to all.

"In the shape of a dog smaller than a greyhound" is a similar description to that given by Dampier to describe a wallaby, and the greyhound analogy was also used by Cook. Although Mr. Calaby and Mr. Marlow disagree on this point I am inclined to weight small greyhound four points in favour of *Thylogale* and one each for *Phalanger* and *Hypsiprymnodon*. *Hypsiprymnodon* only scores one on this round for being too small and more likely to have been described as jerboa-like since this too was a common analogy. *Phalangers* had been described as monkeys by other visitors and it seems difficult to apply a greyhound description to it.

This revised estimate arrived at by ruthless weighting gives a big overall majority to *Thylogale*, twelve, with *Phalanger* eight or nine points, *Hypsiprymnodon* eight, and *Uromys*, with two, out of the running. *Thylogale* was always my favourite competitor so I can be charged with weighting in its favour. Weighting being a matter of opinion and not mathematically determinable in such cases, other authors can apply a different system and arrive at a different answer. In the meantime I think we are all agreed that there is little doubt that Prado's description is at present the earliest European record of a marsupial, and two of us are agreed that the description fits *Thylogale* best. Before making a choice between *T. stigmatica* and *T. brunii* which I considered together in my original report I would like to know whether one of them has a more castor-like odour than the other.

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<sup>1</sup> Sokal, R. R., and Sneath, P. H. A., *Principles of Numerical Taxonomy* (San Francisco, 1963).

<sup>2</sup> Gould, J., *Proc. Zool. Soc. Lond.*, 375 (1860).

<sup>3</sup> Ramsay, E. P., *Proc. Linn. Soc., New South Wales*, 1, 307, 395 (1877).

<sup>4</sup> Alston, E. R., *Proc. Zool. Soc. Lond.*, 126 (1877).

<sup>5</sup> Tate, G. H. H., and Archbold, R., *Amer. Mus. Novit.*, 804, 810 (1935).

<sup>6</sup> Tate, G. H. H., *Bull. Amer. Mus. Nat. Hist.*, 97, 189 (1951).

<sup>7</sup> Dampier, W., *A Voyage to New Holland, etc., in the Year 1699* (London, 1703).

<sup>8</sup> Cook, J., *Journals. The Voyage of the Endeavour 1768-1771*, edit. by Beaglehole, J. C. (Cambridge, 1955).

### Germination of *Rosa arvensis*

It appears that dormant rose achenes of many species may be put in the same category as those 'two-year' seeds which require a warm period in the soil to permit disintegration of the coat, followed by a period at low temperature to after-ripen the embryo. On the other hand, Harrison<sup>1</sup> reports that achenes of *R. arvensis* germinated in good numbers in the first year after planting in the autumn under natural conditions. This behaviour appears to be characteristic of members of the section *Synstylae*<sup>2</sup> and also to some extent of *R. rugosa*<sup>3</sup>.

It has been shown<sup>4</sup> that the lack of immediate germination in the achene of *R. arvensis* is not due primarily to the massive pericarp acting as a barrier to the movement of water or gases. That a similar conclusion may be applied to the action of the underlying testa is shown

Table 1. GERMINATION AT 26° C OF SEEDS OF *R. arvensis*

	% Germination after 14 days
Entire seeds (embryo + testa)	10
Cap of testa over radicle removed	20
Only cap of testa left: remainder removed	30
Whole of testa removed	50

by the results in Table 1, obtained by the methods previously described. It thus appears that the mere presence of the whole or part of the testa inhibits germination to some extent.

The dormant achenes of *R. rugosa* contain substances (soluble in water, ethanol and ether) which inhibit the straight growth of *Triticum* coleoptile sections and the germination of *rugosa* embryos<sup>4</sup>. These two tests were therefore used on eluates of the segments of chromatograms run from the acidic fraction (separated on an activated alumina column) of the ether-soluble material present in the diffusate obtained by steeping dormant achenes of *R. arvensis* in 80 per cent ethanol for five days at 2° C. Fig. 1 shows the result of the embryo test. When the embryos in the highly inhibitory eluates from the  $R_F$  segment 0.4-0.5 were then washed for a few minutes in water and returned to a clean germinating dish containing water, 60 per cent germination occurred in the next 24 h; there seems little doubt that the inhibitors were not toxic in the concentrations used here and may be readily leached from the embryo. Since it had also been shown that the dormancy imposed by the testa on the embryo in *R. arvensis* could be alleviated almost completely by soaking the seeds in the kinetin analogue, 6-benzylaminopurine (10 µg/ml.), or to a slightly lesser extent by gibberellic acid (150 µg/ml.), a duplicate experiment was carried out in which the inhibitory segments  $R_F$  0.3-0.4 and 0.4-0.5 were eluted with the purine solution instead of water. Fig. 1 shows that the inhibition was again removed almost completely. The wheat coleoptile, straight-growth assay also showed the presence of inhibitors around the middle of the chromatogram, and they may therefore be compared in some respects with the phenolic inhibitors which Varga<sup>5,6</sup> found in the outer parts of many fruits and equated with the β-inhibitor complex of Bennet-Clark and Kefford<sup>7</sup>.

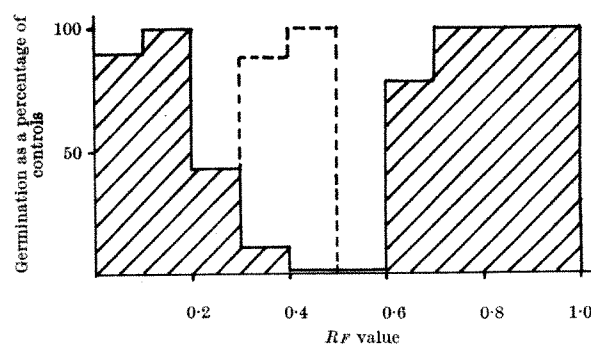


Fig. 1. The germination after 6 days of naked embryos in eluates from a chromatogram of the acidic fraction of the ether-soluble substances present in an ethanolic diffusate of dormant achenes of *R. arvensis*. Chromatogram run in butanol/ammonia/water: 10/1/1. —, Segments eluted with water; ---, segments  $R_F$  0.3-0.5 from a duplicate chromatogram eluted with 6-benzylaminopurine

Although leaching of the inhibitors from the achenes by soil water probably occurs under natural conditions, there is some evidence<sup>4</sup> that cold-stratified embryos of *R. rugosa* are able to germinate even in the presence of inhibitors from dormant achenes of the same species; it appears likely that the state of the embryo is controlled by the balance between growth-inhibitors and promoters (such as kinins and gibberellins) as well as by the absolute amount of the former.

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<sup>1</sup> Harrison, J. W. H. (personal communication).

<sup>2</sup> Rowley, G. D., *Amer. Rose Annual*, **41**, 70 (1956).

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### Ammonium Requirement for Embryogenesis in vitro

PREVIOUS work on tissue cultures of the wild carrot has shown that the embryogenesis which occurs in such cultures is not fundamentally different from the same process in the ovule<sup>1,2</sup>. It has also been shown that undifferentiated pro-embryos, sieved from callus, will continue to grow in a disorganized non-polar fashion if they are exposed to a concentration of 2,4-dichlorophenoxyacetic acid (2,4-D) which is higher than 0.1 mg/l. (ref. 3). Lowering the 2,4-D concentration permits histological differentiation and polarized growth to occur. It is thus possible to establish a crude control over the process of embryogenesis by suitable alterations of the 2,4-D concentration. While this technique has provided an experimental system in which the simultaneous development of numerous embryos can be examined, it has provided no information on the morphogenetic factors which initially cause cultured cells to behave as zygotes.

Recently, the relative effectiveness of different nitrogen sources on the growth of a strain of wild carrot callus which was originally derived from storage root phloem was examined. This strain had been in culture for more than one year on a simple medium consisting of minerals, sucrose, vitamins, kinetin, and 2,4-D. It was found that callus grown in the absence of ammonium would not form embryos when transferred afterwards to a medium which would normally permit (by lowering the 2,4-D level) development of any pro-embryos present in the tissue. Phloem-derived tissue will grow indefinitely on nitrate as the sole nitrogen source, with the optimal nitrate concentration about 60 mmoles/l. Addition of as little as 5 mmoles/l. ammonium, while inhibiting growth slightly, permits embryogenesis to occur. Microscopic examination of callus grown exclusively on nitrate nitrogen revealed only single cells and a virtual absence of multicellular structures (Fig. 1a). Examination of the same strain of callus grown on ammonium-containing media revealed numerous multicellular filaments which were recognizable as pro-embryos (Fig. 1b).

In subsequent experiments which were designed to test the validity of this phenomenon with other strains of wild carrot callus, it was found that petiole segments proliferated very slowly if they were placed on a medium containing nitrate as the sole source of nitrogen and 2,4-D as the sole hormone. If ammonium was added to this medium, a healthy rapidly growing callus developed. It was found that addition of kinetin to the medium eliminated the ammonium requirement shown by explanted petiole segments. By using both kinetin (0.5 mg/l.) and 2,4-D (0.5 mg/l.) it was thus possible to obtain petiole callus which grew as well on media containing only nitrate as it did on nitrate plus ammonium.

To test systematically for the presence of pro-embryos in such callus, a method was used which was a modification of techniques previously described<sup>3</sup>. The 45–75  $\mu$  cell fraction from petiole callus (after 60 days in culture) was used to inoculate liquid culture tubes (18 mm  $\times$  150 mm rimless test-tubes sealed with polyethylene) which were

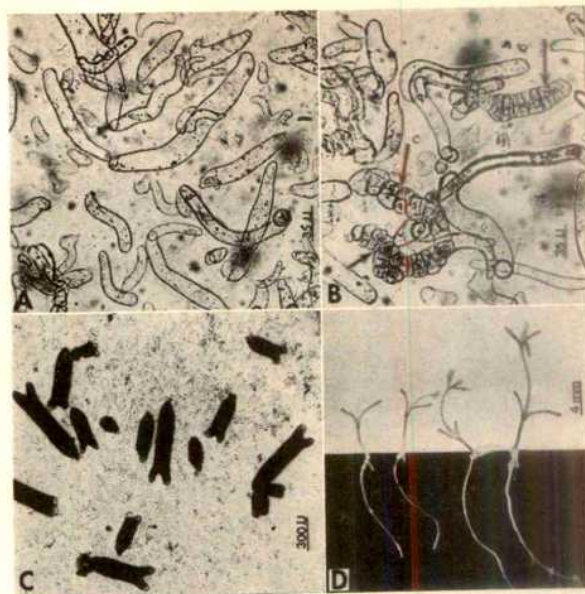


Fig. 1. A, cells from phloem-derived callus which has been grown on a medium containing nitrate as the sole nitrogen source. B, cells and pro-embryos from phloem-derived callus grown on media containing ammonium. Arrows indicate proembryos. C, embryos which formed in rotating liquid cultures inoculated with the cell fraction shown in B. D, normal plantlets from embryos shown in C.

rotated at 2 r.p.m. in a position about 10° from the horizontal. It should be pointed out that in the process of removing this fraction from callus, the cells are washed by approximately one-half litre of fresh medium. The test medium used was the basal medium previously described<sup>3</sup> modified to contain either 60 mmoles/l. potassium nitrate, or 55 mmoles/l. potassium nitrate plus 5 mmoles/l. ammonium chloride. Kinetin was supplied at 10<sup>-3</sup> mg/l. and 2,4-D at 5  $\times$  10<sup>-4</sup> mg/l., levels low enough to permit development of any pro-embryos present in the inoculum to be tested.

The cell fraction obtained from nitrate-grown petiole callus failed to produce embryos in either of the two liquid media (nitrate, or ammonium plus nitrate). In the case of the cell fraction from callus grown on ammonium-containing media, many embryos were observable in the cultures within two weeks of inoculation. Embryos were as numerous in the tubes which contained no ammonium as they were in those with ammonium. While ammonium is thus required for initiation of embryo-genesis in cultured cells, nitrate alone may be sufficient for the further development of pro-embryos in the undifferentiated state (Fig. 1B) to the mature state (Fig. 1C). However, the part played by conditioning of the medium requires investigation. It is possible that young pro-embryos require reduced nitrogen and obtain it in these cultures by absorbing the nitrogen released in various molecular forms by living and dead cells in the suspension. Raghavan and Torrey have recently shown that undifferentiated pro-embryos of *Cattleya*, an orchid, require ammonium. The failure to grow on nitrate was correlated with low nitrate reductase activity<sup>4</sup>.

Fig. 1C is a drop of suspended material taken from a liquid culture tube 13 days after inoculation. These embryos are quite normal. If the embryos are left in the rotating culture tubes longer than 2 weeks, the primary root grows out and mature leaves appear (Fig. 1D). Mature leaves appear in both the presence and absence of ammonium. Growth of the plantlets is definitely slower, however, in the medium lacking ammonium.

A major problem concerns the total failure of pro-embryos to form in the liquid culture tubes which contained ammonium and which received nitrate grown callus. We assume from this fact that new pro-embryos also failed to form in the tubes inoculated with ammonium-grown



petiole callus, and that the embryos found in these tubes developed only from the pro-embryos already present in the inoculum.

Preliminary results, using the phloem-derived callus, indicate that other sources of reduced nitrogen (glutamine, aspartate, a mixture of 14 amino-acids, coconut milk) will not substitute for ammonium.

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### Capillary Movement in Fungal Hyphae

SOME years ago I observed<sup>1</sup> capillary movement in the hyphae of *Mucor mucedo*; the phenomenon was not photographed at the time and subsequent attempts to repeat the observation were unsuccessful. Recently, however, the observation has been repeated, in fungi cultivated on dishes of sugar-agar. Capillary movement was observed after the addition of a drop of guinea-pig blood to the culture medium, but it is uncertain whether the relation between the observation and the addition of blood was causal or merely fortuitous.

Serial photomicrographs have been prepared, demonstrating the phenomenon, and Fig. 1 shows part of such a series. The movement of the hyphal contents was slow but continuous; displacement in the large hypha shown in the photograph was from left to right and the rate of flow was variable. Transport from 'A' to 'A'', as shown in Fig. 1, took from 0.25 to 10 min. Movement within the smaller hyphae was towards the larger one.

The stream carried with it the protoplasts<sup>2</sup> and smaller particles, and movement was continuous over a period, usually of hours, but in one case for several days. The oval or lozenge-shaped segments seen in the photographs

are broken up or deformed from time to time, especially as they enter the large hypha from a tributary, somewhat in the way that erythrocytes may be deformed in capillary transport in animals<sup>3</sup>.

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### Inhibition of the Perfect Stage of *Venturia inaequalis* (Cooke) Wint., by Urea

EPIDEMICS of apple scab (*Venturia inaequalis*) originate from ascospores discharged from perithecia present on over-wintered leaves on the ground. Hutton<sup>1</sup> has shown that post-harvest pre-leaf-fall applications of organo-mercurial compounds greatly improved the level of disease control in that they suppressed perithecial formation and hence reduced the amount of ascospore inoculum in the following spring.

Recently there has been growing interest in the use of post-harvest pre-leaf-fall urea sprays as a means of supplying nitrogen to fruit trees<sup>2</sup>. As *in vitro* investigations had suggested that excess nitrogen could suppress perithecial formation<sup>3</sup>, we studied the effects of urea on the development of *Venturia inaequalis* in overwintering leaves.

In October 1963 scab-infected apple plants (clone M.III) were sprayed in the field with either 5 per cent or 2 per cent urea solutions. 48 h later samples of 200 leaves from each treatment, and a control sample from trees sprayed with water, were placed in 14 × 14 mesh nylon envelopes and allowed to overwinter in an exposed situation on the ground. Analysis of leaves immediately after collection showed the percentage dry weight of nitrogen to have increased from 1.81 to 2.06 with 2 per cent urea and to 2.49 with 5 per cent urea. In November further leaf samples were taken from non-treated M.III plants and from the scion variety 'Granny Smith', dipped in 5 per cent urea or in water (control) and then similarly overwintered. All urea solutions contained 0.01 per cent of the wetting agent 'Agral LN' which has been shown to be biologically inactive in the context of the present work.

During the next 4 months, observations were made on the state of the leaves and, at intervals, random samples of 10 dipped and 10 non-treated M.III leaves were tested for microbial activity. Two 1-cm. disks were cut from each leaf and the bulk samples of 20 disks macerated in 10 ml. of sterile distilled water in a Measuring and Scientific Equipment microhomogenizer for 4 min. After allowing large pieces of leaf debris to settle, 1 ml. of the supernatant liquid was withdrawn and suitable dilutions plated into nutrient agar containing 5 per cent sucrose. Bacterial colonies were counted after 3 days at 25° C.

The 5 per cent urea had a noticeable effect on the decomposition of the leaves. Five weeks after treatment the laminae of many of the sprayed leaves had partially or completely disappeared, leaving a veinal skeleton. The dipped leaves remained intact until the following spring, but were observed to be a darker brown than the corresponding controls. This colour change was associated with striking differences in microbial activity. In mid-December when the first estimate was made there were approximately 170 times as many bacteria per unit area on the treated compared with the non-treated leaves (Fig. 1). By early January the difference had fallen to times 48; thereafter it diminished progressively but residual effects of the urea treatment were still apparent in the spring.

In early March, all leaves were brought indoors to prevent the release of mature ascospores by rain, and their

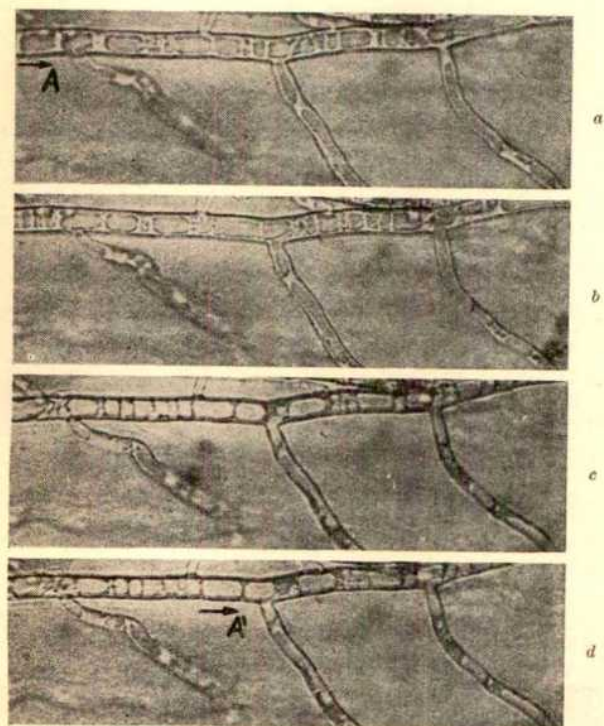


Fig. 1. Capillary movement in hyphae of *Mucor mucedo*



## MICROBIOLOGY

Preparation of Fluorescent Polyvalent *Salmonella* antisera

IMMUNOFLUORESCENCE methods for detecting *Salmonellae* in meat<sup>1</sup> and egg products<sup>2</sup> have been described recently. In both investigations the indirect immunofluorescence technique<sup>3</sup> was used, involving treatment with rabbit polyvalent *Salmonella* agglutinating antisera, followed by staining with fluorescein conjugated goat anti-rabbit antiserum. This technique takes about two or three times as long as the direct technique using conjugated rabbit antisera originally described by Coons *et al.*<sup>4</sup>.

We therefore attempted to produce several fluorescent polyvalent *Salmonella* antisera, and to compare their staining characteristics with their agglutinating properties.

*Salmonella* O antigens were prepared by a process of heating and drying<sup>5</sup>. To obtain polyvalent antisera mixtures of equal weights of several antigens were injected into groups of rabbits. Each rabbit received nine 1-ml. intravenous injections at 3-4 day intervals, the first injection being equivalent to about 10<sup>6</sup> organisms and the last, 10<sup>10</sup> organisms. A week before the first injection small blood samples were obtained from each rabbit and checked for absence of agglutinins for the *Salmonellae* involved.

Three groups of rabbits were immunized in this way. The first group received O antigens 4; 7; 8; 9; 3, 10; 11; 3, 19; 13, 22, the second group received O antigens 4; 7; 8; 9 and the third group O antigens 3, 10; 11; 3, 19; 13, 22. Seven days after the last injections blood was drawn from each rabbit, held at room temperature for 5-7 h, and the serum collected. The sera from each group of rabbits (that is, those that received the same antigen mixture) were bulked. The O agglutination titres of the three bulked sera were determined against individual *Salmonellae* possessing the various antigens injected.

The  $\gamma$ -globulins of the three antisera were then precipitated by 50 per cent saturation with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, centrifuged, and redissolved in phosphate buffered saline (pH 7.4). After dialysis against fresh buffered saline the globulins were conjugated with fluorescein isothiocyanate, the fluorochrome being added as a solid to the reaction

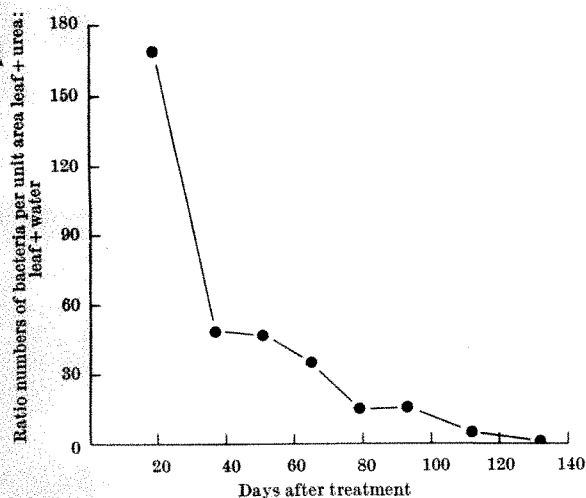


Fig. 1. Effect of 5 per cent urea on microbial activity in overwintering apple leaves

ascospore productivity determined by the method of Hutton and Burchill<sup>4</sup>. This consisted of taking random samples of 100 standard disks of leaf tissue from each treatment and agitating them in aerated water to discharge the ascospores from the perithecia. The resulting spore suspensions were concentrated by centrifugation and counted in a haemocytometer. The procedure was repeated on five successive occasions with each sample, with intervals to allow for further maturation of ascospores. The ascospore productivity was expressed as total ascospores liberated per cm<sup>2</sup> of leaf tissue (Table 1).

Table 1. EFFECTS OF UREA TREATMENTS ON THE ASCOSPORE PRODUCTIVITY OF APPLE LEAVES INFECTED WITH *Venturia inaequalis*

Treatment	Ascospore productivity total spores/cm <sup>2</sup> leaf	Percentage reduction
Sprayed M.III leaves		
Water	1,980	
2 per cent urea	906	55
5 per cent urea	57	97
Dipped M.III leaves		
Water	9,200	
5 per cent urea	280	97
Dipped 'Granny Smith' leaves		
Water	2,600	
5 per cent urea	84	97

Higher urea concentrations consistently reduced ascospore productivity by approximately 97 per cent compared with the water-treated controls. This was comparable with results obtained in other experiments with organo-mercurial fungicides. Although less marked, there was some inhibitory action by 2 per cent urea.

Decreased spore productivity seems to be due to the failure of the fungus to produce perithecia in the leaves, suggesting that inhibition may occur at an early stage in its development. Whether this is a direct effect of the increased nitrogen in the substrate or an indirect effect resulting from increased microbial activity is not yet known. Whatever the mode of action of urea, its use as a post-harvest spray suggests an alternative to organo-mercurial fungicides, and a possible approach to the control of apple scab by biological means.

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Table 1. AGGLUTINATION TITRES AND STAINING CHARACTERISTICS OF *Salmonella* POLYVALENT ANTISERUM 1 AND EQUIVALENT CONJUGATE

O antigens in mixture injected	Corresponding O agglutination titre in antiserum	Fluorescent staining by conjugate
		Bright staining of:
4	1/250	<i>S. typhimurium</i> (0:4)
7	1/100	<i>S. oranienburg</i> (0:7)
8	1/250	<i>S. virginia</i> (0:8)
9	1/500	<i>S. gallinarum</i> (0:9)
3, 10	1/250	<i>S. anatum</i> (0:3, 10)
11	1/100	<i>S. aberdeen</i> (0:11)
3, 19	1/250	<i>S. senftenberg</i> (0:3, 19)
13, 22	1/100	<i>S. poona</i> (0:13, 22)

Table 2. AGGLUTINATION TITRES AND STAINING CHARACTERISTICS OF *Salmonella* POLYVALENT ANTISERA 2 AND 3 AND EQUIVALENT CONJUGATES

O antigens in mixtures injected		Corresponding O agglutination titre in antisera		Fluorescent staining by conjugates
Anti-serum 2	Anti-serum 3	Anti-serum 2	Anti-serum 3	Stained brightly by anti-serum 2, but not at all by anti-serum 3:
4	—	1/250	<1/25	<i>S. typhimurium</i> (0:4)
7	—	1/250	<1/25	<i>S. oranienburg</i> (0:7)
8	—	1/250	<1/25	<i>S. virginia</i> (0:8)
9	—	1/500	<1/25	<i>S. gallinarum</i> (0:9)
				Stained brightly by anti-serum 3, but not at all by anti-serum 2:
—	3, 10	<1/25	1/500	<i>S. anatum</i> (0:3, 10)
—	11	<1/25	1/500	<i>S. aberdeen</i> (0:11)
—	3, 19	<1/25	1/500	<i>S. senftenberg</i> (0:3, 19)
—	13, 22	<1/25	1/100	<i>S. poona</i> (0:13, 22)

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mixture<sup>6,7</sup>. The globulin conjugates were freed of unconjugated dye by passing them through 'Sephadex G25', and concentrated to their original volumes by dialysis in polyethylene glycol (mol. wt., 20,000). The conjugated antisera were then tested for staining of *Salmonellae* from each of the *Salmonella* groups for which antigens had been prepared.

Tables 1 and 2 show the agglutination titres and staining characteristics of each of the three polyvalent antisera. Reasonable titres were obtained against each antigen, and in each case this corresponded with strong specific fluorescent staining of the relevant *Salmonellae*. For example, the polyvalent serum No. 1 stained all the organisms tested, but the polyvalent sera Nos. 2 and 3 stained only those organisms belonging to O groups for which the antisera showed agglutinating powers.

The conjugated polyvalent antisera described here are being assessed in routine immunofluorescence *Salmonella* tests on raw meat. Experience so far confirms that the direct technique may have worth-while advantages over the indirect technique used in our earlier investigations<sup>1</sup>. If necessary a series of such conjugated polyvalent antisera could be prepared, using group O antigens from all existing *Salmonella* groups.

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### Colicin 15: Possibly a Defective Bacteriophage

COLICINS, bactericidal agents elaborated by many strains of the Enterobacteriaceae, have been classified as antibiotics<sup>1</sup>. Purified preparations of four colicins have been identified as lipocarbohydrate proteins<sup>2-5</sup>; a fifth apparently lacks the lipid component<sup>6</sup>. This communication presents evidence that an inducible colicin from *Escherichia coli*, WT15 (ref. 7), may be a defective bacteriophage.

*E. coli* WT15 produces colicin, with concomitant lysis of the cells, only after exposure to ultra-violet light or to certain chemicals. Azaserine is a convenient inducing agent for large quantities. Cells were grown in two portions of 2 l. each of glucose-ammonium salts medium<sup>8</sup> in 4-l. Erlenmeyer flasks on a rotary shaker at 37° C. When the concentration reached about 10<sup>8</sup> cells/ml., azaserine, in concentrated aqueous solution, was added until the final concentration was 0.4 µg/ml. While incubation, with shaking, continued, small samples were removed aseptically at intervals to determine their optical densities in the Coleman Universal Spectrophotometer at 540 mµ. When maximum lysis was achieved (about 4 h), the culture was saturated with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and stored at 5° C overnight. After refrigerated centrifugation at 3,900g for 30 min, the precipitate was resuspended in 73 ml. deionized H<sub>2</sub>O. During the next 48 h at a maintained temperature of 5° C the mixture was dialysed twice against 6 l. of deionized H<sub>2</sub>O with stirring. Insoluble debris was removed by centrifugation for 20 min at 14,350g. The supernatant fluid was chromatographed on diethylaminoethanol-

cellulose (DEAE) by a variation of the procedure of Goebel<sup>9</sup>. The sample (19 ml.), adjusted to pH 8.0 with 1 ml. M tris(hydroxymethyl)aminomethane, was added to the column (1.1 cm × 21 cm) of DEAE-cellulose, previously equilibrated to pH 8.0 with 0.05 M tris buffer. Gradient elution was achieved by the dropwise addition of 150 ml. of 0.3 M tris-0.5 M NaCl at pH 7.2 to a mixing chamber containing 150 ml. of 0.05 M tris at pH 8.0. Fractions of 1.5 ml. each were collected automatically at the rate of 0.1 ml./min and their optical densities determined at 280 mµ in a Beckman DU spectrophotometer. They were assayed for ability to inhibit the growth of a sensitive strain of bacteria. Uniform drops of two-fold serial dilutions of each fraction were placed on the surface of agar plates seeded with *E. coli* WT15. The plates were then incubated at 37° C for 18 h. The reciprocal of the greatest dilution producing a clearly defined zone of inhibition was arbitrarily assigned as the activity factor for the preparation. Results (Fig. 1A) indicate considerable purification of colicin, most of the biologically active material being eluted in Fractions 52-59. Before and after elution of the major part of the colicin some fractions showed relatively high absorption at 280 mµ, but little or no biological activity.

Fractions 52 through 59 (Fig. 1A) were combined and applied to a second column of DEAE-cellulose. Gradient elution was performed as previously described (Fig. 1B). Biological activity closely followed intensity of absorbance at 280 mµ, and single peaks of activity and absorbance were obtained. The fractions, negatively stained with phosphotungstic acid, were examined in the electron microscope. The quantity of bacteriophage-like bodies

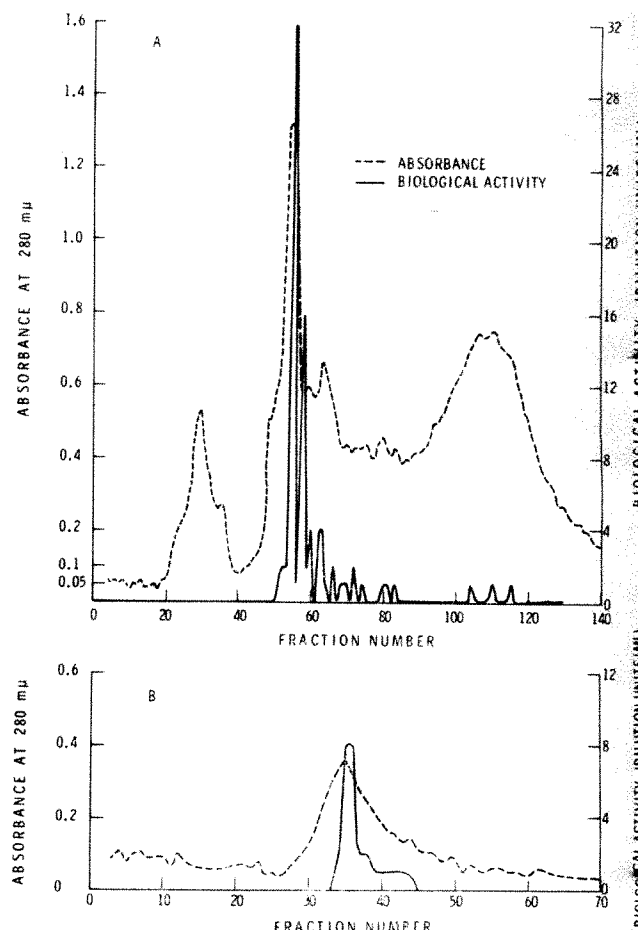


Fig. 1. Colicin 15 chromatographed on DEAE-cellulose by gradient elution from 0.05 M tris buffer, pH 8.0, toward 0.3 M tris-0.5 M NaCl, pH 7.2. A, Fractionation of crude concentrate; B, second chromatogram of fractions 52 through 59 from A.



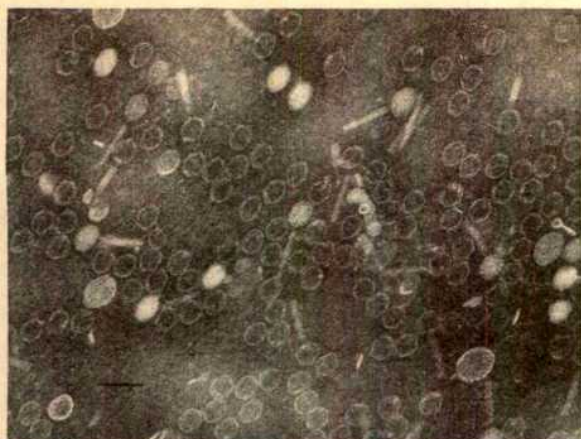


Fig. 2. An electron micrograph of purified colicin 15, negatively stained with phosphotungstic acid, showing empty heads, tails and apparently intact phage particles. The scale line represents 100 mμ. (x c. 59,300)

seemed to be correlated with the degree of biological activity of the preparation. Fraction 35, which exhibited a high degree of antibacterial activity, contained an abundance of phage elements (Fig. 2), consisting predominantly of empty heads but also showing tails and particles that appear to be intact phage. Fractions 30 and 51, which had little biological activity, contained few phage-like structures.

The identity of phage and colicin was confirmed by sedimentation of the pooled active fractions 35 and 36 (Fig. 1B) in high-density  $\text{NaNO}_3$  in deuterium oxide, density 1.28 g/ml. The sample (1.15 ml.), mixed with 3.15 ml. of the  $\text{NaNO}_3$  in  $\text{D}_2\text{O}$  to a density of 1.21 g/ml., was centrifuged in the SW 39 rotor (Spinco Model L ultracentrifuge) at 38,000 r.p.m. (159,000g) for 17 h. Five fractions (0.8 ml. each) were removed sequentially from top to bottom of the tube. Each was assayed microbiologically for colicin and examined in the electron microscope. Biological activity and phage were found only in the bottom fraction.

The particulate nature of this colicin was further evidenced by differential centrifugation and gel filtration. A concentrate of colicin (150 ml.), prepared by precipitation with  $(\text{NH}_4)_2\text{SO}_4$ , was thoroughly dialysed and centrifuged at 90,000g for 2 h. The lowest fifth of the total volume was removed and recentrifuged at 31,890g for 2 h. After the lower half of this preparation was dialysed overnight at 5° C against 0.1 M phosphate buffer at pH 7.0, 0.2 ml. was applied to a column of 4.3 ml. Sephadex G-200 in a 5-ml. serological pipette. The column was washed with 0.1 M phosphate buffer, pH 7.0. The excluded phase contained nearly all the biologically active material as well as an abundance of phage-like components. Since Sephadex G-200 has an approximate exclusion limit of a molecular weight of 200,000, colicin 15 apparently has a large molecular size.

Electron microscopic examination disclosed apparently intact phage particles in concentrates of colicin with high bactericidal activity against *E. coli* WT15. However, attempts to demonstrate typical lytic phage were unsuccessful. No plaques were produced by various preparations of colicin when tested in the usual manner against thirteen different strains of Enterobacteriaceae, including *E. coli* WT15.

Although colicins and temperate phages have long been thought to be related<sup>10</sup>, direct evidence has been lacking. In an electron microscope examination of colicinogenic strains, Kellenberger and Kellenberger<sup>11</sup> could not identify structural components that could be considered colicins. That colicins may represent the products of defective lysogeny has recently been suggested<sup>10,12</sup>. If so, colicins may range from relatively simple proteins to

completely formed phage particles. The identity of phage and colicin from *E. coli* WT15 suggests that bacterial lysis after induction may be a function of the development of the defective phage particle. Possibly smaller units, such as proteins, are secreted without lysis, whereas synthesis of discrete structures, such as heads or tails, results in cell disruption. These data strongly suggest that *E. coli* WT15 is a defective, lysogenic strain and that colicin 15 is a bactericidal component of its phage.

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## ENTOMOLOGY

### Enforced Occurrence of a Mosaic Type Neurosecretory Cell in the Chrysomelid Beetle, *Galeruca tanacetii* (Linn.)

*Galeruca tanacetii* (Linn.) has a summer reproductive diapause followed by autumnal reproductive activities in the same year. A univoltine species, the adults of *Galeruca* emerge about the first week of June and remain in diapause until the middle of August when the ovarioles are completely undifferentiated. Development and maturation of the terminal oocytes are completed by mid-September, after which the females oviposit successive batches of eggs to the end of November. The interval between successive ovipositions varies between 7–10 days.

It has been found that photoperiod and temperature are the primary factors controlling diapause and reproduction. This beetle is a short-day species. The critical photoperiod is about 12 h/day at 20° C; at 15° C is probably about 2 h longer. With a 12 h photoperiod at 20° C, reproductive diapause is terminated in newly emerged females, which oviposit within 40 days. At photoperiods of 16 h and more, diapause is sustained and there is virtually no development of ovarioles within 70 days.

In the course of investigating the effect of transferring ovipositing and maturing females to a photoperiod of 16 h at 20° C, to examine the reproductive activities, two observations were made on the cytology of the neurosecretory cells of the pars intercerebralis of the brain. Ovipositing females laid an average of 1.8 batches of eggs per beetle and maturing females laid an average of 1.1 batches of eggs per beetle when transferred to these conditions in 30 days. They then reverted to



diapause within the experimental period. Post-mortem dissections revealed undeveloped eggs in the ovarioles.

Histological observations made on the neurosecretory cells of these experimental beetles using the alcian blue/phloxine technique<sup>1</sup> revealed that (a) the neurosecretory cells were comparable in cytology to those of diapause females, and (b) the A-cells contained within them phloxinophil granules. These mosaic type cells are comparable to the 'castration cells' of the suboesophageal ganglion of *Leucophaea maderae*<sup>2</sup> and in some hymenopterous species in which Thomsen<sup>3</sup> found both red and blue granules in the same cells using the chrome-haematoxylin/phloxine technique. I have used the alcian blue/phloxine and the chrome haematoxylin/phloxine techniques in cytological investigations of the neurosecretory cells and have not found any phloxinophil granules in the A-cells. In the control in this series of experiments, when maturing females were transferred to a photoperiod of 12 h at 20° C, they continued to lay eggs and their A-cells had no phloxinophil granules. It appears that the enforced occurrence of these mosaic type cells can be attributed to an altered metabolism of the beetle from a physiologically active reproductive phase to a non-active diapause phase as a result of changing its external environment.

This work was carried out at Silwood Park, Imperial College Field Station, Sunninghill, Berkshire. I thank Dr. N. Waloff for her supervision.

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## CYTOLOGY

### Labelled Cytoplasmic Organelles in *Allium cepa* Var. 'White Lisbon' after Administration of Tritiated Thymidine

THE specificity of tritium- or carbon-14-labelled thymidine as a precursor for deoxyribonucleic acid (DNA) appears to be good in many organisms<sup>1,2</sup>. Autoradiographs examined by light and electron microscopes have demonstrated that most of the label is incorporated into nuclear chromatin during the DNA synthetic phase of cell reproduction or during an exchange process in differentiated cells<sup>3-5</sup>. Occasional evidence for the cytoplasmic incorporation of thymidine has been obtained with animal cells after treatment with deoxyribonuclease<sup>6</sup>. In egg cells and developing embryos of vertebrates there is biochemical evidence for the presence of extranuclear DNA<sup>7-9</sup>.

In recent experiments, seedlings of *Allium cepa* were germinated on filter paper moistened with sterilized tap-water for 6-7 days at room temperature and then placed in tap-water containing 2.5 µc./ml. of tritiated thymidine (specific activity 2.7 c./mmole) for 1 h or 24 h. Root tips about 1-3 mm long were fixed in potassium permanganate<sup>10</sup> or osmium tetroxide<sup>11</sup> for 15 min-5 h, dehydrated in ethanol and embedded in 'Araldite'. Thin sections cut on a Porter-Blum MT2 ultramicrotome were transferred to 'Formvar' membranes<sup>12,13</sup> and coated with 'L4' emulsion (Ilford, Ltd.). Sections, 1-2 µ, placed on glass slides were coated with the same emulsion and the preparations were stored in light-proof boxes at approximately 4° C. 23-84 days later the autoradiographs were developed in 'D19b' (Kodak) for 2 min and fixed in 20 per cent sodium thiosulphate for 5 min at 20° C. The thin sections and superimposed autoradiographs were mounted on 3 mm copper grids<sup>12,13</sup> and examined either unstained or after heavy metal staining<sup>14,15</sup> in a Siemens Elmiskop I electron microscope. The autoradiographs of

Table 1. LIGHT MICROSCOPE AUTORADIOGRAPHS

Time of fixation	Auto-radiograph exposure time	Distribution of developed grains				Total cells
		Labelled nucleus and cytoplasm	Labelled cytoplasm only	Labelled nucleus only	Unlabelled	
1 h	23 days	158	146	13	24	341
	51 days	148	130	9	34	321
	Per cent	46.3	41.7	3.3	8.7	100
24 h	23 days	399	19	—	2	420
	51 days	468	40	—	—	508
	Per cent	93.3	6.4	—	0.3	100

Table 2. ELECTRON MICROSCOPE AUTORADIOGRAPHS

Grain distribution over root tips fixed after 1 h in <sup>3</sup> H-thymidine							
Auto-radiograph exposure time	No. of cells	Grain distribution					Total grains
		Nucleus	Mito-chondria	Pro-plastids	Rest of cytoplasm	Cell wall	
23 days	15	461	18	31	15	4	529
	Per cent	87.1	3.4	5.9	2.8	0.8	100
84 days	15	743	61	52	23	16	895
	Per cent	83.0	6.8	5.8	2.6	1.8	100

thick sections were investigated using a phase contrast microscope. Control sections of unlabelled root tips were prepared in the same way.

Heavily labelled nuclei were observed in thick sections examined by light microscopy, together with some cytoplasmic labelling which in many cells was localized around the outside of the nuclear membrane or over groups of organelles distributed elsewhere in the cytoplasm. Nucleoli were not labelled in any of the preparations. The cytoplasmic labelling was observed over most meristematic cells and cells which were differentiating.

In sections of seedlings exposed to thymidine for 1 h before fixation, 46.3 per cent of the cells had labelled nuclei and cytoplasm and 41.7 per cent had labelled cytoplasm only. A smaller proportion of cells were unlabelled (Table 1). After 24 h nearly all the meristematic cells and differentiating cells had labelled nuclei and cytoplasm. The cells with labelled cytoplasm only were mainly, but not exclusively, situated in the boundary zone between root cap and meristem at 24 h.



Fig. 1. Electron microscope autoradiograph of a root tip meristem cell exposed to tritiated thymidine for 1 h. Developed photographic grains occur over nuclear chromatin. ( $\times 18,000$ )



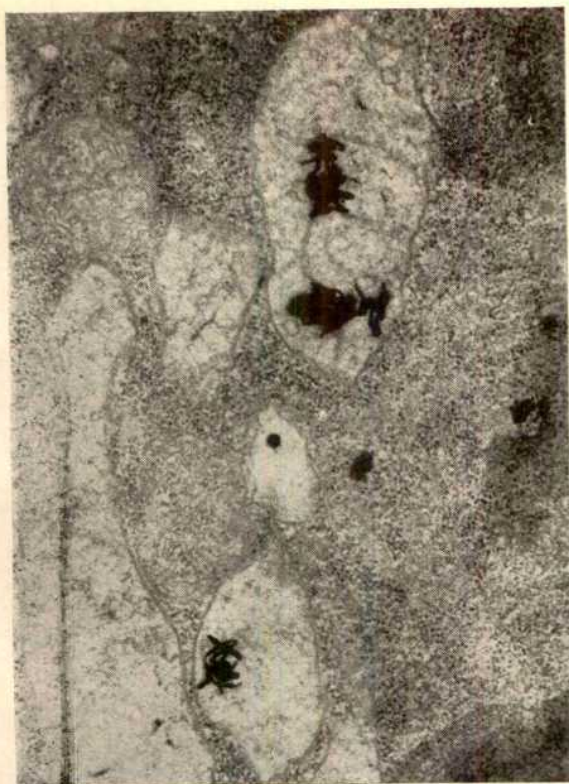


Fig. 2. Electron microscope autoradiograph of root tip exposed to thymidine for 1 h. Developed photographic grains are visible over the internal matrix of proplastids. ( $\times 30,000$ )

Electron microscope autoradiographs showed the same distribution of developed silver grains over nuclei and cytoplasm as the light microscope preparations, with most grains occurring over nuclear chromatin (Fig. 1 and Table 2). Over the cytoplasm, developed grains were seen over mitochondria, and also over structures termed 'proplastids'<sup>16</sup>, but which could be immature mitochondria<sup>17</sup>, in considerably greater numbers than over the remaining cytoplasm and cell walls (Table 2). The grains were more frequently observed over the internal matrix and membranes of the mitochondria and proplastids than over the boundary membranes (Fig. 2). In many cases grains were associated with fibres similar in appearance to those shown by Nass and Nass<sup>20,21</sup> to be removed by deoxyribonuclease.

These results are interesting in view of recent electron cytochemical evidence<sup>18-21</sup> supporting earlier suggestions<sup>6,22</sup> that mitochondria and plastids may contain DNA.

The high concentration of grains over nuclear chromatin and, to a lesser extent, over proplastids and mitochondria, taken in conjunction with the lack of grains over nucleoli and ribosomes, suggest that in our autoradiographs the thymidine has been incorporated into DNA and not into ribonucleic acid (RNA). Enzymatic experiments using nucleases are in progress.

The results included in Table 1 indicate that during the first hour of thymidine administration label is incorporated into the mitochondria and proplastids of some cells which are not in the nuclear DNA synthetic phase. In view of the high proportion of cells with labelled cytoplasm only at this time (Table 1) it seems likely that mitochondria and proplastids are labelled independently from nuclei which are in the DNA synthetic phase, and they probably do not become labelled by passage of already labelled DNA out of the nucleus. It is possible that mitochondrial and proplastid DNA synthesis is required for the production of new organelles in the cytoplasm<sup>23</sup> or as a stage in the addition of material to

nuclear DNA<sup>6</sup>. Further experiments which are in progress should throw more light on these possibilities.

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### A Cytoplasmic Organelle of Melanocytes

DURING his classical investigations of intracellular organelles Claude isolated melanin granules from the liver of *Amphiuma tridactylum*<sup>1</sup>. Sections of these granules were later examined by Drochmans, who showed them to possess definite structural detail<sup>2</sup>. Other investigations have shown the existence of a highly regular, almost crystalline lattice in melanin granules from other tissues<sup>3</sup>. Melanin itself was suggested by Longuet-Higgins as having properties of a one-dimensional semi-conductor<sup>4</sup>, and this was agreed on by Pullman<sup>5</sup>. Semiquinone free radicals have been demonstrated in melanin granules and the characteristic signals<sup>6</sup> became more pronounced during irradiation of such granules with light<sup>7,8</sup>.

Evidence such as that summarized here suggested that melanin granules might play some part in the energy metabolism of the cells in which they abound<sup>9,10</sup>. This seemed to be particularly pertinent to the pigment cells of the human nervous system since depigmentation of these cells is a frequent finding in some extra-pyramidal diseases<sup>11,12</sup>.

Electron microscopic investigations of osmium-fixed *Amphiuma* liver showed that the hepatocytes contained large numbers of mitochondria. In contrast to these cells, the melanocytes contained either few or no mitochondria. Instead, the dominant cytoplasmic organelles of the melanocytes were uniform round bodies measuring approximately  $2.2\mu$  in diameter. Each of these was bounded by a membrane which, in some of the micrographs, appeared to be double. This membrane enclosed several dense melanin granules as well as many concentrically laminated sub-units. While the melanin granules were of the same uniformity as that demonstrated by Claude<sup>1</sup>, the concentrically laminated sub-units varied in size, in shape and in density. However, they were always less dense than the melanin granules and were always oval or round.

Examination of formalin-fixed paraffin sections stained with hæmatoxylin and eosin showed different degrees of pigmentation of the granules from one cell to the next, but apparent uniformity of pigmentation within each cell. The granules were clearly visible and were seen to occur



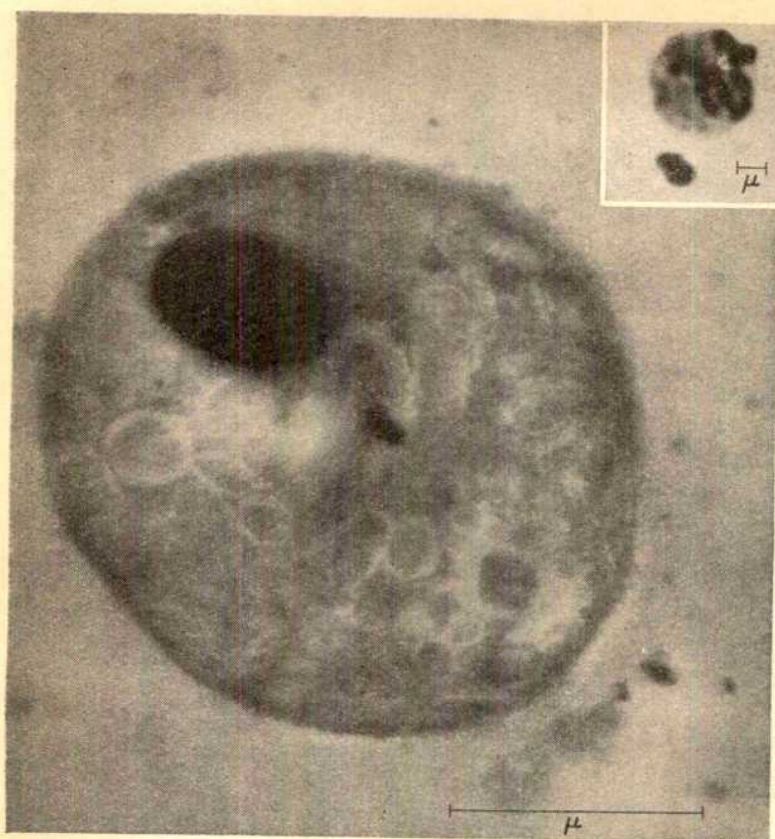


Fig. 1. The large electron-micrograph shows a thin section of an osmium-fixed, uranyl-acetate-stained intracellular organelle like those described in the text ( $\times c. 33,200$ ). The inset is a photomicrograph of an entire organelle, from a homogenate in 0.25 M sucrose stained with toluidine blue. Two melanin granules attached to each other are seen outside this organelle ( $\times c. 3,600$ ).

mostly in clumps. As opposed to the melanin granules, the larger body in which the granules were embedded could not be seen. After incubation of de-paraffinized sections in 30 per cent hydrogen peroxide for 24 h at room temperature the melanin granules could no longer be seen but the cytoplasm was now studded with vacuoles. These were larger than the melanin granules but it was difficult to measure their size because their boundaries were not clear. Furthermore, the cytoplasm of the melanocytes was ash coloured and showed a foamy appearance. Neither the cytoplasm of the hepatocytes nor the nuclei of all the cells investigated had changed their appearance following incubation with hydrogen peroxide. In addition, the cell membranes of the melanocytes were much more prominent after this incubation than they had been before. It was therefore improbable that the vacuolated appearance of only the cytoplasm of the melanocytes was due to an artefact. On the contrary, in view of the earlier description of phagocytic 'vacuoles'<sup>13</sup> in melanophages<sup>14</sup> this finding could have indicated that the intracellular organelle under examination was merely a vacuole.

A critical experiment was thought to be one in which the integrity of these bodies could be demonstrated following the rupture of the cell membrane. It was assumed that these bodies must be fragile otherwise they would have been isolated earlier. Therefore, liver suspensions in 0.25 M sucrose were made gently, namely, by a single passage through a tissue press followed by a single excursion of a slowly rotating 'Teflon' pestle through a Potter-Elvehjem homogenizer. Such suspensions showed an abundance of the bodies we were seeking among unbroken cells, cellular debris and free melanin granules. The bodies were round in shape and contained one or more melanin granules embedded in a pale refractive matrix. However, these bodies were not so uniform as we had expected them

to be from the electron micrographic examinations. Indeed, some were seen to swell during prolonged observation of suspensions in sucrose solution. These organelles were not observed to take up free melanin granules. The latter often adhered to each other in strands or in clumps. The matrix of the large organelles was readily stained by toluidine blue and more slowly by janus green B.

It was pertinent to ask whether this intracellular organelle was peculiar to the *Amphiuma* liver. Preliminary observations were therefore made on an osmium-fixed sample of *Substantia nigra* obtained from a human autopsy 2 h after death (BNL Hospital No. A-201-64). Electron microscopic investigation revealed the presence of many cytoplasmic bodies containing primarily dense melanin granules. These bodies were round or polygonal, about one-third the size of those seen in *Amphiuma* liver, and were arranged in clusters within the cytoplasm. Neither the boundaries of these bodies nor their fine structure could be examined accurately because autolysis had already set in. However, the resemblance between these bodies and those described in *Amphiuma* liver was close enough to make it likely that they are the same type of organelle.

It is of interest that arrangements of melanin granules bearing some similarities to those shown by our electron microscopic investigations have been encountered by earlier workers in a variety of tissues. These have been variably designated as phagocytic vacuoles<sup>15</sup>, as conglomerate granules<sup>16</sup>, as melanin-building centres<sup>17</sup>, as melanin spherules<sup>18</sup>, as irregular inclusions<sup>19</sup>, or as compound granules<sup>20</sup>. We would expect that some of these might maintain their structural integrity after completion of the homogenization necessary for their isolation in bulk.

The demonstration of this discrete integrated intracellular organelle indicates that the metabolism of melanins is a much more complex process than we thought it to be during the initial phases of this work<sup>11,12</sup>.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 1

SOCIETY OF ENGINEERS (in the Apartments of the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Inaugural Meeting for the year 1965. Mr. C. L. N. Laing: Presidential Address.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Members' Discussion on "The Society's Publications". Discussion Leader: Prof. W. G. Overend.

INSTITUT FRANCAIS DU ROYAUME-UNI (at Queensberry Place, South Kensington, London, S.W.7), at 8.15 p.m.—French Scientific Films.

## Tuesday, February 2

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. K. Wolfe: "The Refurbishing of Engineers in the U.S.A."

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5.30 p.m.—Dr. K. McCarthy: "Studies with Rubella Virus". (Sixth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. A. L. Ashton and Mr. J. E. H. Hayward: "Newer Thermosetting Materials".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the University College Chemical and Physical Society, at University College, Gower Street, London, W.C.1), at 6.30 p.m.—Prof. A. J. Birch, F.R.S.: "Some New Synthetic Reactions Applied to Steroids".

ROYAL PHOTOGRAPHIC SOCIETY OF GREAT BRITAIN (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 7.15 p.m.—Dr. Harold E. Edgerton: "Electronic Flash Photography".

## Wednesday, February 3

ROYAL MICROSCOPICAL SOCIETY, and the BRITISH MICROCIRCULATION SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), from 11 a.m. to 7 p.m.—Symposium on "Living Microscopy".

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, Prince Consort Road, London, S.W.7), at 2 p.m.—Meeting on "Methods of Assessing Colour Rendering". Speakers: Dr. J. L. Ouweltjes, Dr. B. H. Crawford and Dr. D. A. Palmer.

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. D. Penington: "Regulation of Bone Marrow Activity".\*

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (joint meeting with the Spectroscopy Group of the Institute of Physics and the Physical Society, at the Wellcome Building, Euston Road, London, N.W.1), at 3 p.m.—Meeting on "New Light Sources in Spectroscopy".

GEOLOGICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. G. P. Butler, Mr. C. G. St. C. Kendall, Dr. D. J. J. Kinsman, Mr. D. J. Shearman and Sir Patrick A. d'E. Skipworth, Bt.: "Recent Anhydrite from the Trucial Coast of the Arabian Gulf".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Dr. M. B. Priestley: "Evolutionary Spectra and Non-Stationary Processes".

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Dr. Edwin Clarke: "Nicolas Stensen and the Brain".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. C. R. Major and Mr. A. C. Moore: "The Economics of Motor Gasoline Quality".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. J. Bolton: "A Seasonal Tariff for Domestic Supplies".

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. C. Kendrew: "The Genetic Determination of Protein Function".\*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E./I.E.E. Medical Electronics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Safety of Operating Theatre Equipment".

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "Data Recovery".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2) at 6 p.m.—Mr. Cecil J. Allen: "The Future of Railways".

SOCIETY OF ENVIRONMENTAL ENGINEERS, PACKAGING GROUP (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. D. B. Osborn: "Cushion Design".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Chelsea College Chemical Society, at Chelsea College of Science and Technology, Manresa Road, London, S.W.3), at 7 p.m.—Sir Cyril Hinshelwood, O.M., F.R.S.: "The Evolution of Kinetics".

## Thursday, February 4

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. R. H. Kay: "Bioluminescence of a Planktonic Crustacean"; Mr. B. E. B. Moseley and Mr. H. Laser: "Repair of Ionizing Radiation Damage in Radiation Resistant Bacteria".

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. Gordon Ratray Taylor: "Biological and Botanical Books Old and New".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. W. B. Douglas: "Health and School Achievement". (Seventh of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

CHEMICAL SOCIETY (in the Physics Lecture Theatre, Imperial College of Science and Technology, South Kensington, London, S.W.7), at 6 p.m.—Prof. F. Sondheimer: "Syntheses in the Cardiac-Active Steroid Field". (Tilden Lecture.)

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Dr. A. J. Barnard: "Refrigeration Plant for the Quick-Freezing Industry".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Major-General B. D. Kapur: "Development of Electronics in India".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "Synthesizing Hydraulic Control Systems from Available Components".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Prof. J. H. Argyris: "Current Work in the Structures Field".

## Friday, February 5

TELEVISION SOCIETY (in the I.T.A. Conference Suite, 70 Brompton Road, London, S.W.3), at 7.45 p.m.—Dr. N. Mayer (Munich): "The N.T.S.C. Colour Television System Using Additional Reference Transmission".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. D. M. MacKay: "The Eye and the Brain".

## Monday, February 8

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Organic Mental Syndromes".\*

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. Bondi, F.R.S.: "The Disproof of Theories".

UNIVERSITY OF LONDON (in the Physiology Department Theatre, St. Bartholomew's Hospital Medical College, West Smithfield, London, E.C.1), at 5.30 p.m.—Prof. K. J. Ullrich: "Characteristics of Renal NaCl-transport, and Its Modification by Aldosterone and Saluretic Sulphonamides".\*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. B. W. Leyland: "Earth Return Applied to High Voltage Systems".

ROYAL AERONAUTICAL SOCIETY, HISTORICAL GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Sqn./Ldr. S. C. Winfield Smith: "British Aviation Fifty Years Ago".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. J. B. Ward Perkins, C.B.E.: "The Changing Historical Landscape of Italy".

## Monday, February 8—Tuesday, February 9

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2)—Conference on "Electronics Design".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PRINCIPAL LECTURER (with high academic qualifications, and preferably industrial or research experience) IN MATHEMATICS—The Registrar, Lancaster College of Technology, Priory Street, Coventry (February 2).

ASSISTANT LECTURERS OR LECTURERS (2) IN THE DEPARTMENT OF MATHEMATICS—The Registrar, University of York, Heslington, York (February 12).

ASSISTANT LECTURER (preferably with special interests in fungi) IN THE DEPARTMENT OF BOTANY; an ASSISTANT LECTURER IN ORGANIC CHEMISTRY and an ASSISTANT LECTURER IN INORGANIC CHEMISTRY; an ASSISTANT LECTURER IN GEOLOGY; a LECTURER/ASSISTANT LECTURER IN THE HISTORY AND PHILOSOPHY OF SCIENCE; ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF PHYSICS; an ASSISTANT LECTURER IN THE DEPARTMENT OF PSYCHOLOGY; an ASSISTANT LECTURER IN THE DEPARTMENT OF ZOOLOGY; and an ASSISTANT LECTURER IN BIOLOGY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (February 11).

LECTURER IN NUMERICAL ANALYSIS, and an ASSISTANT LECTURER IN MATHEMATICAL STATISTICS—The Registrar, The University, Leicester (February 13).

LECTURER IN THE DEPARTMENT OF PSYCHOLOGY; and LECTURERS (2) IN THE DEPARTMENT OF SOCIOLOGY—The Registrar, The University, Leicester (February 13).

ASSISTANT LECTURER (preferably with an interest in developmental research) IN PSYCHOLOGY—The Registrar, The University, Sheffield (February 15).

LECTURER (preferably with a special interest in the nutritional aspects of biochemistry) IN BIOCHEMISTRY—The Secretary of the University Court, The University, Glasgow (February 15).

SENIOR LECTURER and a LECTURER (specialists in one or more of the following fields: econometric theories, applied econometrics, mathematical programming, input-output analysis, sources of economic statistics) IN THE DEPARTMENT OF ECONOMETRICS—The Registrar, The University, Manchester, 13 (February 17).

EXPERIMENTAL OFFICER (with some experience in organic microanalysis) for microanalysis IN THE DEPARTMENT OF CHEMISTRY—The Registrar, The University, Manchester, 13 (February 19).

LECTURER (with an honours degree and experience of control engineering and/or electrical machines) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, The University, Leeds, 2 (February 22).

**PROFESSOR AND HEAD** (with a higher degree, preferably a doctorate, research experience in the field of plant science, and preferably experience in university teaching) of the Department of Botany, University of Queensland, Australia—Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, February 26).

**TURNER AND NEWALL RESEARCH FELLOWS IN ENGINEERING, INORGANIC CHEMISTRY, PHYSICS** and allied subjects—The Registrar, The University, Manchester, 13 (February 27).

**ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS**—The Registrar, University College of Wales, Aberystwyth (February 28).

**CHAIR OF BIOLOGICAL SCIENCE** at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (February 28).

**LECTURER AND AN ASSISTANT LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY**—The Secretary, University College, Gower Street, London, W.C.1 (March 1).

**READER IN ANTHROPOLOGY**—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 1).

**READER IN ELECTRICAL ENGINEERING SCIENCE IN THE NEW DEPARTMENT OF ENGINEERING SCIENCE**—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 1).

**SENIOR LECTURER AND A LECTURER IN THE DEPARTMENT OF STATISTICS**—The Secretary, The University, Aberdeen, Scotland (March 1).

**CHAIR OF VETERINARY ANATOMY OR VETERINARY PHYSIOLOGY** at the University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 2).

**LECTURERS** (preferably with special qualifications and experience in cell physiology, plant biochemistry, experimental taxonomy, cytogenetics or microbiology) in **BOTANY** at the University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1, or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (March 3).

**POST-DOCTORAL RESEARCH ASSISTANTS IN ORGANIC CHEMISTRY** for synthetic work related to cephalosporin C and the penicillins—Dr. G. Lowe, The Dyson Perrins Laboratory, The University, South Parks Road, Oxford (March 6).

**LECTURER OR ASSISTANT LECTURER IN SOCIAL ANTHROPOLOGY** in the School of African and Asian Studies—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (March 10).

**LECTURER OR ASSISTANT LECTURER** (with special interests in economic geology) in the **DEPARTMENT OF GEOLOGY**—The Registrar, University College of Swansea, Singleton Park, Swansea (March 20).

**DEMONSTRATOR** (preferably with postgraduate research experience and a special interest in fungal genetics or cytology) in the **DEPARTMENT OF AGRICULTURAL BOTANY**—The Registrar, University College of Wales, Aberystwyth (March 31).

**POST-DOCTORAL FELLOW** to work on new methods of peptide synthesis in collaboration with Dr. C. J. M. Stirling—Prof. H. B. Henbest, Department of Chemistry, Queen's University, Belfast, Northern Ireland (March 31).

**PROFESSOR AND HEAD OF THE DEPARTMENT OF FORESTRY**—The Secretary and Registrar, The University College of North Wales, Bangor, North Wales (March 31).

**ASSISTANT LECTURER OR LECTURER IN ORGANIC CHEMISTRY**—The Registrar, University College of Swansea, Singleton Park, Swansea (April 5).

**CHAIR OF PHYSICAL CHEMISTRY**—The Registrar, University College of Swansea, Singleton Park, Swansea (May 1).

**ASSISTANT LECTURER (Demonstrator) IN THE DEPARTMENT OF ANATOMY** (Prof. J. Z. Young, F.R.S.) to undertake teaching and research—The Secretary, University College, Gower Street, London, W.C.1.

**ASSISTANT MASTER** to teach Mathematics to scholarship level—The Headmaster, Merchant Taylors' School, Sandy Lodge, Northwood, Middlesex.

**D.S.I.R. RESEARCH ASSISTANT** (with some experience of electro-physiological techniques) in the **ZOOLOGY DEPARTMENT**, to work with Dr. E. J. Binyon on the neuromuscular physiology of certain echinoderms—The Professor of Zoology, Royal Holloway College (University of London), Englefield Green, Surrey.

**RESEARCH ASSISTANT** (preferably with chemistry, physics or biochemistry qualifications) for a M.R.C. project on ion transport across living membranes—Prof. H. E. de Wardener, Department of Medicine, Charing Cross Hospital Medical School, Fulham Hospital, London, W.6.

**RESEARCH FELLOW** (graduate in electrical engineering, physics or mathematics) in the **DEPARTMENT OF ELECTRICAL ENGINEERING**, for studies in the synthesis of lumped linear electrical networks—The Registrar, The University, Leicester.

**SENIOR LECTURER OR READER** (academically well qualified and with lecturing as well as significant research and/or industrial experience) in the **DEPARTMENT OF INDUSTRIAL CHEMISTRY**—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 1/G.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

The Gas Council. Research Communications. GC105: The Gas Council Basic Research Group—Its Aims and Objectives. By Dr. J. A. Gray. Pp. 8. GC104: Report on the Gas Council Research Scholarships, 1964. Pp. 4. GC106: The Catalytic Gasification of Petroleum Feedstocks. By Dr. R. G. Cockerham and T. A. Yarwood. Pp. 15. GC107: Recent Field and Laboratory Investigations Into Domestic Warm Air Heating. By E. W. G. Dance, J. A. Harris and D. R. Wills. Pp. 34. GC108: Forced-Convection Techniques for Gas-Fired Rapid Billet Heating. By M. N. Lawrence and J. Spittle. Pp. 16. GC109: The Design and Control of a Range of Self-Propportioning, Nozzle-Mixing, Tunnel Burners. By M. L. Hoggarth and P. F. Aris. Pp. 24. GC110: Air/Gas Proportioning Techniques for Industrial Burners. By R. A. Hancock. Pp. 16. GC111: 55th Report of the Joint Refractories Research Committee, 1963-64. Pp. 27. GC112: Further Experiments with a Slagging Pressure Gasifier. By Dr. D. Hedden, Dr. J. A. Lacey and A. G. Horsler. Pp. 16. GC113: The Gasification of Coal in an Experimental Rummel Double-Shaft Slag-Bath Gasifier. By Dr. M. MacCormack and J. Wrobel. Pp. 14. GC114: Applications of Modern Analytical Techniques in the Gas Industry. Part 2: The Complete Analysis of Fuel Gases by Gas Chromatography. By Prof. A. L. Roberts and Dr. C. P. Ward. Pp. 10. GC115: Domestic Aerated Burners for Methane. By J. A. Harris and J. A. Prigg. Pp. 19. GC116: The Use of New Analytical

Techniques in Control and Research. By G. R. Boreham and W. E. Armstrong. Pp. 22. (London: The Gas Council, 1964.) [2511]

Building Research Station Digest (Second Series), No. 52: Space and Water Heating in Local Authority Flats. Pp. 4. (London: H.M. Stationery Office, 1964.) 4d. [2511]

General Register Office. Census 1961: England and Wales. Housing National Summary Tables. Pp. v+14. (London: H.M. Stationery Office, 1964.) 2s. 6d. net. [2511]

Ciba (A.R.L.), Ltd. Technical Notes, No. 262 (October 1964): Church Furniture Bonded with Aerolite. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1964.) [2511]

### Other Countries

Institut für Atomenergie, Kjeller Research Establishment. Kjeller Report No. 79: Quarterly Progress Report, April-May-June, 1964. Pp. iv+25. (Kjeller: Institut für Atomenergie, Kjeller Research Establishment, 1964.) [2511]

Population Reference Bureau, Inc. Population Bulletin, Vol. 20, No. 6 (October, 1964): United Nations: Population Opinion Survey. Pp. 141-172. (Washington, D.C.: Population Reference Bureau, Inc., 1964.) 50 cents. [2511]

Transactions of the American Philosophical Society. New Series, Vol. 54, Part 6: John Daly Burk—Irish Revolutionist and American Patriot. By Joseph I. Shulim. Pp. 60. (Philadelphia: The American Philosophical Society, 1964.) 2 dollars. [2511]

The National Parks of Queensland, Australia. Pp. 32. (Brisbane: State Public Relations Bureau, Premier's Department, 1964.) [2511]

Proceedings of the United States National Museum, Smithsonian Institution. No. 3475, Vol. 114: Moths of the Family Acrolophidae in America North of Mexico (Microlepidoptera). By Frank F. Hasbrouck. Pp. 487-706. No. 3494, Vol. 116: A Review of the Ophioid Fish Genus *Oligopus* with the Description of a New Species from West Africa. By Daniel M. Cohen. Pp. 1-22+5 plates. No. 3496, Vol. 116: A Survey of Vertebral Numbers in Sharks. By Victor G. Springer and J. A. F. Garrick. Pp. 73-96+1 plate. No. 3498, Vol. 116: One New Species and Two Redescriptions of Catfishes of the South American Cichlid Genus *Corydoras*. By Stanley H. Weitzman. Pp. 115-126. No. 3499, Vol. 116: Osteology and Relationships of South American Characid Fishes of Subfamilies Lebiasinidae and Erythrinidae with special reference to Subtribe Nannostomina. By Stanley H. Weitzman. Pp. 127-170. (Washington, D.C.: Government Printing Office, 1964.) [2511]

Arctic Institute of North America. Technical Paper No. 15: The Lemming Cycle at Baker Lake, Northwest Territories, During 1959-62. By Charles Krebs. Pp. 104. (Montreal, Washington, D.C., and New York: Arctic Institute of North America, 1964.) 2 dollars. [2511]

World Meteorological Organization. Technical Notes. No. 53: The Effect of Weather and Climate Upon the Keeping Quality of Fruit. By G. C. Green. Pp. xxi+180. (WMO-No. 137. TP.63.) 8 Sw. francs. No. 55: The Influence of Weather Conditions on the Occurrence of Apple Scab. (Report of a working group of the Commission for Agricultural Meteorology, prepared by J. J. Post, C. C. Allison, H. Burckhardt and T. F. Preece.) Pp. xi+41. (WMO-No. 140. TP.65.) 5 Sw. francs. No. 56: A Study of Agroclimatology in Semi-Arid and Arid Zones of the Near East. By G. Perrin de Brichambaut and C. C. Wallén. Pp. xv+64. (WMO-No. 141. TP. 66.) 6 Sw. francs. No. 59: Windbreaks and Shelterbelts. (Report of a working group of the Commission for Agricultural Meteorology, prepared by J. van Eimera, R. Karschun, L. A. Razumova and G. W. Robertson.) Pp. xv+188. (WMO-No. 147. TP.70.) 10 Sw. francs. No. 60: Meteorological Soundings in the Upper Atmosphere. By W. W. Kellogg. Pp. vii+46. (WMO-No. 153. TP. 73.) 8 sw. francs. (Geneva: World Meteorological Organization, 1963 and 1964.) [2511]

National Academy of Sciences—National Research Council. Ground-Based Astronomy: a Ten-Year Program. (A Report prepared by the Panel on Astronomical Facilities from the Committee on Science and Public Policy of the National Academy of Sciences.) Pp. 105. (Washington, D.C.: National Academy of Sciences—National Research Council, 1964.) [2511]

Nuclear Structural Engineering, Vol. 1, No. 1 (January, 1964). Edited by Thomas A. Jaeger. Pp. 1-140. Published bi-monthly. Subscription price per volume of 500 pages, 90 D.fl.; 180s.; 25 dollars. (Amsterdam: North-Holland Publishing Company, 1964.) [2511]

World Health Organization. Protection Against Ionizing Radiations: a Survey of Existing Legislation. (Offprint from Vol. 15, No. 2 of the *International Digest of Health Legislation*.) Pp. 170. (Geneva: World Health Organization; London: H.M. Stationery Office, 1964.) 6 Sw. francs; 10s.; 2 dollars. [2511]

European Atomic Energy Community—Euratom. Euratom's Biology Programme 1961-1964—Report and Perspectives. By R. K. Appleyard. Pp. 37. (Eur. 1884.e.) (Brussels: European Atomic Energy Community—Euratom, 1964.) [2511]

Instituto Antártico Argentino. Publicación No. 10: Nomenclatura del Hielo en el Mar. Por Rodolfo N. Panzarini. Pp. 103. (Buenos Aires: Instituto Antártico Argentino, 1963.) [2511]

Report and Accounts of the National Botanic Gardens of South Africa (Kirstenbosch, Newlands, Cape; Karoo Garden, Worcester; and Harold Porter Botanic Reserve, Betty's Bay), for the financial year ended 31 March 1964. Pp. 56. (Kirstenbosch, Newlands, C.P.: National Botanic Gardens of South Africa, 1964.) [2511]

The Journal Literature of Physics: a Comprehensive Study Based on *Physics Abstracts* (Science Abstracts) (Section A), 1961 Issues. By Stella Keenan and Pauline Atherton. Pp. 156. (New York: The American Institute of Physics, 1964.) [2511]

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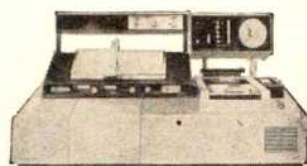
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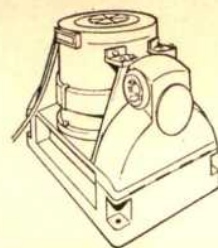
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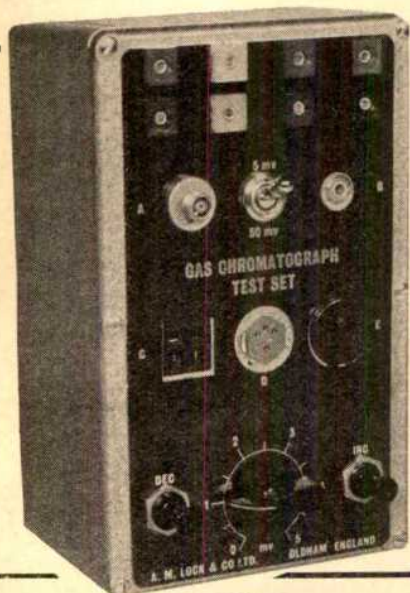
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## MINISTERS OF THE CROWN ACT, 1964

THE Machinery of Government Bill\*, which received an unopposed second reading in the House of Commons on November 19, provides the usual structure for the new Departments of State. These are the Ministry of Land and Natural Resources, the Ministry of Overseas Development and the Ministry of Technology. The Bill also amends the statutory restrictions on the number of Ministers who may sit in the House of Commons. The Attorney-General, in moving the second reading, described it as a purely technical Bill, but his assertion that it involved no questions of constitutional principle was challenged. It provides a limit of 91 in place of the 70 prescribed by the House of Commons Disqualifications Act of 1957 for Ministers who may sit in the House of Commons. While it is concerned with the establishment of new Ministries, it does not deal with their functions: these are to be dealt with by subsequent measures. (During the debate that followed, the Chancellor of the Duchy of Lancaster assured the House that there would be opportunity to debate these functions at a later stage.) The Attorney-General explained the reason for the several clauses, including the abolition of the limit of 27 on the number of Ministers holding senior offices who may sit and vote in the House of Commons.

Mr. Selwyn Lloyd, who followed, nevertheless asked for information regarding the function of the new Ministries and pointed out the constitutional issue which the Attorney-General had evaded. Quoting the report of the Herbert Committee in 1941, he reminded the House that that Committee had laid down as a principle the need to limit the control or influence of the executive Government over the House of Commons by means of an undue proportion of office holders being members of the House. He thought that the present figure of 91 was a clear breach of the principle laid down by that Select Committee, which did not contemplate one higher than 60 and took a serious view of the possible effect on the constitutional development of the House of a considerable increase in, for example, the number of Parliamentary Private Secretaries. Sir Derek Walker-Smith also insisted that for such reasons the Bill went to the very heart of the function of Parliament, the genesis of which lay in seeking to exercise control over the Executive.

Mr. J. Grimond considered that the House of Commons had far too few opportunities for discussing the machinery of Government, and thought that it was time that the changes that had occurred piecemeal for some time were fully examined, including the recasting of the Ministry of Defence by the previous Government. He complained that the Attorney-General had not met the need, and quoted the view of the Haldane Committee that any improvement in the organization of the Departments of State which was so marked as to increase substantially their efficiency should have as its correlative an increase in the power of the Legislature as the check on the acts and proposals of the Executive. Further, the duties of a member of Parliament were changing, and unless he

accepted the new role, his influence would continue to diminish. It was essential to find methods of being better informed, and Mr. Grimond thought that the first function of the debate was to obtain as much information as possible from the Government about the new Ministries and the need for their formation. He was sceptical of the proliferation of Ministries as well as about the absolute increase in the number of Ministers who could sit and vote in the House of Commons.

Sir Kenneth Pickthorn, who had been a member of the Herbert Committee, also emphasized the constitutional issue, and Mr. J. Percival questioned whether the object in increasing the number of Ministers allowed to sit in the House of Commons was to increase the control of the Executive over Parliament. Mr. W. R. van Straubenzee insisted that the first requirement was that Ministers should be responsible to the House of Commons, and he was particularly concerned over the increase in the number of paid Government Whips. He thought that the general public were also uneasy at the greater hold of the party machine on the average back-bencher. The constitutional issues were also recognized by Mr. A. Buck, Mr. E. M. Taylor and some others, while Mr. R. A. Butler put as the first issue the undesirable increase in the Executive in relation to the Legislature, supporting Mr. Grimond's plea for further enquiry, for example, by a Select Committee following up the Haldane Report.

Replying for the Government, the Chancellor of the Duchy of Lancaster, Mr. D. Houghton, admitted that some aspects of the machinery of Government and of the structure and functions of departmental organization might require more consideration. He brushed aside constitutional issues rather brusquely with the suggestion that justification for the new Ministries was a matter for the Government, which must shape the mechanism of Government to suit its purposes and policies. That such a dictatorial claim should pass unchallenged illustrates the weakness and shallowness of the debate, and although Mr. Butler quoted Dr. B. Crick's *The Reform of Parliament*†, that book in itself shows up the superficiality of the debate. Indeed, the whole debate illustrates the correctness of much of Dr. Crick's arguments, and the book especially indicts the Government's refusal to face the constitutional issue.

Early in his book Dr. Crick deals specifically with this issue of the continued strengthening of the Executive and its implications. While from 1900 until 1960 the number of Cabinet Ministers fluctuated below the level of 19 which it reached in both those years, it never exceeded that figure. During this period the number of other Ministers doubled from 10 to 20, and junior Ministers increased from 15 to 35. Although this was offset by a decrease in Royal Household appointments from 16 to 9, the total rose from 60 to 83—the number of M.P.s in the Government rising from 33 to 65, while the number of Parliamentary Private Secretaries increased from 9 to 36. There were thus in 1960 some 101 M.P.s involved in the Government compared with 42, and it is thus clear that the

\* *Machinery of Government Bill*. Pp. ii+7. (London: H.M.S.O., 1964.) 1s. 3d.

† *The Reform of Parliament*. By B. Crick. (London: Weidenfeld and Nicolson, 1964.) 36s.

increase with the formation of the present Government merely marks a tendency which has characterized the whole of the past sixty years.

The actual figures in the Machinery of Government Bill and the composition of the present Government, which has been largely responsible for the present concern, are not material, but it is worth noting that at the Committee stage in the House of Commons on December 10 the Attorney-General gave a figure of 137 for office holders in the present administration, of whom 116 are among the 317 supporters of the Government in the House of Commons. In the House of Lords on December 21 the number in the House of Commons was put at 122, including 31 Parliamentary Private Secretaries. That may not be a new situation, but with almost 40 per cent of the Government party in the House of Commons directly involved in the Government, the implications call for reconsideration.

The constitutional issue was not explicitly discussed at the Committee stage on December 9 nor when the Bill received its third reading in the House of Commons on December 10, the main criticism centering on the functions or the need for the proposed new Ministers. As might be expected, the constitutional issue figured more prominently when the Bill reached the House of Lords, both at the second reading on December 15 and in Committee on December 21. The Earl of Longford, in moving the second reading, admitted the importance of this constitutional issue, at least by implication, when he referred to the possibility that the Government might be acquiring too large an influence over Members of the House of Commons. While deprecating any idea that the House of Lords should attempt to set the limit, he conceded that there was obviously some limit to the number of Ministers that it is desirable to appoint in relation to the number of Members of Parliament.

Strong support for the view that the Bill was fundamentally a constitutional Bill came from Lord Dilhorne, Lord Morrison of Lambeth (who thought that the growth in the number of those in the House of Commons who could not speak and vote freely was unhealthy) and Lord Alport, though the Earl of Longford's reluctance to dogmatize on issues primarily concerned with the House of Commons was generally shared. Only the Lord Chancellor seriously argued that constitutional issues were not involved, but his defence was unconvincing, and earlier in his speech he indicated that the previous Government had also been careless on this matter, as Dr. Crick has shown so well in his book. The major feature of the Committee stage, however, was the acceptance by the Government of an amendment by Lord Dilhorne to change the title of the Bill to "Ministers of the Crown Bill", thus bringing it into line with the very similar Ministers of the Crown Act of 1937. This amendment was accepted by the House of Commons on December 22 and the Bill received Royal Assent as the Ministers of the Crown Act, 1964, on December 23.

Dr. Crick believes that this situation whereby the governing body gains in executive power presents grounds for serious concern. "A modern Prime Minister", he writes, "now has a patronage beyond the wildest dreams of political avarice of a Walpole or a Newcastle", and he directs particular attention to the increase in the number of Parliamentary Private Secretaries—an increase which he does not regard as justified by the loss of prominent back-benchers, when much of the most effective Parliamentary control of the Executive takes place not in the

official proceedings of Parliament but in the committees of the Government party itself.

Dr. Crick is thoroughly realistic, and two of the outstanding features of his book are the clarity with which he discusses this question of control of the Executive and the way in which he demonstrates the reluctance of the House of Commons itself to face the real issue—a feature which characterized the debate on November 19 just as much as earlier debates. The full weight of his argument is in support of strengthening the critical capacity of the House of Commons against the Executive, and he demonstrates the absurdity of any fear that this would necessarily weaken Britain's system of Cabinet Government. Equally important, he shows how mistaken is the common belief that the prime function of Parliament is to check or overthrow a Government: its prime function, on the contrary, is to put relevant facts and issues before the electorate which does sit in judgment on Governments. This critical and informative function is even more important than when Bagehot discussed it as the fourth main function.

It is essential that Dr. Crick's point should be clearly grasped if the Government's proposals for changing the structure of Government are to be assessed fairly and impartially. This is also true if sound proposals are to be made for meeting the difficulties arising out of the growing complexity of public affairs to-day and in determining the most effective means of bringing expert advice to bear whether in scientific or technical matters or in any other sphere. Dr. Crick is convinced that Parliament as well as the Executive needs to be better served in this respect, and his discussion bears very pertinently on two present issues. The first is the place and use of the Parliamentary Question; the second is the payment of Members of Parliament. Both are closely interlocked with the fundamental issues involved in the relation between science and Parliament and science and Government.

On the second issue, Dr. Crick argues convincingly that the first step, before Members' salaries are raised, should be to provide from public funds the essentials, such as secretary, office, postage, telephone and travel, that they require if they are to do their job efficiently. Salaries would still need raising, but this could perhaps be done selectively. As it is, the rejection of the recommendations of the Lawrence Committee regarding facilities, coupled with the increase in the proportion of the Government party involved in the Government, carries sinister implications. These the Government would be wise to dispel by positive measures, thus making up for its blatant brushing aside of the constitutional issues implicit in the Machinery of Government Bill.

On the issue of Parliamentary Questions, Dr. Crick is equally critical of the inertia or blindness of the House of Commons itself. He refuses to accept that shortage of Parliamentary time is any adequate explanation. The real difficulty is the refusal of the House of Commons to put first things first, and he is particularly severe on the missed opportunities in the debates of March 15 and August 1, 1963. He believes, for example, that if the existing Question Time was realistically used it might be a most effective means of controlling the Executive, and that the House of Commons has only itself to blame for allowing the period to shrink well below the full hour.

Here Dr. Crick takes his stand with Mr. D. N. Chester and Miss N. Bowring as opposed to Mr. S. A. Walkland in his recent article "Science and Parliament" in *Parlia-*



mentary Affairs for the Summer and Autumn of 1964. Mr. Walkland was discussing more particularly the origins and influence of the Parliamentary and Scientific Committee, but challenged the view that the Parliamentary Question could have any educational influence so far as science or technology is concerned. He seems to assume that such questions might be used to assess or criticize the technical basis of particular decisions or to probe official policy, but it is scarcely conceivable that such could ever be the intention. They could indeed bring to light issues or questions of fact that have been neglected. However, short of a revolution in the facilities or accommodation provided for Members of Parliament, it is most improbable that the Parliamentary Question by itself could do anything to check the increasing disparity between Parliament and the Executive in the matter of advice with which Mr. A. Albu, chairman of the Parliamentary and Scientific Committee, is much concerned. Mr. Albu, like Dr. Crick, believes that members have the remedy in their own hands and that no Government would be able to resist determined pressure from a sufficient number for the supply of more information and adequate research services to Members of Parliament. Nevertheless, Dr. Crick's examination scarcely justifies Mr. Walkland's pessimism, though it must be admitted that the way in which questions in Parliament have been used since a Ministry for Science was created does afford some justification. The extreme triviality and time-wasting character of many of the questions are well calculated to bring Parliament into discredit, but demonstrable abuse is not evidence that wise and effective use is not just as possible. Lord Mancroft's firm advocacy of the Parliamentary Question as a really effective weapon in both Houses, in the debate on the Address in the House of Lords on November 10, should not be overlooked, nor should the publication by D. N. Chester and N. Bowring, *Questions in Parliament* (Nature, 198, 321; 1965). Possibilities for good as well as for abuse need to be examined in due perspective, and while Dr. Crick never attempts to outline any scheme of Parliamentary reform he indicates what needs to be done to assess the adequacy or shortcomings of any particular proposals and the criteria which any satisfactory scheme should seek to satisfy.

His point that Parliament serves primarily to inform the electorate, not to overthrow Governments, is of cardinal importance; as a corollary, while a Government exists to govern, strong Government needs strong—but that does not mean factious—opposition. Government, he points out, must plan and must control finances, both in broad terms and in detail, but Members of Parliament are elected to serve their country, their party and their constituency, not themselves. Controls on any Government are all ultimately political, and in actual fact no Government can be responsible, or be held responsible, for every error or act of maladministration, nor will the House of Commons itself ever be able to look at everything that needs attention.

That much understanding of the political process is fundamental before approaching the question of technical and expert advice, but Dr. Crick comments also that much of politics is the art of being present at the right time. It is also worthy of note that he gives a reasoned and impressive rejection of the idea of an Ombudsman at present being considered by the Government, which is implicit in his assertion of the distinctive character of British Government and politics. In the present context of the Ministers of the Crown Act, the refusal to provide

Members with accommodation and facilities means that the proposals take on implications which would make the Government seek more distinctively British means of providing safeguards against administrative abuse. Here a remark of Dr. Crick is pertinent: "If anything useful and significant is to be done in a free society, it must be done publicly and in such a way as to consult, involve and carry with it those affected".

The main constructive proposal that flows from Dr. Crick's study is exactly in line with that which emerges from the studies of Mr. Albu and Mr. Walkland. Apart from emphasizing the value of the House of Lords in relieving pressure on the House of Commons in certain ways, Dr. Crick suggests that a rational use of specialist committees might provide an effective means for the House of Commons to fulfil more particularly its critical and educational functions. This has been convincingly demonstrated by the way in which the Select Committee on the Nationalized Industries has functioned. Mr. Walkland suggests, but much more diffidently, the establishment of a Select Committee on Scientific Policy, possibly to replace the Parliamentary and Scientific Committee, to whose influence he seems inclined to attribute less weight than is really due. However, the reality of this issue of power and influence is unmistakable, and it is too important to be discussed here and will be more appropriately considered in the context of the Science and Technology Bill and the actual functions of the new Ministries. For the present it must suffice to add that Mr. Walkland builds his hopes partly on the attraction into political life of more representatives of those professions whose attitudes are embodied in the Parliamentary and Scientific Committee. The improvement in the salary of members of Parliament might well act in that direction as he suggests; an improvement in facilities and accommodation is more likely to be decisive. Over and above either, however, the position of science in relation to Government and the structure of advice are likely to be critical, if not determining, factors.

## TWO CULTURES IN ACTION

### Science and the Shabby Curate of Poetry

Essays About the Two Cultures. By Martin Green. Pp. xiii + 159. (London: Longmans, Green and Co., Ltd., 1964.) 25s. net.

**E**VEN more than a discussion or critique, *Science and the Shabby Curate of Poetry* is a consequence of Lord Snow's "The Two Cultures". That lecture induced Mr. Green, who was then teaching English in the United States, to take mathematics courses at Harvard and the Massachusetts Institute of Technology, physics at Boston and later, on his return to England, to attend lectures at Cambridge in biochemistry and the history and philosophy of science. The present book sets forth the reflexions inspired by Snow's lectures and Mr. Green's subsequent experiences, including a year teaching English in a college of advanced technology. The outcome could have been a book of outstanding interest for, as this approach indicates, Mr. Green, while accepting Snow's thesis in outline, is entirely unbiased, though his primary concern is with the aspects of the subject which constitute a problem for the literary man. Snow's argument is treated fairly but not uncritically: however, it is curious that in almost his first paragraph Mr. Green shows that he has entirely missed what Snow had to say about the bearing of the two cultures on the gap between the rich and the poor countries.

These high expectations are unfortunately sadly disappointed. Mr. Green does not display Dr. Leavis's delightful talent for making the comprehensible incomprehensible, and there is too much careless writing, if not jargon, for either lucidity or forceful exposition: the general style of the book and the use of italics convey an impression of patchwork that is distracting and does not make easy reading. Mr. Green has grasped Lord Snow's essential message, but his attempt at further interpretation and application is scarcely effective or felicitous. He begins with a firm defence of Snow's thesis against Dr. Leavis's attack, in which he recognizes that science itself is an expression of human creativity, but one which requires more generous development if it is to make its full contribution to culture. He then discusses an essay by Prof. Trilling which he regards as far more stimulating and constructive than the outburst by Dr. Leavis. He regards the contribution which science has to offer as far more constructive than that which literature usually offers to-day, and while he suggests that more humility and realism about educational problems are to be found among scientists than among humanists, it is something of a paradox for him to state that we should be more concerned with how to make training in literature narrower and more logically astringent than to make training in science broader.

There may be two opinions also as to the extent to which in the past fifty or a hundred years both science and humanism have produced a greater literature than ever before—though it is probably true that our culture never lacks an integrated body of intellectual experiences, that includes both the scientific and the literary categories. It is when he is concerned more specifically with exposition and interpretation that Mr. Green is most disappointing: the achievement does not measure up to his aim and this is, at least in part, a matter of poor technique. He obviously grasps the root of the problem in education, the need for university teachers to be humanists and for the humanist to have some understanding of the world in which he lives.

The value of Mr. Green's book lies in its stimulus to thought, in the way in which he sets other minds examining imaginatively some of the problems which he brings to light. No essay on even a particular aspect of communication could hope to have any real effect unless a high standard of effective communication was set by example. Here, the argument is too often left loose, unconsolidated in the distraction of jargon. If Mr. Green had considered more carefully the reader he is addressing, his comments on Snow, on Leavis, and on Trilling, his accounts of his university courses and teaching experience, his survey of scientific exposition, could scarcely have failed to coalesce into a brilliant, suggestive and satisfying account. But for that a higher price must be paid in discipline as well as in an imaginative understanding of his readers.

R. BRIGHTMAN

## PRIMITIVE SYSTEMS OF MOVEMENT

### Primitive Motile Systems in Cell Biology

Edited by Robert D. Allen and Noburo Kamiya. (Proceedings of a Symposium on the Mechanism of Cytoplasmic Streaming, Cell Movement, and the Saltatory Motion of Subcellular Particles, held at Princeton University, April 2-5, 1963.) Pp. xix + 642. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 157s.

**D**URING April 1963, nearly one hundred scientists carrying out research in the biological field gathered at Princeton University to discuss the subject of primitive motile systems in cell biology, and this book comprises

thirty papers of the symposium and edited versions of discussions which followed their verbal presentation. Each paper was discussed separately, and four groups of topics which make up the four parts of the book were given "free discussion" by experts invited to make authoritative comments on the main topics.

In an introduction, the editors indicate that "primitive motile systems" include pseudopodial, ciliary and flagellar types of motion, the gliding of cells, the streaming of cytoplasm, the movements of chromosomes in cell division, the contraction of myonemes, the saltatory movements of cytoplasmic inclusions and some other kinds of movements in the cells of plants and animals. Despite great improvements in methods of investigation which have arisen out of technical advances, the basic mechanisms underlying these various kinds of movement have long remained obscure. Concepts such as gel contraction have appeared attractive but proved practically unacceptable.

The most fundamental kind of motion seen within living cells, the erratic zigzag displacements known as Brownian movement, occurs at molecular level, whereas saltatory motion involves cytoplasmic particles of very much greater size. Cytoplasmic streaming also involves visible particles, but these are grouped and move in unison as if transported by the flowing movements of the ground cytoplasm. Amoeboid movement has been regarded as a special kind of protoplasmic streaming in which locomotor organs (pseudopodia) are formed and alternately disappear, having little in common with the sort of cytoplasmic streaming which is seen in plant cells. Diversity of movement is enhanced in more highly organized motile systems involving cilia and flagella. All such forms of motion are produced by forces which are described as contractile, electrical, osmotic and so forth and which must have their sites localized objectively by direct observation. A further problem which is explained by the editors is the determination of the nature and availability of the chemical fuel—whether it is invariably adenosine triphosphate (ATP), how it is used and how withheld during cellular inactivity, and of what units it is composed.

In the introduction to Part 1 of the book, which deals with cytoplasmic streaming in plants and Myxomycetes (Mycetozoa), K. V. Thimann states that this kind of movement involves ATP, which produces characteristic effects when injected into living cells—causing the Myxomycete plasmodium to liquefy, inducing the cytoplasm of amoebae to flow away from the site of injection, stimulating streaming in *Acetabularia* and reactivating sperm tails after storage in the cold. Present also in Myxomycetes is a myosin-like protein capable of acting like the enzyme ATPase, stimulated by calcium in an effect which is antagonized by magnesium. Light, especially if it is blue, alters protoplasmic viscosity and there are ionic effects and other effects of a physiological nature. There is also evidence that streaming in Myxomycetes derives from anaerobic processes, and in plants it will continue for long periods in an atmosphere of nitrogen. Opinions concerning the mechanism involved converge on the importance of a sol-gel interface, which in amoebae is constantly breaking down and re-forming.

Hayashi considers the cortical gel layer in cytoplasmic streaming, concluding that rotational streaming in cells of Characeae ceases when this layer is destroyed by any means and recommences when it is re-formed. In discussion, the extreme thinness of the layer was indicated and the fact was disclosed that what is important in streaming is an organized inner surface and not the thickness of the layer. Asked how the cortical gel can be distinguished from endoplasm, Hayashi made the point that the former is stationary and the latter moving. Asked if the difference between these two parts is a matter of aggregation, he replied that they are structurally stable and different in function but probably transformable,

one from the other. Once the cortical gel layer is damaged it can no longer produce streaming, and the essential requisite is probably the organized inner surface of this layer.

In the introduction to Part 2, which is concerned with cytoplasmic streaming and locomotion in free-living amoebae, P. P. H. De Bruyn indicates that although we still do not know how an organism without visible locomotor organelles moves, various ideas have been rejected—that surface tension is responsible for amoeboid movement, that movement is due to elastic recoil from tension, that gelation itself is the cause of cytoplasmic contraction. It is suggested that terms borrowed from colloid chemistry may have been detrimental in some respects: the terms 'sol' and 'gel', 'solation' and 'gelation', serve to compare visible changes but do not tell us much about changes in the cytoplasmic proteins that are responsible for cytoplasmic movements, and it might be helpful to revert to the terms 'endoplasm' and 'ectoplasm'. The term now used to denote the motor force in cytoplasmic movement is 'contractility', and we have to remember that the contractile materials are not localized but are widely distributed throughout the cell. In amoeboid organisms there is morphological evidence of fibrillar organization, and the vital question is whether or not contraction can be localized in the fibrillar components. Are these invariably present when contraction occurs or are they formed when the need arises from a three-dimensional molecular reticulum?

Leucocytes of the blood form an interesting series. The lymphocyte moves like a monopodial amoeba, but in the granulocyte or macrophage this type of movement gives way to the more depolarized type and is accompanied by the properties of phagocytosis and pinocytosis, a development which points to a developmental effect on internal molecular architecture. Later on, the macrophage may develop into a fibroblast with limited motility but a marked ability to form fibres. Such changes must be accompanied by profound alteration in the morphoplastic or contractile proteins.

Wolpert and colleagues prepared a specific antibody against isolated surface membrane of an amoeba, labelled it with fluorescein to mark the surface, observed its behaviour during locomotion, and concluded that the surface flows forward during movement. This surface membrane seems to have a passive role in locomotion, suggesting that the forces which cause movement must reside in the cytoplasm. An isolated cytoplasmic fraction showed motility similar to that seen in intact cells, and induction of motility seems to be ATP-determined and sensitive to temperature. This fraction is capable of gelation streaming and syneresis, and electron micrographs of the gel resulting from syneresis show oriented fibres which may be responsible for motility. This paper produced lively discussion, and Goldacre expressed his views on the mechanism and control of amoeboid movement, proceeding from the familiar general view, based largely on the work of Pantin and of Mast, that locomotion in an amoeba is the result of contraction of one end of a tube of 'plasmagel' which encloses 'plasmasol', to his own opinion that in *Amoeba proteus* the motor mechanism resides in the rear end ('tail') rather than the front or advancing end. This region behaves like cytoplasm into which ATP has been injected, and a theory of amoeboid movement explained how a continuous supply of ATP could be provided at the cytoplasmic contraction in the amoeba's tail by membrane-plasmagel contact.

That this is not the final answer is shown by the findings of Kamiya that in capillary tube experiments an external hydrostatic pressure gradient can counterbalance endoplasmic streaming. So delicate is the balance that pressure change by less than 0.5 mm water can reverse the flow. A new pseudopod can be formed artificially by externally applied pressure gradients which force the endoplasm to flow, when the advancing end of an amoeba has a smooth

surface and often a hyaline cap and the rear end develops a wrinkled surface and a tail-like appearance. In free discussion, Allen, who gave a paper in Part 3 on cytoplasmic streaming and locomotion in marine Foraminifera, contrasted the two main hypotheses, from contraction and tail contraction, and produced some highly technical discussion, which will prove heavy going to all but the specialists in this mechanism.

Part 3 deals with cytoplasmic streaming, locomotion, and the behaviour of specialized amoeboid cells such as the pseudopodia of sea-urchin larvae, cells involved in inflammatory processes, neurones, filapodia and axopodia. In his introduction to this section, D. Marsland discusses briefly the two main types of hypotheses in respect of amoeboid movement, tube-wall contraction and fountain-zone contraction dealt with by Allen. What is referred to as the Mast-Marsland tube-wall contraction hypothesis of amoeboid movement can be summarized briefly. The tubular plasmagel-plasmasol system of an amoeba is not to be regarded as an inert system. In a complex interplay of stimulation and response, any part of one physical system may be converted into the other. The entire system tends to be tubular and either unbranched or branched, but local changes may result in a variety of structural reorganizations. The lumen of the tube may become occluded by localized gelation at any point, or it may increase or decrease by alteration of the sol/gel ratio. The contractile tension in the wall may also increase or decrease generally or locally, or the wall may give way because of localized solation, allowing another branch (pseudopodium) to grow out. Contractile tension may be maximal in the rear of an active amoeba, but the organism must be allowed a latitude of variation in satisfying the complex requirements of locomotion.

In introducing Part 4, D. W. Bishop refers to a fascinated non-participant witnessing heroic efforts to unify a chaotic situation which might be better regarded as a parcel of processes. He argues that while an underlying basis for movement in many cells may be attributed to muscle-like proteins, it is perhaps not to be expected that a single operational process is functional at all times throughout a cell, regardless of the intricate and experimental state of the cytoplasm. He emphasizes the comments of others that control and regulation mechanisms may act variously and continuously throughout the amoeba's body. This part of the book deals with the motion of subcellular particles and with mitotic movements, and there is considerable free discussion. The whole book contains a great wealth of highly technical information, much of it experimental, about the numerous areas of research on primitive motile systems in protoplasm, supported by many references to the literature and enhanced by discussions. In spite of innumerable observations and numerous hypotheses, however, it cannot be said that clearer ideas have emerged than existed already, although there are many pointers to extensions of research for the specialist. This seems to be indicative of progressive increase in difficulties of interpretation as a result of technical advances which have favoured greater and more detailed observation of relevant minutiae.

BEN DAWES

## PHYSIOLOGY AND BIOCHEMISTRY OF HERBICIDES

The Physiology and Biochemistry of Herbicides

Edited by L. J. Audus. Pp. xix+555. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 110s.

THE replacement of mechanical by chemical energy as the principal mode of continuing the "controversy with weeds" alluded to by a certain bishop is now a widely accepted and welcomed fact of agricultural life.



Nearly all the herbicides in use to-day have been discovered by industry in the course of screening new chemicals for biological activity, an empirical approach that is unlikely to be superseded for a long time to come. Nevertheless, the biochemical and physiological approach is likely to be increasingly fruitful in exploiting more fully the potentialities of existing herbicides and designing new ones. Both in this context and as a contribution to a better understanding of the selective effects of herbicides on plants, *The Physiology and Biochemistry of Herbicides* is especially timely. In seventeen chapters by various authorities, it reviews the present knowledge of all the major aspects of the behaviour and mode of action of herbicides which have a bearing on the nature of their phytotoxic effects.

The book opens with a chapter by R. C. Brian on the history of the development of herbicides, their practical uses and modes of action in plants. This is followed in Chapter 2 by a review of methods of analysis and determination by V. H. Freed.

Chapters 3, 4 and 5 provide perhaps the centre of gravity of the book. Chapter 3, by A. S. Crafts, reviews the penetration of herbicides into various plant surfaces, their movement out of leaves and roots, local accumulation, binding, persistence and ultimate fate. The various transport pathways of different herbicides receive thorough treatment, and the great complexity of routes of movement, sites of action and modes of eventual detoxication is well brought out.

In Chapter 4, G. S. Hartley reviews the present knowledge of the behaviour of herbicides in the soil in terms of the various physical factors influencing their movement and action. Aspects considered include the diffusion of herbicides in the air and water phases of soil, their transport and dispersion in moving water, and the mechanism of their adsorption by clay and organic matter. This is an impressive attempt to provide a mathematical-physical framework within which the great complexity of behaviour exhibited by herbicides in the soil can be interpreted, and is especially welcome in view of the practical importance the soil-acting herbicides have now assumed.

In Chapter 5, L. J. Audus reviews the interactions between herbicides and micro-organisms in the soil and indicates the dominating role of bacteria in the detoxication of herbicides. There is a report of apparently unpublished work on the relation between structure in the phenoxy herbicides and the ease with which they are attacked by bacteria. Biochemical pathways of herbicide degradation and the effect of herbicides on various classes of micro-organism are also considered.

The next chapters pursue the theme of the action of herbicides within the plant. In Chapter 6, O. Kiermayer reviews the effect of auxins and growth-regulator herbicides on cell division and elongation. Ch. J. Gorter and W. van der Zweep in Chapter 7 review the morphogenetic effects of growth-regulator herbicides. In Chapter 8, F. T. Addicott and H. R. Carns consider abscission responses to herbicides; and in Chapter 9, D. J. Wort reviews the effect of herbicides on plant composition and metabolic systems. In a further chapter, he also considers the effect of sub-lethal concentrations of 2,4-D on plants with and without the addition of minor nutrient elements.

In their second contribution, H. R. Carns and F. T. Addicott in Chapter 11 review the effects of herbicides on various endogenous regulator systems, with an interesting discussion on the role of unsaturated lactones in the damage caused by growth-regulator herbicides. In Chapter 12, R. C. Brian considers the effect of herbicides on biophysical processes in plants, and concludes that, of these, changes in permeability brought about by the use of growth-regulator herbicides are the most likely to result in morphological changes. In Chapter 13, J. van Overbeek summarizes the knowledge of the mode of

action of herbicides operating on the growth processes and photosynthetic activities of plants.

The next three chapters are devoted to aspects of the selective effects of herbicides. E. Åberg in Chapter 14 considers the influence of stage of development, vigour, previous history and variety on the response of crops and weeds. In Chapter 15, K. Holly reviews selectivity in relation to chemical structure, spray retention and penetration into plant surfaces and soil factors which influence the soil-acting herbicides. He stresses the relative neglect of the study of mechanisms of root uptake. R. L. Wain in Chapter 16 ends this section with a review of the behaviour of herbicides within the plant in relation to their selective effects. The book concludes with a final chapter by H. Linser on the design of herbicides in the light of present biochemical knowledge, in which he stresses the need for more knowledge of the sites and modes of action of herbicides and the special metabolic characteristics of species on which selectivity can be based.

The individual fields are on the whole well separated from each other and between them provide an impressive overall picture both of the great range of interactions between herbicides and plants and of progress made in their unravelling.

Despite the vast amount of experimentation on natural and synthetic auxins, the primary sites of action of growth-regulator herbicides such as 2,4-D are still not known; nor are the reasons for the differing reactions of the Gramineae and dicotyledonous plants well understood. These and many other problems remain. The field is an active one and is also likely to reflect the increasing significance of the soil-acting herbicides. As both a source of reference and a commentary, this volume will be widely welcomed by advanced students and research workers to whom it is primarily addressed. There is an index of authors of bibliographic references and a subject index. There are a few misprints, such as Knüsh for Knüsli on p. 17 and some apparent confusion between pyridiazine and pyridazine on p. 492.

P. J. BOYLE

## BRITISH CENTIPEDES

### Centipedes of the British Isles

By E. H. Eason. Pp. x+294+5 plates. (London and New York: Frederick Warne and Co., Ltd., 1964.) 63s. net.

CENTIPEDES are among the commonest animals on the surface of the Earth. European soils, woodland, grassland and arable alike, support on the average more than one hundred of these animals per square metre and yet many people are scarcely aware of their existence. Centipedes have never been a fashionable group, but there will be many for whom the publication of a British monograph will have been an eagerly awaited event. Once again our small collection of British Faunas has been added to by an amateur, that is to say by one who combines his natural history with other professional duties. There is nothing amateurish about *Centipedes of the British Isles*; it is an authoritative account of the entire British fauna. Forty-four species are described with great clarity and meticulous attention to detail.

Each species is accorded about four pages of text, of which at least one, frequently two, are devoted to very clear line drawings that adequately illustrate the text. In addition, there are five excellent photographs by Dr. Manton. Everything necessary for reasonably certain diagnosis of adults is set forth. Unfortunately, it is a little difficult to find one's way about the book; there is no distinction in the index between the most pertinent page references to the species and incidental mentions in the body of the keys and in the general introductory matter. There is some advantage in having the spinulation

tables for each species of *Lithobius* gathered together in an appendix, but they would be better with species headings instead of just numbers.

The immature stadia of one species of *Lithobius* are described in detail from the author's own work and more general remarks on the stadia in other groups are included. Although references are given for other life-history investigations, the author does not include much detail from these. Since he mentions that such authorities as the late K. W. Verhoeff have been misled by sub-adult specimens, he might have used a little more space to help others not to make the same mistakes. For example, the post-larval *Lithobius forficatus* with four teeth on the coxosternum of each maxilliped might be mistaken for a smaller species which does not run down in the key.

Our knowledge of the distribution of centipedes in Britain is still sadly incomplete—witness the lists of county records at the end of each species description. This will give much encouragement to those for whom the prospect of a new county record is exciting. In a general chapter on distribution, the paucity of the Scottish and Irish faunas is noted and species with restricted distributions are mentioned. It would have been helpful had the species concerned been listed; for example, one cannot discover at a glance which are the three littoral species without glancing through the notes on all forty-four. A foretaste of the interest which will accrue from detailed mapping of distribution is available for *Lithobius variegatus*. This must be our most interesting centipede and appears to be endemic to Britain. Dr. Eason noticed that the existing records were all from the west side of the 38° F January isotherm, and his collecting in some critical localities confirmed this pattern of distribution.

One cannot expect clear statements of habitat preferences when such a small number of people have been able to recognize species. As a consequence most of the habitat notes are disappointingly vague. Two of the more explicit notes refer to *Lithobius calcaratus* and *L. crassipes*—"the majority of records... are from woodland" and "common in moorland and mountainous country as well as in grassland and woodland". I think I detect here a bias towards woodland for *calcaratus* and open land for *crassipes* which is the very reverse of some published habitat notes. However, such impressions concerning distribution are necessarily very subjective. By providing the ecologist and naturalist with reliable means for diagnosis, Dr. Eason has ensured that more objective descriptions of ecological distribution will be possible in the near future.

J. G. BLOWER

## USER—COMPUTER LANGUAGE

Numerical Methods and Fortran Programming with Applications in Engineering and Science

By Daniel D. McCracken and Dr. William S. Dorn. Pp. xii + 458. (New York and London: John Wiley and Sons, Inc., 1964.) 57s.

TO quote Sir Edward Bullard on the subject of the language of machines: "Most machines do only a few things, and the problem of telling them what to do is not difficult. An electronic computer is an unusual machine in that it can undertake a very wide range of operations. This variety of possible behaviour raises very severe problems of communication between the user and his machine and has led to the development of the art and profession of computer programming...". *Numerical Methods and Fortran Programming* deals with two associated topics—the problem of communication between the user and the computer mentioned by Sir Edward Bullard and the prior business of converting mathematical

models into a step-by-step form ready for communication.

In fact, three main tasks must be undertaken when solving a physical problem using a computer. The problem must first be given a mathematical model which illustrates the crux of the matter to be solved. Secondly, having decided on the model, a method of numerical investigation is chosen and the problem is coded, that is to say, a list of instructions in some language is prepared, which will eventually uniquely direct the computer to perform what calculation is required. Thirdly, the programme is fed into the computer and 'de-bugged', and eventually the required results are obtained. Put in this way, the book covers the second task outlined here adequately and very well.

An important point which is sometimes missed, and which is scarcely mentioned in the book (perhaps because it is an introductory work), is that when one approaches the computer with a programme, the computer is already armed with a very large programme, the compiler, which is waiting to deal with coding work. The compiler translates the programme as language into a form which the computer can deal with, and it is only when the whole programme has been translated in this way that the real business of computation can proceed.

Some of the art and profession of computer programming mentioned by Sir Edward Bullard is to do with spelling and the grammar of the language, but some of it is concerned with getting to know what the compiler will make of a particular piece of coding.

In these days of ever increasing use of electronic digital computation, the book will satisfy two main requirements. For mathematicians, physicists and engineers, not in any way familiar with a computer language, four chapters and an appendix take the subject of a particular language, Fortran II, from the fundamentals, through the basics of programme design to the use of sub-routines and format procedure for input and output. Interleaved between these chapters are six other chapters dealing with the numerical analytical detail which one must tackle sooner or later if one is serious about using computers to solve serious problems. The numerical analysis topics range from power series, iteration for the roots of algebraic equations, numerical integration, simultaneous linear equations, to the integration of sets of ordinary and partial differential equations. The material of the two main topics is matched, so that the reader has usually seen enough of the particular language to cast his numerical analysis into language, and has usually seen enough numerical analysis to put his equations into a language that can be solved by a computer. Each chapter is followed by a good number of well-chosen exercises, answers to which are provided; but more important is the use made of 'case studies' which occur at the close of each chapter. The case study poses a practical problem on the material so far introduced and then carefully goes through the detail of its solution. While being useful to the extent that the reader is made to appreciate the necessity for intense attention to accuracy in detail, which is vital for successful computer work, the case studies may be too discursive for some readers and might well be missed on a first reading. Readers who begin to programme some examples, however, will find themselves going back to the detail for vital information.

For advanced undergraduates and for research workers in almost any field of science who intend using computers, this book will be welcome, particularly if they are aimed at an I.B.M. machine or at least a computer with a Fortran compiler; it must be said, however, that not all computers have one, and that some computers have rather poor ones. The book is well produced and makes an excellent general purpose introduction to the whole business of getting answers from digital computers.

G. BLACK

## "THE FRIEND OF MANKIND"

### A PORTRAIT OF COUNT RUMFORD

Substance of a Friday Evening Discourse delivered at the  
Royal Institution on November 13, 1964

By A. H. EWEN

THE year 1964 marked the one hundred and fiftieth anniversary of the death of Sir Benjamin Thompson, Count Rumford. During his lifetime his colourful career in the United States, England, Bavaria and France as a soldier, statesman, scientist and social reformer made him one of the best-known names of his generation. As a scientist he achieved international renown, and was awarded the Copley Medal of the Royal Society for his contributions to the science of heat; in both Europe and the United States his name was literally a household word, thanks to the Rumford stoves and Rumford roasters which were all the vogue in both aristocratic and middle-class homes; his cure for the domestic evil of smoky chimneys added a new word to the English language—to 'rumfordize'—while the famous Rumford soups, prepared according to his economical recipes in public kitchens, were well known to the poor of many countries.

But to-day Rumford's name and his work have lapsed into obscurity, and even into disrepute. Both the importance of his contribution to the science of heat, so strongly stressed by Tyndall, and the originality of his observation of convection currents have been questioned. Despite President Franklin Roosevelt's dictum that Rumford together with Benjamin Franklin and Thomas Jefferson are the three greatest minds that the United States ever produced, his reputation in America suffered a severe blow from the discovery in the 1930s of positive proof of his spying activities on behalf of the British in the American War of Independence. This revelation of his disloyalty to the Republican cause has led some recent biographers, for example, J. A. Thompson (1935) and Sanborn Brown (1962), to assert that his present obscurity is fully deserved because of his dishonourable and anti-social conduct, and they point to his difficulties in Bavaria and England as evidence of his arrogant and dictatorial personality, which in their view not only disgusted and antagonized his contemporaries but also made him impossible as a colleague.

This charge of dishonourable conduct is primarily based on Thompson's work as an intelligence agent in the War of Independence; but on this point his American critics fail to appreciate that their unfavourable judgment of his conduct is a purely subjective opinion. At this period Thompson held a commission as a major in the New Hampshire Provincial Regiment, and his activities can equally well be interpreted as the skilful execution of a highly dangerous mission which he undertook in obedience to the orders of his superior officers. Thompson did not conceal his unwillingness to join in the rebellion against British rule, and however unwelcome the discovery of his spying may be to American opinion, willing obedience to the orders of superior officers cannot justifiably be termed dishonourable.

Nor can the difficulties which he experienced in Bavaria be simply attributed to his own faults of character. Acting as the executive Minister of the Elector Charles Theodore, Thompson embarked on a carefully planned campaign for modernizing the army and abolishing mendicancy. Before putting his scheme into operation he made full use of his scientific ability to determine the best material for army uniforms, the most nutritious and economical diet for his work-people, and the most efficient design for fuel-saving cooking-ranges; in all his scientific

work he was always more concerned with finding a scientific answer to a practical problem than with pursuing a disinterested enquiry into the secrets of Nature. This closely integrated group of reforms constitutes one of the first instances in history of the application of scientific method to the solution of social problems, and in his emphasis on the technological aspects of applying scientific knowledge to the common purposes of everyday life Thompson was a true pioneer.

But it was not the merit or the method of his reforms that aroused opposition so much as their political implications, and this opposition took the form of attacks on his personal character and integrity. One of the chief causes of contention was his creation, on swampy ground on the banks of the River Isar, of a public park, known as the English Garden, for the benefit of the citizens of Munich. This operation was completely successful, but brought a major clash with the Town Council, who resented the political popularity which accrued to the Elector from the creation of the park. Another cause of trouble was his attempt to open commissioned rank to all classes of the community by founding a Military Academy which made education available even to the poorest; but this enlightened reform provoked the hostility of the privileged caste of aristocratic army officers. In both these instances it was the political implications of his reforms, not his defects of character or his methods of administration, that created his difficulties, and ultimately led to his withdrawal from active political life after the Elector had granted him a life pension, and created him a Count of the Holy Roman Empire as a reward for his services.

In the same way the difficulties which he experienced during his stay in England, when he was associated with the project of establishing the Royal Institution, were primarily political rather than personal. His outspoken attacks on the luxury and self-indulgence of the aristocracy, and their torpid indifference to science and scientific improvements, and his advocacy of social reforms such as popular technical education and the abolition of 'jobbery' in public administration, rendered him an object of suspicion to the Tory Government, who regarded him as a revolutionary agent, and refused to accept him as Bavarian Minister in London. He was excluded from Court, and rumours that he was a French spy were fostered by high Government officials. The satirical poet Peter Pindar reflects the prevailing hostility in his poetical "Letter to Count Rumford", in which he taunted Rumford with his exclusion from Court, decried his scientific work as quackery, and ridiculed his arrogance and self-conceit.

The cordial warmth of his reception in Paris during a seven-weeks visit contrasted sharply with the cold-shouldering which he had experienced in London. He was accorded precedence as a Minister, a distinction which had been denied him in London; his eminence as a scientist and a philanthropic pioneer was publicly recognized, and a personal attachment was formed with Madame Anne Lavoisier, the widow of the great chemist. Rumford had no hesitation in deciding to wind up his affairs in England, including his association with the Royal Institution, and to exchange the aloof hostility of London society, which showed little or no interest in science, for the more congenial atmosphere of the *bourgeois* society of Paris with its high regard for science.



But in France his rigid insistence on maintaining a strict neutrality in the conflict between France and Britain aroused the hostility of the strongly nationalist members of the Institute, who also displayed jealousy of his scientific work. After the failure of his marriage with Madame Lavoisier he purchased a property at Auteuil, where he lived in increasing isolation until his death on August 21, 1814.

Superficially, Rumford's persistent failure to achieve lasting social success and personal happiness in three such widely different countries as feudal Bavaria, patrician England and republican France would seem to be a decisive indictment of his own character. Although his American critics attribute this failure to his lack of moral principle, which they insist antagonized his contemporaries, it was, in fact, Rumford's rigidity of principle and unwillingness to compromise that caused his social difficulties. If he had been more pliable and less scrupulous, he could easily have adapted himself to the winds of political change, and he would then have escaped social isolation in his lifetime, and historical disrepute after his death. But he was never mercenary, and always resolutely opposed to 'jobbery'. Although he could have made a considerable fortune by the commercial exploitation of his scientifically improved heating appliances, he made his designs freely available for the public benefit.

An important influence in his life was the fact that he was always a 'foreigner'. In his native America his support of the British made him a renegade and an exile; in Bavaria he was a 'Yankee Interloper'; in England many thought that he was a German, and there was a widespread belief that he was a French agent; in France no one quite knew whether he was an American, an Englishman or a German, but he was certainly not a Frenchman. This lack of 'roots' created a sense of psychological insecurity, which he tried to conceal under a mask of excessive self-assertion, which his critics construed as arrogance and self-conceit.

But this foreignness was also a source of strength, as it enabled him to accomplish tasks which would never have been undertaken by a native of the country. Only a foreigner would have attempted to create an English Garden in the heart of Germany; only a foreigner would have succeeded in launching an enterprise like the Royal Institution in the middle of a major war and at a time of acute financial stringency.

But this bland disregard of his environment also made him an easy target for the attacks of his opponents; he was always open to the charge of lack of patriotism. For Rumford gave his loyalty to mankind as a whole rather than to any one single country. As a German newspaper once said of him: "Rumford is a citizen of the world in the great meaning of that word, for wherever Fate puts him, he acts with equal vigour. Not his own country, but the whole world, will one day erect his monument". That day has not yet come, and in the meantime the chief consequence of his lack of nationality has been that no country wishes to claim him and sing his praises.

The sheer diversity of his achievements as a soldier, statesman, scientist and social reformer inevitably prevented him from attaining the highest rank in any one single field, but in all these spheres of activity his work reached a high level of distinction. But Rumford's chief claim to fame is that he belonged to that very small group of men who have possessed the rare combination of imaginative vision and the administrative ability to realize that vision in a permanent and socially viable form. The proof that Rumford possessed this rare combination of qualities to a high degree is shown by the enduring vitality of his creations 150 years after his death.

Few men have bequeathed such a diverse set of benefactions to posterity. His endowments for the encouragement of science, which were very generous in proportion to his means, have borne notable fruit. The Rumford Medal of the Royal Society numbers among its recipients many of the world's greatest scientists. His emphasis on the technological aspect of science in his endowment of the Harvard professorship played an important part in the foundation of the Massachusetts Institute of Technology in 1865, when the first Rumford professor delivered the inaugural address.

The Royal Institution, "originally and chiefly my work" as Rumford once justifiably claimed, has made a contribution of outstanding magnitude to the advancement of science, both in the field of original research and in the sphere of scientific education. On his memorial in the English Garden at Munich, which has recently celebrated the one hundred and seventy-fifth anniversary of its opening, Rumford is described as "The Friend of Mankind", a title well deserved by his diverse contributions to human knowledge and human happiness.

## RECENT TRENDS IN THE LEVEL OF FALL-OUT CAESIUM-137 IN MAN

By DR. S. H. COHN, DR. E. A. GUSMANO and DR. R. A. LOVE

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**B**ODY-BURDENS of caesium-137 ( $^{137}\text{Cs}$ ) in the population reflect the level of fall-out from previous nuclear weapon tests. Since the  $\gamma$ -rays of  $^{137}\text{Cs}$ , as well as those of naturally occurring potassium-40, can readily be measured in people by use of the whole-body  $\gamma$ -spectrometer, many data have been adduced on the level of  $^{137}\text{Cs}$  in order to ascertain its distribution in the world, as well as its attendant potential radiation hazard<sup>1,2</sup>. During 1948-61 world-wide data on the levels of  $^{137}\text{Cs}$  in man, as well as in food, have been collected and reported by a United Nations Scientific Committee<sup>3</sup>. The  $^{137}\text{Cs}$  content of man increased continuously to a maximum in 1959, followed by a continuous fall during the period 1959-61. This article reports an investigation on ten adults for the period June 1960-September 1964.

Measurements of the internally deposited  $^{137}\text{Cs}$  and  $^{40}\text{K}$  were obtained with the Brookhaven whole-body counter. An 8-in.  $\times$  4-in. sodium iodide crystal detector used in a standard counting geometry previously described was

used<sup>4</sup>. The subjects (7 males and 3 females), ranging in age from 25 to 45 years, are all members of the scientific staff of Brookhaven. These people are all in good health and have not been exposed to radioactive contamination in the course of their normal work. A 30-min count was obtained from each subject at monthly intervals for the four-year period.

The  $^{137}\text{Cs}$  data are expressed as pc./g potassium, the conventional manner of reporting such data. (The primary advantage of this unit is that it tends to minimize the spread in values due to variations in body-weight.) The area under the appropriate spectral photopeaks of  $^{137}\text{Cs}$  (0.663 MeV) and  $^{40}\text{K}$  (1.46 MeV) was integrated with an IBM-7094 computer programme, and the absolute levels of the radionuclides determined by calibration against a phantom (REMAB, Alderson) containing known levels of a standardized solution of  $^{137}\text{Cs}$  and potassium. As a further check, the  $^{137}\text{Cs}$ -levels of five of the male subjects were calibrated against a standardized  $^{137}\text{Cs}$

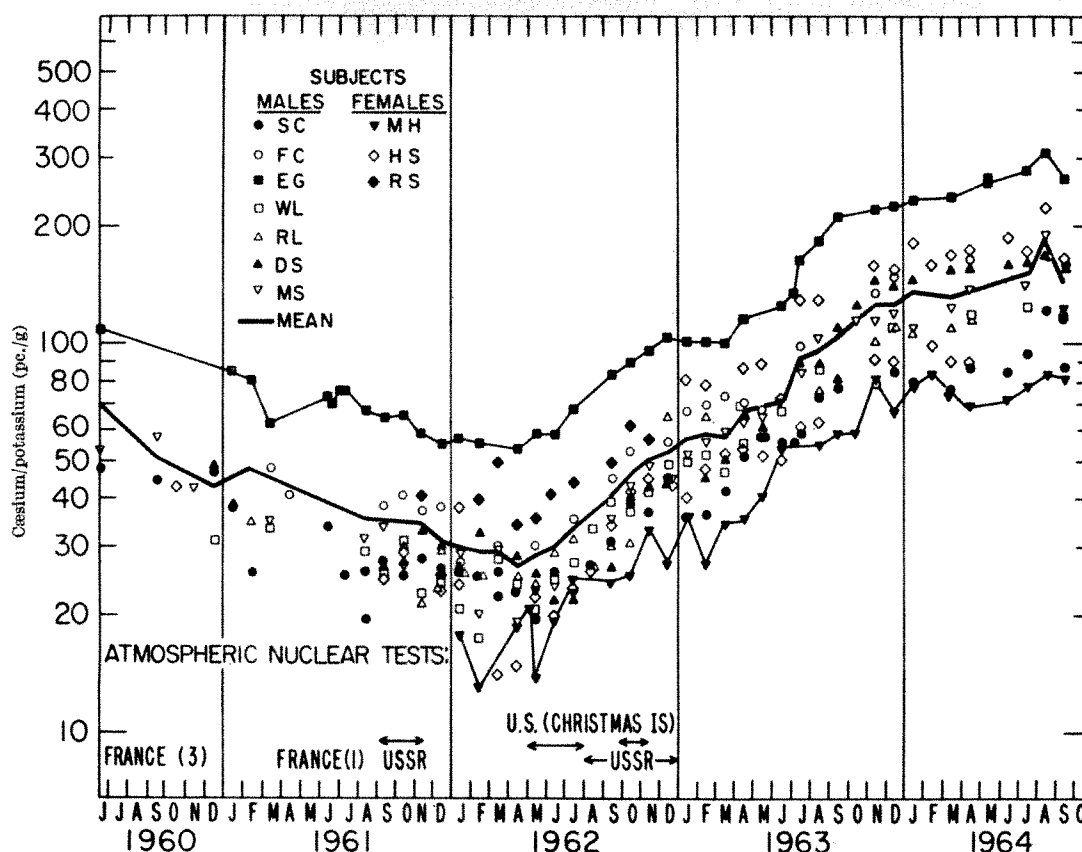


Fig. 1. Body-burdens of cesium-137 in a selected population (1960-64)

solution obtained from the International Atomic Energy Agency and administered intravenously. The absolute  $^{137}\text{Cs}$  values obtained with this standard solution check quite closely ( $\pm 4$  per cent) with the calibration made with the phantom.

The profiles of the  $^{137}\text{Cs}$ -level in each of these 10 subjects are shown in Fig. 1. There was a continuous fall in  $^{137}\text{Cs}$ -levels from June 1960 until the spring of 1962, when the curves show an upward inflexion. From December 1958 until the autumn of 1961 the only reported nuclear detonations were the four small French detonations in the spring of 1960 and 1961. The  $^{137}\text{Cs}$ -levels continued to rise to August 1964. The mean  $^{137}\text{Cs}$ -level in August was 180 pc./g potassium, which corresponds to a dose-rate of 3 mr./y., or 2.2 per cent of that from natural radiation sources. The values of the most recent measurements in mid-September 1964 are lower in each subject than those recorded in August. It is necessary to continue these measurements to ascertain whether the maximum level was reached in August followed by the beginning of a downward trend. In each subject the increase in the  $^{137}\text{Cs}$  from the minimum point in early 1962 to August 1964 is approximately six-fold. The August 1964 mean  $^{137}\text{Cs}$ -level is more than 2.5 times the previous maximum mean value observed in 1959. This increase in the level of  $^{137}\text{Cs}$  is opposed by the normal biological loss of  $^{137}\text{Cs}$ . The biological loss of  $^{137}\text{Cs}$  from the major body compartment varies from 60 to 140 days and accounts in part for the spread in  $^{137}\text{Cs}$ -levels in the population<sup>5</sup>. Within this group there is a fairly consistent spread in values ranging from the male (E. G.) with the highest level to the female (M. H.) with the lowest level.

The most recent fall-out producing nuclear detonations occurred in the latter part of 1962 (Fig. 1). It has been predicted that with tests ending at the end of 1962, the maximum fall-out rate for strontium-90 would occur in 1964, while the maximum deposition of  $^{90}\text{Sr}$  under the

foregoing conditions would occur in 1967 (ref. 3). It appears likely that a similar pattern would hold for  $^{137}\text{Cs}$  (ref. 3). The level of  $^{137}\text{Cs}$  in food and consequently in man is a function of both the fall-out rate and the amount of accumulated deposit. The apparent maximum in the  $^{137}\text{Cs}$  body-burden noted in August 1964 suggests that the  $^{137}\text{Cs}$ -level of milk (the primary food contributor), and consequently of the human population, reflects the present fall-out rate. The fall in the level of  $^{137}\text{Cs}$  in the body during 1960-62 in the face of the rise in the cumulative pool of  $^{137}\text{Cs}$  on Earth further corroborates this dependence of body-burden on the rate of fall-out. Part of the fluctuation in the  $^{137}\text{Cs}$  body-levels as a function of time also undoubtedly reflects the seasonal nature of agricultural practices and afterwards the  $^{137}\text{Cs}$ -level in milk. The sharpest rise in  $^{137}\text{Cs}$ -levels appears in the spring of the year and is associated with heavy rainfall and return of cattle to the pasture.

The small population examined here reflects the level of  $^{137}\text{Cs}$  found in a large changing Brookhaven population counted during the same period. Further, the mean  $^{137}\text{Cs}$ -level of this larger Brookhaven population sampled was very close to the mean of a still larger United States and Western European population. For example, in early 1960 the average  $^{137}\text{Cs}$ -level of 73 Brookhaven personnel was 59.2 pc./g potassium, as compared with the mean value of a large population sample (2,800 people in the United States and Western Europe) of 60 pc./g potassium  $\pm 25$  per cent<sup>3</sup>. The variations in  $^{137}\text{Cs}$ -level have been shown to be surprisingly small over large geographical areas in the United States<sup>4</sup>. That this uniformity in  $^{137}\text{Cs}$ -levels, in fact, extends over the northern hemisphere is suggested by comparing the results of the present investigation with similar work recently carried out in England. Rundo<sup>6</sup> reported a mean for a group of approximately 13 adults of 25 pc./g potassium in April 1962, compared with a mean of 25.8 pc./g potassium at Brook-

haven. The most recent mean  $^{137}\text{Cs}$  value reported by Rundo in April 1964 was 150 pc./g (100–200 range), which compares closely with the value of 136 pc./g (68–250 range) obtained at Brookhaven at the same time. Both these investigations utilized very small samples, and cannot, of course, be extrapolated to determine the  $^{137}\text{Cs}$ -level in the American or Western European population, but the approximate levels of  $^{137}\text{Cs}$  and its pattern with time are clearly indicated. It will be of interest to follow these levels of  $^{137}\text{Cs}$  in larger population samples throughout the world to check on predictions of the  $^{137}\text{Cs}$  inventory and hold-up time for stratospheric fall-out and its rate of transfer to men. Whole-body counting data are valuable in this respect, as there are insufficient data to extrapolate from  $^{137}\text{Cs}$  food levels in order to predict future  $^{137}\text{Cs}$ -levels in man with accuracy. Adequate 'portable' whole-body counters now exist which would permit the collecting of data in parts of the world where at

present no permanent whole-body counting installations exist<sup>7</sup>. Use of these portable counters would greatly extend our knowledge of the distribution pattern of fall-out in man.

We thank Mr. M. Stravino and Dr. C. Constantinides for their assistance and collaboration.

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## AN EXPANDING EARTH?

By DR. K. M. CREER

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**A**MONG the few geologists and geophysicists who have tentatively proposed that the Earth's radius has expanded by a factor of nearly two, Carey<sup>1</sup> has concluded from tectonic investigations that this large expansion has occurred since the Palaeozoic, while Egyed<sup>2</sup> has argued that a slow expansion of about 0.5 mm/yr has been going on throughout the Earth's life. An expansion of this magnitude cannot be accounted for by known physical processes<sup>3</sup>.

I became interested in the expansion of the Earth while using palaeomagnetic data to deduce the distribution of the continents in the upper Palaeozoic, using fibreglass shell models of the continents which could be slid over the surface of a 50-cm diameter globe<sup>4,5</sup>. It has been argued<sup>1,2,11</sup> that the present shapes of the continents are consistent with the supposition that they once comprised a complete outer shell of a smaller Earth. Usually these arguments have been supported by rough sketches.

### Palaeomagnetism and Expansion

Attempts have recently been made<sup>6–10</sup> to use palaeomagnetic measurements to detect changes in the Earth's radius. This is possible because one peculiar feature of all expansion hypotheses is that the area of continental material has remained constant while the bulk of the Earth has expanded. Hence the palaeoradius can be determined from measurements of the difference in palaeolatitude between two sites of known separation on the same continental block. At present the only data which are really adequate for this purpose are for Permian rocks from sites in Western Europe and Siberia, about 5,000 km apart. There is general, though not unanimous<sup>10</sup>, agreement that the data do not support an expansion as rapid as predicted by Carey since the Permian, but they are not sufficiently precise to confirm or reject a slow expansion as proposed by Egyed (Table 1).

### Palaeogeography and Expansion

The continents, down to the 500 fathom line where hypsographic charts show their steepest gradient, now cover about 30 per cent of the Earth's surface. This available sialic continental material might thus have covered a primitive Earth having a radius of about 0.55 of the present value. A simple model experiment could be carried out to investigate the fit by making 'Perspex'

shell models of the continents scaled and moulded to the curvature of the 50-cm globe and then remoulding these to the curvature of a 27-cm diameter globe, 0.55 times the size of the globe modelling the present Earth. These models of the continents would comprise pieces of a jig-saw to be fitted together on the surface of the 27-cm globe which they should cover. In fact, the experiment was carried out by making fibreglass shell models of the continents with great circle distances from a central point increased by a factor of  $0.55^{-1} = 1.82$  and fitting these together on the 50-cm globe. The fit obtained is illustrated in Figs. 1a and 2a.

To aid recognition, coastlines are shown rather than the edges of continental shelves. Most of the space between the continental outlines in the figures is occupied by continental shelf. There is considerable overlap of Patagonia and Antarctica. However, no pre-Mesozoic rocks have been found in Patagonia and it has been suggested that this southern part of South America has existed only since the Mesozoic. There is also an overlap in the region of the East Indies; but this is not apparent in Figs. 1a and 2a because they do not show the edges of the continental shelves.

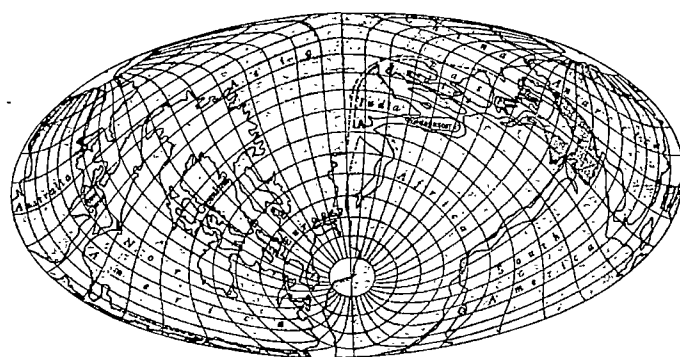
Even if continental material did once comprise a complete outer skin, one should not expect a perfect fit because of the following sources of distortion: (1) That produced by the radial expansion method used. This could well be different from the way in which the shapes of the continents would be distorted if the Earth expanded. (2) That due to tectonic movements, mountain building and a variety of other geological processes such as erosion. This point is considered in a later section of this note.

The problem of judging the accuracy of fit by exact mathematical treatment is thus complicated by the large number of variables. Nevertheless, suppose we take a number of pieces of spherical shell, of about continental size and complexity of shape, the total area of which equals that of the sphere having their curvature. The probability of finding a fit as good as found for the model continents may be computed. This I am now doing.

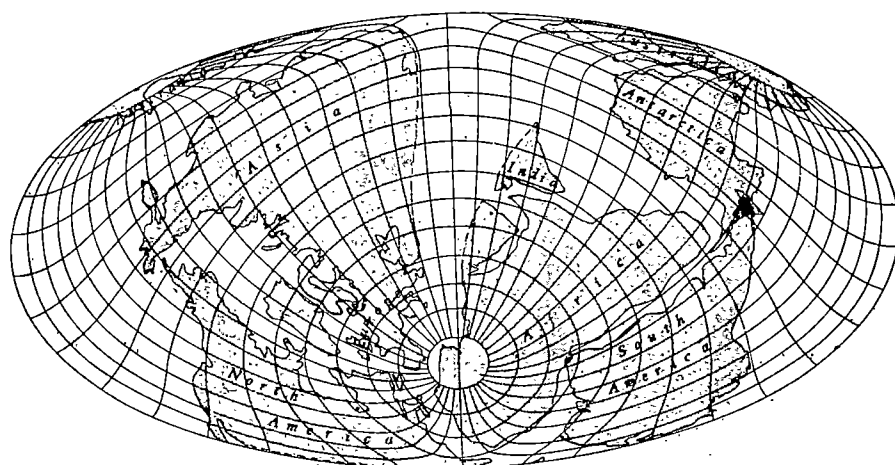
Table 1

Ref.	Ratio of Permian to present radius	Method
Carey (ref. 1)	0.83	Tectonic studies
Egyed (ref. 2)	0.99 – 0.97	Palaeogeography
Cox and Doell (ref. 8)	0.90 ± 0.03	Palaeomagnetic
Ward (ref. 9)	0.94 ± 0.04	Palaeomagnetic
van Hilten (ref. 10)	0.83 ± ?	Palaeomagnetic

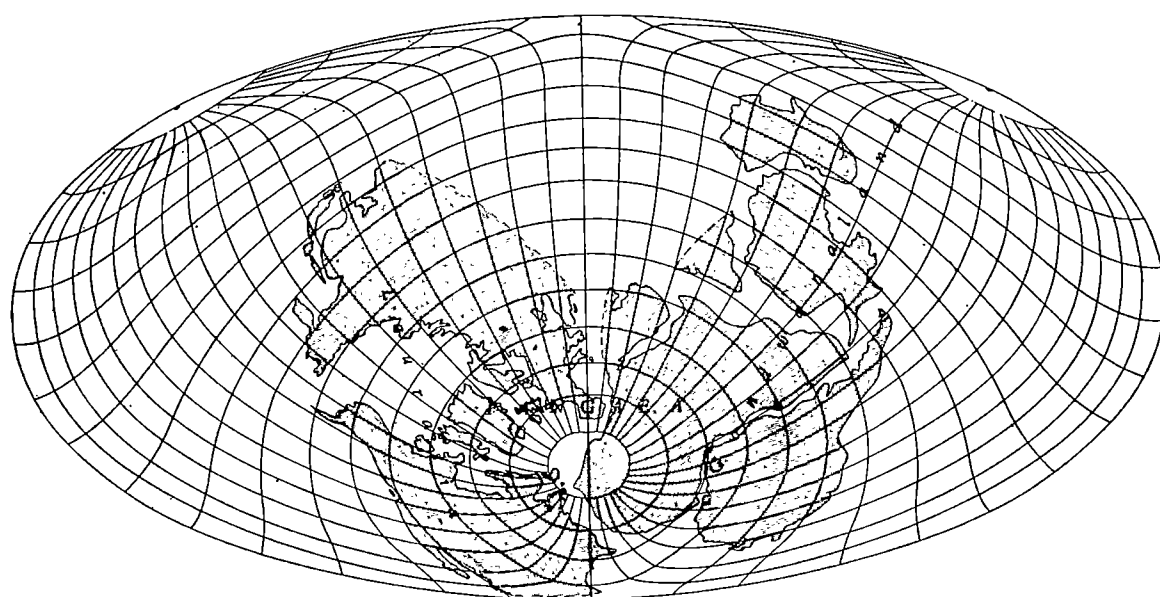




(1a)



(1b)



(1c)

Fig. 1. To illustrate how the shapes of the continents are consistent with evolution on an expanding Earth. Radius of Earth in (a) is 0.55, in (b) 0.77 and in (c) 1.0 of the present radius

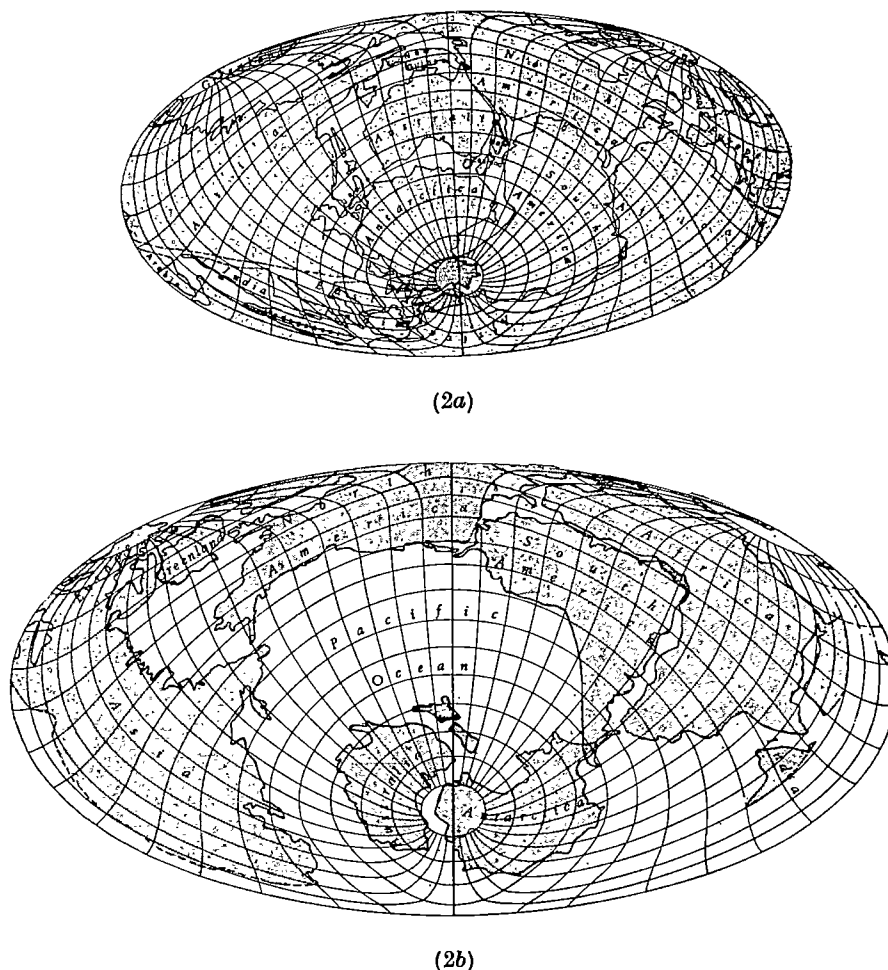


Fig. 2. To illustrate how Australia and Antarctica fit between eastern Asia and western America on an Earth the radius of which is 0.55 of the present value (a). On expansion of the interior of the Earth a U-shaped crack appears to have developed round the Pacific Basin. (b) illustrates Earth reduced to 0.77 of its present radius. (Note Figs. 1a and 2a and Figs. 1b and 2b respectively show the same continental distributions viewed from different angles and moved with respect to the grid)

At this stage, however, I have formed the impression that the fit of the continents on a smaller Earth illustrated in Figs. 1a and 2a appears too good to be due to coincidence and hence requires explaining. The simplest explanation appears to be expansion of the Earth, and one purpose of this article is to suggest that this hypothesis should be taken seriously.

#### Development of Pangaea

Some of the earlier workers in continental drift noticed that the profiles of the coastlines of the continents were such that they could be fitted together on an Earth of present radius to form a single supercontinent which they called 'Pangaea' (Fig. 1c). Recently it has been shown by precise methods<sup>12</sup> that the profiles of the continental shelves match even better than the coastlines.

To test whether the distribution of continents on a primitive Earth of little more than half the present radius is consistent with their grouping in Pangaea, a set of 'Perspex' shell models of the present continents on the 50-cm globe could have been remoulded to the curvature of an intermediate-sized globe, say 37 cm diameter. In fact, as before, the 50 cm globe was used and fibreglass continents prepared with great circle distances from the same central point increased by a factor of  $0.75^{-1} = 1.33$  above the present.

A grouping of continents transitional between the primeval grouping (Figs. 1a and 2a) and the grouping in

Pangaea (Fig. 1c) was easily obtained and is illustrated in Figs. 1b and 2b. Thus, if the Earth has expanded, it appears to have done so in such a way that the sialic skin first developed a U-shaped crack between Australia and America and between Australia and Asia. Subsequent expansion then appears to have been taken up largely in this initial crack, which widened to form the Pacific Basin.

As the slow expansion proceeded, the sialic skin would have to adjust itself to the decreasing curvature and the various parts of Pangaea would experience considerable changes in latitude and longitude. This might be considered as a kind of continental drift but of completely different origin and nature from that of the more rapid and familiar kind to which palaeomagnetic evidence relates and which occurred during the past few hundred million years.

#### Distortion produced on Change of Curvature

The amount of distortion in shape produced by the radial expansion method depends on the great circle distance of an element of coastline from the centre of expansion. Let us consider how a circular spherical cap subtending a semi-angle  $\theta$  at the centre of the sphere might adjust itself to the decreasing curvature of an expanding sphere while maintaining a constant surface area. Circular elements of area could expand or contract by an amount depending on the angle subtended with

Table 2a. SEMI-ANGLES SUBTENDED BY CONTINENTS

Continent	Maximum	Minimum
South America	35°	10°
Africa	40°	10°
Australia	20°	10°
Europe	20°	10°
Asia	45°	20°
North America	30°	15°
India	10°	5°
Antarctica	20°	10°
Pangaea	90°-100°	10°-20°

TABLE 2b. SPHERICAL CAP OF CONSTANT AREA ON AN EXPANDING GLOBE

Semi-angle subtended by circular spherical cap on a globe of		Ratio of circumferences (2)/(1)
(1) Radius $R$	(2) Radius $2R$	
45°	22°	1.08
60°	29°	1.16
70°	33°	1.19
80°	37°	1.22
100°	45°	1.43
118°	50°	1.71
135°	55°	2.32
180°	90°	∞

the centre. The semi-angle  $\theta'$  subtended on the larger sphere is related to  $\theta$  by  $2\pi R^2(1 - \cos \theta) = 2\pi R'^2(1 - \cos \theta')$ . However, the length of the circumference must change because the new circumference  $2\pi R' \sin \theta'$  is different from the old unless  $\sin \theta = \theta$ , which is certainly not true for angles subtended by the continents (Table 2a).

Factors by which the circumference increases for different initial and final angles  $\theta$  and  $\theta'$  are given in Table 2b. Since the various parts of the coastlines of continents subtend different angles  $\theta$  from the respective chosen centres of expansion, the shape of continents deduced for the smaller Earth using the model will differ in some measure from their present shapes.

The model of expansion used supposes that the shell of continental material adjusted itself to the new curvature by plastic flow. However, if the continents developed from a spherical shell on an expanding Earth, the shell would presumably have cracked. For a large number of very small cracks the result would be that for plastic flow, for the cracks would have been filled in by continental material transported from regions near the centre, where crustal thickening and hence uplift would have occurred.

However, as pointed out in the previous section, a large U-shaped crack seems to have developed round what is now the Pacific Basin. A subsidiary crack also appears

to have developed along the line of the Tethys Basin. If afterwards, smaller cracks formed, adjustments in curvature may possibly have developed in independent units of fairly small  $\theta$  of about the same size as the present continents. Thus the distortion produced by the radial expansion method need not be very different from that which could have occurred if the continents have developed on an expanding Earth. For large values of  $\theta$  such as we would have to consider if we tried to adjust the whole of Pangaea to the greater curvature of an initially smaller Earth the distortion would be impossibly great (Table 2). For values of  $\theta$  typical of the present continents, however, the distortion may at worst be about 20 per cent, this being the difference between the columns in Table 2, that is, for maximum and minimum  $\theta$  for each continent. Redistribution of continental material within Pangaea during most of its history before it broke up is irrelevant to the problem of fit, because the Earth had grown almost to its present size before disintegration commenced.

### Supercontinental Drift

In the Devonian and for the remainder of the Palaeozoic there is varied evidence that two supercontinents, Laurasia and Gondwanaland, existed<sup>4,5</sup>. The reconstruction based on palaeomagnetic data is illustrated in Fig. 3.

It thus appears possible that a phase of continental drift occurred in pre-Devonian times when Pangaea broke into two parts, Laurasia and Gondwanaland, which afterwards drifted apart, leaving the Tethys Sea between them. Palaeomagnetic data for the lower Palaeozoic and the late Pre-Cambrian are insufficient at present to deduce when this predicted 'supercontinental' drift occurred.

### Rate of Expansion

The expansion hypothesis provides a possible explanation for the development of Pangaea, the periphery of which is so shaped that it could conceivably have covered the entire surface of a smaller Earth. However, the fit of the edges of the continents which comprised Gondwanaland is better for an Earth of the present size than for the smaller Earths, as can be seen by comparing Figs. 1a, b and c. Everitt<sup>12</sup>, who has recently used an electronic

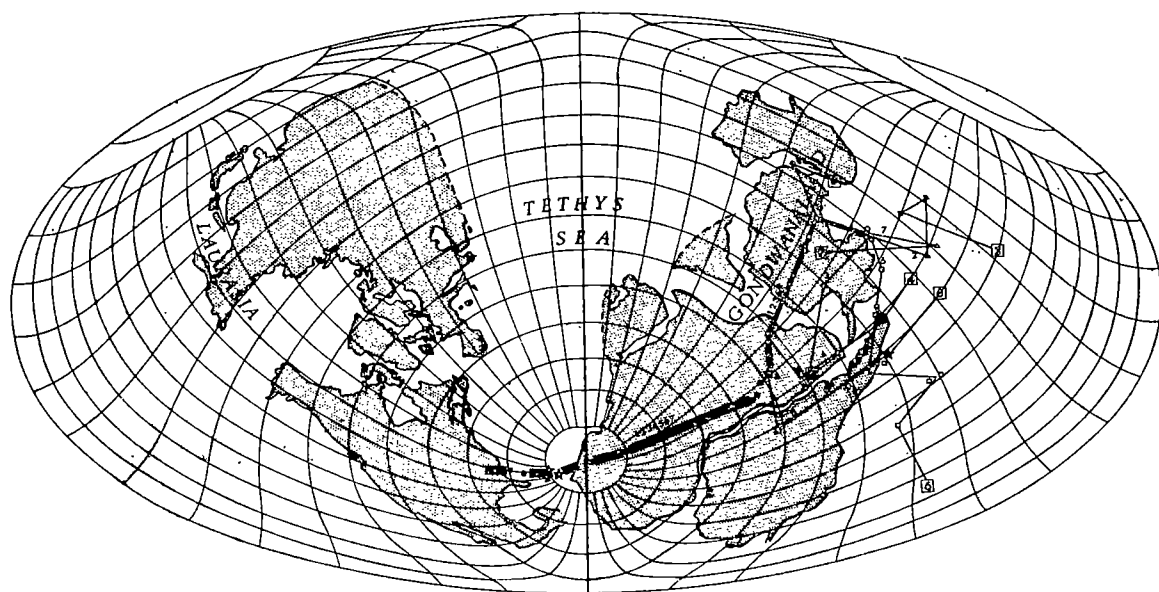


Fig. 3. Reconstruction for the Devonian deduced from palaeomagnetic data on present-size Earth. The polar paths for the Upper Palaeozoic and subsequent geological time are numbered as follows: (1) South America, (2) Africa, (3) Australia, (4) Europe and West Russia, (5) North America, (6) India, and (7) Antarctica. Curve 5 diverges from the main curve in the Pennsylvanian, Curve 4 in the Permian and curves 1, 2, 3, 6 and 7 diverge in the Upper Permian or Triassic



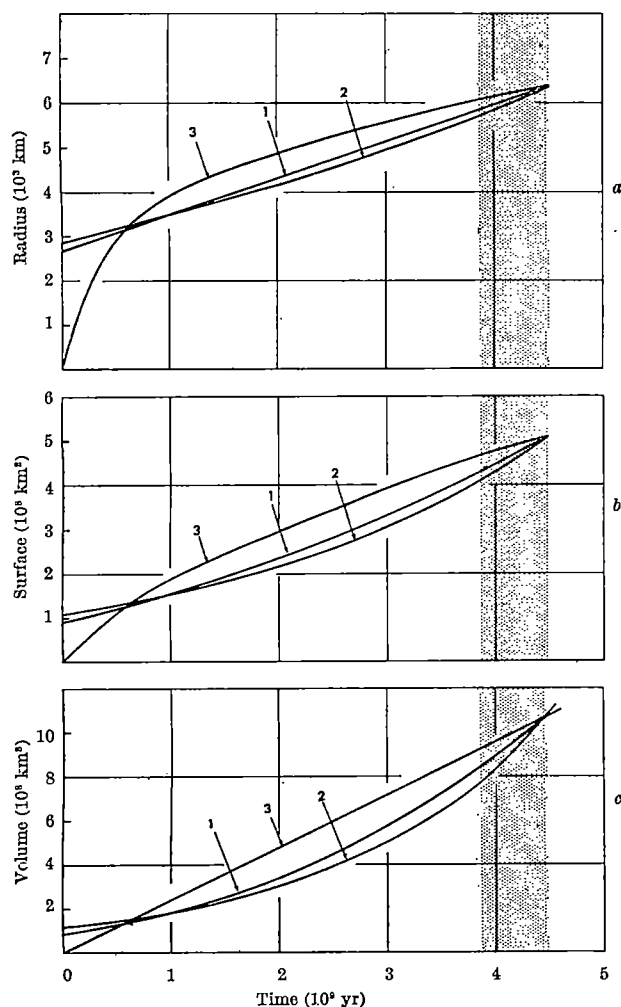


Fig. 4a, b and c. Earth expansion according to following laws: (1)  $R = R_0 (1 + at)$ , (2)  $V = V_0 \exp \beta t$ , and (3)  $V = V_0 (1 + \gamma t)$ . The shaded part corresponds to Phanerozoic time

computer to adjust the continents into their best-fitting positions, has drawn maps which exhibit an almost perfect fit of: (1) South America and Africa; (2) North America, Greenland and Western Europe. These fits could certainly not be bettered by recomputation for a smaller Earth. The fit of Australia and Antarctica into the Pacific is not so good for shell models of the present Earth and appears rather better for a smaller Earth.

Thus it can be argued that the Earth's radius was not appreciably different from its present value when the fragmentation of Laurasia and Gondwanaland commenced<sup>4,5</sup> in the Permian or Triassic. This conclusion is supported by the palaeomagnetic evidence given in Table 1. Van Hilten<sup>10</sup>, who, alone among those who have considered the palaeomagnetic data, argues that the Permian radius was appreciably less than the present, has on this basis deduced a rather strange reconstruction of the continents for the Permian. I think his conclusions are wrong since the Permian palaeomagnetic data are consistent with du Toit's reconstruction<sup>4,5</sup> on an Earth of the present radius. Hence a rapid and recent Earth expansion as advocated by Carey<sup>1</sup> appears most improbable.

Let us now suppose that the expansion has been occurring throughout the Earth's life and hence try to draw some conclusions about possible present rates of expansion. In an Earth formed by the accretion of cold bodies, radioactive heating would enable chemical evolution to take place fairly quickly. It has been estimated<sup>13</sup> that a proto-core would form in about 500 million years.

The process of chemical evolution might have been approaching completion after about 1,000 million years, although it may still be happening on a small scale even to-day. Thus, the sialic continental material may have entirely covered the Earth when it was about 1,000 million years old. Its radius would then have been about 0.55 times its present value. The Earth is now thought to be about 4,500 million years old.

In the absence of an explanation of the supposed expansion let us consider three simple laws: (1)  $R = R_0 (1 + at)$ , a steady increase of radius with time; (2)  $V = V_0 \exp \beta t$ , in which the incremental increase in volume at any time is proportional to the volume at that time; (3)  $V = V_0 (1 + \gamma t)$ , a steady increase in volume with time. These laws are illustrated in Figs. 4a, b and c, from which we see that (1) and (2) are nearly identical. If we consider law (3) we find that  $V_0$  would have to be negative if  $R = 0.55 R$  (present) at  $t = 10^9$  years and  $V_0$  would equal 0 at  $t = 0$  if  $R$  had the foregoing value at  $t = 0.74 \times 10^9$  years. In Table 3, Earth radii, surface areas and volumes are given for all three cases for 200 million years ago (Permo-Triassic) and 500 million years ago (lower Palaeozoic).

Table 3

Expansion law	Radius (km)	
	200 m.y. ago	500 m.y. ago
$R = R_0 (1 + at)$	6,205 (2.6%)	5,960 (6.4%)
$V = V_0 \exp \beta t$	6,180 (3.0%)	5,840 (8.0%)
$V = V_0 (1 + \gamma t)$	6,270 (1.6%)	6,110 (4.1%)
(Present radius 6,370 km)		
	Surface area ( $10^6 \text{ km}^2$ )	
	200 m.y. ago	500 m.y. ago
$R = R_0 (1 + at)$	485 (4.9%)	448 (12.6%)
$V = V_0 \exp \beta t$	476 (6.6%)	430 (15.8%)
$V = V_0 (1 + \gamma t)$	497 (2.5%)	474 (7.1%)
(Present surface area $510 \times 10^6 \text{ km}^2$ )		
	Volume ( $10^6 \text{ km}^3$ )	
	200 m.y. ago	500 m.y. ago
$R = R_0 (1 + at)$	1.02 (5.6%)	0.90 (16.7%)
$V = V_0 \exp \beta t$	0.98 (9.3%)	0.83 (23.2%)
$V = V_0 (1 + \gamma t)$	1.03 (4.6%)	0.96 (11.1%)
(Present volume $1.08 \times 10^6 \text{ km}^3$ )		

Expansions in percentages of present values are given in brackets.

### Earth Expansion, Continental Drift and Mountain Building

In Table 4, typical times which might be associated with each of the foregoing processes are listed. Because of the comparatively long typical time associated with Earth expansion, I think it unreasonable to invoke it as the principal cause or source of energy for drift or orogeny. Even so, a few geologists have recently attempted<sup>1,14</sup> to do this. I think that expansion should be regarded as something which may have been gently, but persistently, occurring in the background. There may be little obvious geological evidence of expansion: most of this could easily have been obscured by more vicious and rapid processes such as continental drift and orogeny.

Table 4

Phenomenon	Typical time ( $10^9$ years)	Presumed source of energy
Earth expansion	Time for earth radius to increase by a factor $e$	4,000-5,000 Cosmological
Polar wandering	Time for $180^\circ$ of polar wander at the observed rate of $2/3^\circ$ per million years	250 Planetary
Continental drift	Duration of a phase of drift—approx. one geological era (for example, Mesozoic)	200 Planetary (in mantle)
Orogenesis	Duration of Alpine orogeny	20 Regional (in crust and upper mantle)

If Pangaea developed along the lines described, two questions arise. First, why did continental drift not occur until such a late stage in the Earth's development; and secondly, why was its shape not distorted out of all recognition by mountain building processes and erosion?

On this hypothesis of development, the first phase of continental drift occurred before the Devonian when Pangaea bisected to form Laurasia and Gondwanaland. The second and main phase occurred during the Mesozoic. It is noted that some geologists have pointed out that orogenic activity has slowly increased in frequency during geological time, that is, the period for which there is a continuous geological record. Thus, orogenic activity, and hence erosion, may have played a much smaller part in the development of the crust in the remote past than is indicated by geological evidence for recent periods. This possibly explains why it appears that sections of the edge of Pangaea retained their initial shapes so well.

It may well be a long time before sufficient evidence pertinent to the expansion hypothesis is found. Careful investigations of Pre-Cambrian rocks should yield useful results. Geological examinations on other Earth type planets may be revealing, particularly if one is found where neither orogenic activity nor mantle convection has occurred. For an adequate explanation we may well have to await a satisfactory theory of the origin and

development of the universe<sup>16</sup>. In the meantime, we should beware of rejecting the hypothesis of expansion out of hand on grounds that no known sources of energy are adequate. It may be fundamentally wrong to attempt to extrapolate the laws of physics as we know them to-day to times of the order of the age of the Earth and of the universe.

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<sup>10</sup> Van Hiltten, D., *Tectonophysics*, 1, 3 (1964).

<sup>11</sup> Barnett, C. H., *Nature*, 195, 447 (1962).

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## DERMATOGLYPHIC TOPOLOGY

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THE ridges which form the dermatoglyphic patterns on the ventral surfaces of the hands and feet are arranged in lines which are parallel in small fields. Two kinds of discontinuity of pattern are found; these occur at the centres of what are termed loops and triradii. Around the core of a loop (Fig. 1) the direction of the ridges turns through an angle of 180°. The centre of a triradius (Fig. 2) is the point where three different fields of almost parallel ridges meet. The result produces three spokes; the angles between them are greater than 90° and are typically each 120°.

On the fingers and toes a single loop is accompanied by one triradius (see Fig. 3). When two loops are present there are two triradii, and the same applies to a symmetrical whorl in which two loops have become fused.



Fig. 1. Loop pattern

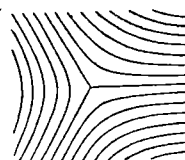


Fig. 2. Triradius



Fig. 3. Configurations on digits; (i) arch, (ii) loop, (iii) whorl

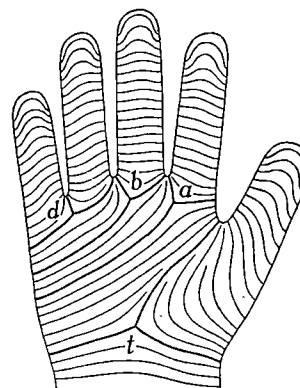


Fig. 4. Minimal number of triradii on hand

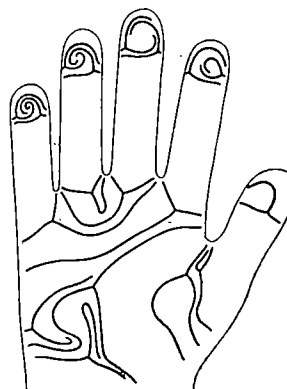


Fig. 5. Left hand with multiple ridge patterns

Curvature of the lines which is insufficient to make a loop is called an arch.

On the palms the minimal number of triradii is four. These are usually in the positions designated *a*, *b*, *d* and *t* (see Fig. 4). For every loop which occurs on the palm there is another triradius. Moreover, it can be shown empirically that, over the whole hand, including the fingers, the number of triradii exceeds the number of

loops by four. In Fig. 5 the hand has 18 triradii and 14 loops. The explanation for this is that, at the junction between the ridged skin on the palmar surface and the thinner skin on the dorsal surface, the ridges run at right angles to the axis of the limb.

An exceptional arrangement occurs at the apex of each digit, where the ridges lie nearly parallel to the edge of the nail; this is equivalent to the presence of a loop pattern the core of which is replaced by part of the nail. The wrist is also exceptional and, again, it corresponds topologically to a loop. Indeed, the whole ridged surface of the hand can be mapped on a circle (see Fig. 6) in which all ridges meet the circumference at right angles. Provided that such an area is covered with ridges parallel to one another in small fields, it is topologically necessary that at least two loops are present somewhere on it (see Fig. 7). For every additional loop (see Fig. 8) there must be a compensating triradius. Thus, the total number of

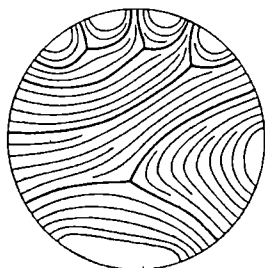


Fig. 6. Circular topological diagram of the hand shown in Fig. 4

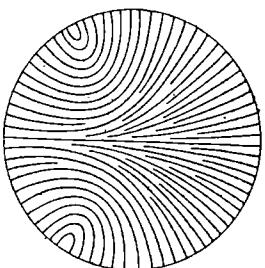


Fig. 7. Circular area with ridges meeting circumference at right angles; two loops are essential

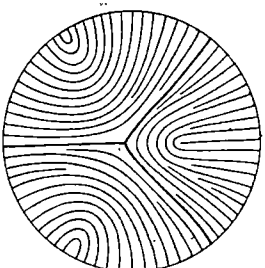


Fig. 8. Circular area as in Fig. 7, but with three loops and one triradius

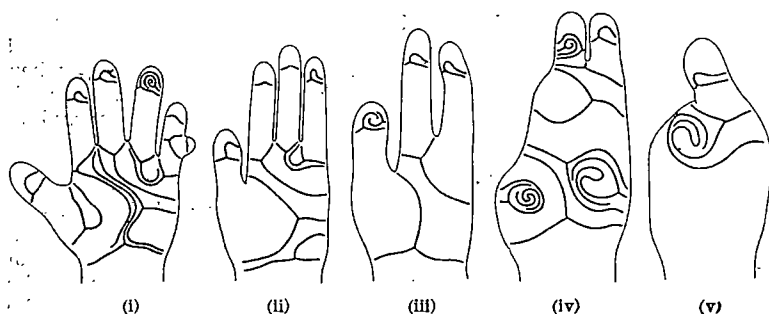


Fig. 9. Examples of palmar dermatoglyphics on malformed right hands with 6, 4, 3, 2 and 1 digits, respectively. (i) Hexadactyly in 13-15 trisomy,  $13+1=8+6$ . (ii) Hereditary ectronychia,  $7+1=4+4$ . (iii) Sporadic deformity,  $6+1=4+3$ . (iv) Hereditary ectrodactyly,  $9+1=8+2$ . (v) Hereditary ectrodactyly,  $3+1=3+1$ .

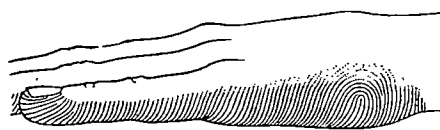


Fig. 10. Indistinct triradius on ulnar edge of the palm

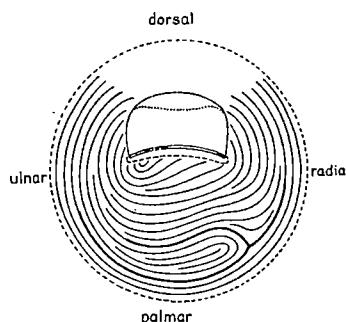


Fig. 11. Diagram of ridges on terminal phalanx of normal thumb which shows a loop vestige near the apex

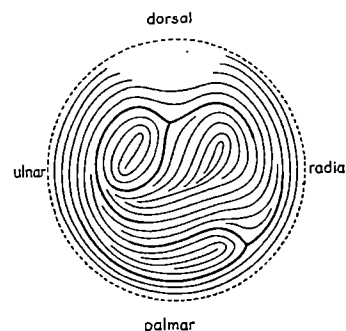


Fig. 12. Diagram of ridges on terminal phalanx of anonychie thumb on which there are four loops but only two triradii

loops will always be two more than the total number of triradii.

Returning to consideration of the hand, remembering that each digit is equivalent to one loop and leaving out the wrist, a general dermatoglyphic formula is obtained. Thus, if  $T$  is the number of triradii,  $L$  the number of loops and  $D$  the number of digits,

$$T + 1 = L + D$$

Had the wrist been included, a unit would have been added to each side of the equation. Obviously, in the normal hand where  $D = 5$ ,  $T - L = 4$ , as already observed. Normal feet are governed by the same formula, but the complete dermatoglyphic configuration is much less easily identified on soles than on palms. The triradii at the bases of the toes are often extremely difficult to find. The rule can be tested by analysing hands with malformations which have deprived them of digits or increased their number. In all examples shown (Fig. 9) the formula is correct.

There are three kinds of conditions which can invalidate the rule. The first type is trivial but common. This is that a triradius or loop fails to be observed on a print although it is actually present. Reference to the equation sometimes shows that the pattern has been incorrectly recorded.

The second source of deviation is that part of a pattern may be so close to the edge, beyond which the ridged skin does not extend, that it may have been incompletely formed. For example, a triradius on the extreme ulnar aspect of the palm, though topologically necessary, may nevertheless be scarcely visible (Fig. 10).



The third origin of exceptions is of critical interest. It concerns the apices of the fingers. On the thumb especially, the ridges do not lie exactly parallel to the hyponychium, and in normal hands occasionally a small loop is actually present towards the ulnar side (Fig. 11). In abnormal circumstances, when the nail is diminished or absent, as in anonychia, the dermal ridges may extend over the area normally covered by the nail. In such instances, as might be expected on topological grounds, there are two loops without accompanying triradii (Fig. 12). The reason for this pattern is that the ridges have trespassed on to the dorsal surface of the hand, where the same rules apply independently as on the palmar surface. The presence of the normal nail indeed is a substitute for two loops, and, from the topological point of view, one belongs to the dorsal and the other to the ventral surface of the digit.

The topological analysis of dermal ridges, besides leading to searches for new configurations, may throw

light on the developmental background. The fact that the ridges run predominantly at right angles to the long axes of the limbs and the digits suggests that they take the shortest courses possible. The symmetrical form of most triradii, with angles of nearly  $120^\circ$ , is also compatible with this view. Observations on the ridge formations on palms and soles of both New World and Old World monkeys indicate that whorls occur on the volar pads<sup>1</sup>. In the human fetus the pattern of ridges is determined at an early stage in the development of the limb when the proportional sizes of digits and pads are different from those in the adult. The permanent configuration is the result of laying a carpet of parallel lines, in some way as economically as possible, over the contours presented by the foetal hand.

I thank Mr. A. J. Lee for assistance in preparing the figures.

<sup>1</sup> Cummins, H., and Midlo, C., *Fingerprints, Palms and Soles* (Philadelphia: The Blakiston Company, 1943).

## NEWS and VIEWS

### The Council for National Academic Awards

H.R.H. PRINCE PHILIP, Duke of Edinburgh, has accepted an invitation to be president of the Council for National Academic Awards. The Council for National Academic Awards is an autonomous body which was established by Royal Charter in September 1964, with powers to award degrees, diplomas, certificates and other academic awards to persons who have successfully pursued courses of study approved by the Council at educational establishments other than universities or who have successfully carried out research work under the supervision of an educational or research establishment other than a university. The Council will award degrees and other academic distinctions, comparable in standard with awards granted and conferred by universities, to students who pursue their higher education in establishments for further education which do not have the power to award their own degrees. The Council will deal with Scottish colleges as well as colleges in England and Wales. Sir Harold Roxbee Cox is chairman of the Council (see *Nature*, 201, 975; 1964).

### The Geological Society of London:

#### Awards

THE following awards for 1964 have been made by the Geological Society of London: *Wollaston Medal*, to Prof. D. M. S. Watson, emeritus professor of zoology and comparative anatomy, University College, London, for his distinguished researches in the field of vertebrate palaeontology; *Murchison Medal*, to Prof. W. F. Whittard, Channing Wills professor of geology, University of Bristol, in recognition of his researches on the stratigraphy and palaeontology of the Silurian and his direction of marine geological investigations; *Lyell Medal*, to Prof. C. F. Davidson, University of St. Andrews, for his petrological work on the rocks of the Hebrides and his mineralogical and economic researches on atomic minerals; *Bigsby Medal*, to Prof. J. Sutton and Dr. Janet Watson (Mrs. J. Sutton), Department of Geology, Imperial College of Science and Technology, for their fundamental contributions to the stratigraphy, structure and metamorphism of the Scottish Highlands; *Wollaston Fund*, to Dr. H. W. Ball, Department of Palaeontology, British Museum (Natural History), for his stratigraphical and palaeontological researches, especially on the Old Red Sandstone and fossil fish; *Murchison Fund*, to Dr. I. Strachan, lecturer, Department of Geology, University of Birmingham, for his work on graptolites and Pleistocene palynology; *Lyell Fund*, a moiety to Mr. S. W. Hester, Geological Survey and Museum, London, for his palaeontological and stratigraphical work on the Upper Carbon-

iferous, and another moiety to Mr. R. Stoneley, geologist, British Petroleum Development, Ltd., in recognition of his work in Antarctica, and for stratigraphical and structural studies in East Africa, New Zealand and Alaska in connexion with exploration for petroleum.

### Biology in the Massachusetts Institute of Technology:

#### Dr. S. E. Luria

IN recognition of the work in bacteriology and public health of the late Dr. W. T. Sedgwick, the William Thompson Sedgwick professorship in biology has been established by the Massachusetts Institute of Technology. Dr. S. E. Luria has been named as the first Sedgwick professor. Dr. Luria received his medical degree at the University of Turin in 1935. He pursued his research in microbiology at the Institute of Radium, Paris, and at various universities in the United States before joining the faculty of the Massachusetts Institute of Technology in 1958, where he established a new programme of microbiological research and teaching within the Department of Biology. Dr. Luria is probably best known for his research on the genetics of bacteria and on the genetic influences exerted by viruses over the bacterial cells they invade. He is the author of more than one hundred technical papers.

### Ministry of Technology

IN a written answer in the House of Commons on January 21 the Prime Minister stated that the branch of the Department of Education and Science concerned with policy for nuclear energy, and a small section of the Ministry of Aviation responsible for sponsoring the electronics industry, had been incorporated within the Ministry of Technology. The greater part of the existing headquarters of the Department of Scientific and Industrial Research, together with most of its stations, and the branch of the Board of Trade concerned with policy for the National Research Development Corporation would also be transferred to the Ministry of Technology when the necessary legislation and Statutory Instrument took effect. In all, rather more than 5,000 staff, including industrial workers, were involved.

### Manpower Resources for Science and Technology

IN answer to a question in the House of Commons on January 21, the then Secretary of State for Education and Science, Mr. M. Stewart, said that a new survey of scientific manpower was now being undertaken. The results of this would probably be available by about the middle of this year and could then be interpreted by the new Committee on Manpower Resources for Science and Technology. In a written answer, also on January 21,

Mr. Stewart added that he expected that the target of 20,000 graduates a year in pure or applied science set for about 1970 by the Barlow Committee would be met during 1965. Rather more than 25,000 boys and girls left school in the year 1962-63 with two or more passes in the Advanced Level Examination for the General Certificate of Education, at least one of which, and mostly all of which, were in science subjects.

### Land and Natural Resources

IN a written answer in the House of Commons on January 19, the Prime Minister stated that the Minister of Land and Natural Resources would be responsible for research into the possibilities of conserving and augmenting national water resources. Accordingly, the new Minister would assume responsibility for those aspects of the Minister of Housing and Local Government's work concerned with the Water Resources Board, including the appointment of members and financial responsibility. He would take a leading part in any major projects, such as estuarial barrages, which involve co-ordination of various different interests in a particular river and with which a number of different Departments are concerned. He would also be responsible for plans for reorganizing the water supply industry under full public ownership. However, the Minister of Housing and Local Government would continue in England to exercise his statutory functions in relation to water supply and river management, particularly executive responsibility for questions of water distribution, minimum acceptable flow in rivers, abstractions, river pollution and sewerage. He would also share with the Secretary of State for Wales his existing statutory responsibilities for the appointment and functioning of river authorities, but the Minister of Land and Natural Resources would also be one of the Ministers who appoint those members of river authorities who are "qualified in respect of public water supply". Appropriate arrangements would be made between the three Ministers about future appointments to the three river authorities concerned with catchment areas in both England and Wales, and these authorities would look to London or Cardiff as appropriate for the problem. The provisions of the Water Resources Act, 1963, were not as yet fully in effect and the position would be kept under review to see whether experience in implementing those provisions called for any change in these arrangements. The position in Scotland was unchanged. In a further answer on January 21 the Prime Minister added that details of the Minister's responsibilities for the establishment of a Land Commission and for future policy relating to the availability of land would be set out in a Transfer of Functions Order to be laid before the House.

### Scientific Advisory Panel to the Ministry of Agriculture, Fisheries and Food

IN a written reply to a question in the House of Commons on January 21 concerning arrangements for securing advice on the scientific aspects of questions arising within the field of agriculture, fisheries and food, Mr. F. Peart, Minister of Agriculture, Fisheries and Food, said that he had gone into this question following the retirement of Sir Harold Sanders from the post of chief scientific adviser (agriculture), and had decided to establish a Scientific Advisory Panel under the chairmanship of Prof. A. C. Frazer, of the University of Birmingham. This Panel would be available to advise him on the scientific aspects of any questions affecting the activities of his Department which might be referred to it. The Panel would also be free to offer its advice on any problems in this field whenever in its view this was desirable. The responsibilities of the heads of the scientific branches in the Ministry would not be affected in any way by these new arrangements. On the other hand, it was hoped that the establishment of the Panel would lead to closer links between the universities and the Ministry.

### The World Weather Centre, Washington

THE World Weather Centre in Washington commenced operation on January 1 and is now providing services on request to all members of the World Meteorological Organization. Essentially it is concerned with gathering, processing and distributing weather observations. It will prepare weather analyses and forecasts for as much of the globe as possible. From the Centre, the processed weather information will be transmitted to regional and national meteorological centres. Opportunities will be provided for training meteorologists from member countries. The Centre will conduct both basic and applied research on large-scale weather problems, and will store weather information for research purposes. The planned activities of the Centre cannot as yet be fully implemented because weather data are not available over much of the Earth's oceanic areas. Moreover, the present meteorological satellite system does not as yet provide global satellite coverage. However, it is hoped that the *Tiros* Operational Satellite System, which is planned to be launched in about a year's time, will eliminate that deficiency. Plans are being developed to implement the Centre in progressive steps. The events leading to the establishment of the World Meteorological Centre began in 1960, when the first two *Tiros* weather satellites were successfully launched. Photographs taken by television cameras on board were used by the U.S. Department of Commerce Weather Bureau in improving analyses, forecasts and warnings. Washington and Moscow have already been designated as World Centres by the World Meteorological Organization, and a third centre in the southern hemisphere is envisaged.

### Annual Growth Rate in Britain

IN the *Westminster Bank Review* for November 1964, an article by Prof. Alan Day, "The Myth of Four Per Cent Growth", estimates the present annual growth of capacity as 3.3 per cent, to which may be added an extra 0.2 per cent to be activated by a regional policy designed to make use of under-employed labour in the North. This suggests that, while the latter policy could also lead to greater inflationary pressures, since the upward trend in the ratio of rate of investment to gross national product appears to have levelled out since 1961, the annual rise of productivity may become stabilized at about 2.7 per cent. Prof. Day also expects a slower rise in the labour force, but nevertheless believes we could achieve a 4 per cent growth, including a big increase in the ratio of investment to national income, though at the price of a severe but temporary setback to the growth of present expenditure. Four per cent could serve a useful social purpose if treated as a target to be achieved only by sacrificing present consumption and severe disturbance to vested interests and economic and social rigidities.

### Careers in Biology

SINCE the first edition of *Biology as a Career* appeared in 1953, there has been a steady expansion of biological science and a perceptibly growing recognition by the public of its value. The fourth edition is published under the conviction that there is now greater need than ever for a general guide describing the training, work and prospects of the professional biologist (Pp. 44. London: Institute of Biology, 1964. 3s. 6d.). In this booklet, the opportunity has been taken to bring facts up to date and to include a new appendix, indicating qualifications necessary for entry as well as giving brief details of the interests of the biological departments of British universities.

### Kindrogan Field Centre

THE Scottish Field Studies Association Field Centre in Garth Memorial Hostel was closed at the end of 1963 and a new, self-contained, residential field centre was opened in March 1964 at Kindrogan, a large country house stand-

ing in 10 acres of ground in Strathardle, Perthshire (The Scottish Field Studies Association. Annual Report, 1963. Edited by B. W. Ribbons. Pp. 27 + 4 plates. Glasgow: The Scottish Field Studies Association, 1964. 4s.). This new permanent field centre affords excellent opportunities for work in the Scottish Highlands on biology, physical geology, physical and human geography and archaeology. Close to the centre are coniferous and deciduous woodland, freshwater river, loch and lochan, dry and wet moorland, glacial moraines, terraces and haughs, and the remains of a small highland village. The Highland Boundary Fault is at hand and a few miles away are Ben Vrackie, Glen Shee, Glen Isla, Beinn A'Ghlo and Killiecrankie. Kindrogan stands at an altitude of 850 feet, and has an annual rainfall of 38 inches. Amateur naturalists and research workers are invited to use the centre for their own private work and to join courses organized by the Association, together with groups of pupils and students accompanied or unaccompanied by their own staff. The standard of such courses is adjusted to suit the needs of those present, and the Association is also prepared to consider arranging courses to suit particular groups. Details of the courses and facilities available may be obtained from the Warden, Kindrogan Field Centre, Enochdhu, Blairgowrie, Perthshire.

### The Quaker Approach to Life

IN her eighteenth Eddington Memorial Lecture, now published, Dr. K. Lonsdale gives a moving and sincere account of the development of her faith as a part of her whole experience of and outlook on life (*I Believe* . . . Pp. 56. Cambridge: at the University Press, 1964. 4s. 6d.). This attitude to life as an experiment calling for commitment, as Dr. J. H. Oldham did, brings out the essential harmony between her work and her faith. Religious truth, she argues, is known to us through religious experience, that of ourselves and of others, and includes scientific experience, while going far beyond it. Even to those who may not follow her on some particular points Dr. Lonsdale's lecture makes refreshing reading and a stimulating challenge to approach life as a whole, much in the spirit of Donald Hankey half a century ago.

### The National Institute of Sciences of India

THE Yearbook of the National Institute of Sciences of India, 1963, constitutes the usual reference work with lists of Fellows, Council and senior office staff, membership of committees, rules and regulations, medals and lecturers, and publications (Pp. vi + 241. New Delhi: National Institute of Sciences of India, 1964. 15 Rs.). The Council's report summarizes the anniversary address of the President, Dr. A. N. Khosla, and gives particulars of the symposia, on endocrinology of reproduction, vitamin metabolism, and fertility of Indian soils, arranged during the year. Proposals are under consideration for development of the Institute on the lines of the Royal Society, and steps are being taken to expedite publication of scientific papers, including removal of the Publications Office from Calcutta to Delhi. Brief reports on researches in mathematics, physics, chemistry and physiology are appended.

### Science and Technology in Poland

THE tentative analysis of the present state of Polish science contributed by H. Jablenski to the *Review of the Polish Academy of Sciences* for April-June 1964 (9, No. 2; 1964) considers successively the position of the biological sciences, medical studies, chemical sciences, mathematical and physical sciences, geology and technical sciences in Poland in relation to the extent to which they meet the needs of Poland, the relations between the several disciplines and gaps which should be filled. The same issue also contains the abridged text of an address by the Deputy Prime Minister, E. Szyr, on the responsibilities, organization and methods of work of the Committee on Science and Technology.

### Roumanian Scientific Abstracts

THE Scientific Documentation Centre of the Academy of the Roumanian People's Republic is now issuing monthly: *Roumanian Scientific Abstracts*. These are to be published in two series, on natural sciences and social sciences, from January/February 1964, in English and Russian, and will provide scientists abroad with complete and rapid information on Roumanian research in these fields. In the issue for the natural sciences, entries are arranged under mathematics and astronomy, physics, chemistry, and biology. Nineteen periodicals are covered.

### New Zealand Department of Scientific and Industrial Research

THE New Zealand *D.S.I.R. Handbook 1964*, which supersedes the 1960 edition, contains in general information compiled by the staff of the Department's Information Service up to March 31, 1964, but since organization and administration of science in New Zealand are at present undergoing important changes some gaps in the coverage are inevitable (Pp. 104. Wellington: Government Printer, 1964). Besides outlining the general organization of the Department, the *Handbook* gives, for each Division or institution, the address, principal officers and an outline of the scope of its work.

### Building Material Properties Classified

ARCHITECTS and builders require a good deal of detailed technical and economic information to permit choice between a number of possible materials or products. For this information reliance is often on technical literature supplied by manufacturers, sometimes supplemented by results of tests or assessments made by independent test houses or research organizations, or on documentation available from building information centres. There is at present no established system for presenting this sort of information, such as could facilitate comparison of similar products on the basis of essential relevant properties of materials and products. To meet this need a report has been prepared, entitled *A Master List of Properties for Building Materials and Products* (CIB Report No. 3. Prepared by CIB Working Commission W. 31. Pp. 20. Rotterdam: General Secretariat, CIB, P.O. Box 299, 1964. 2 Sw. francs). This is an important step towards uniformity of presentation and a systematic framework for setting out values for physical properties and design data without actually specifying any particular standards. Under the heading 'Properties', this *Master List* gives guidance on general information; composition and method of manufacture; shape, dimensions, weight; general appearance; physical, chemical and biological properties; durability; special technical properties for installations, mechanical plant, equipment, etc.; working characteristics; and functional properties of common types of building elements in which the material or product is incorporated. Under the heading 'design considerations and details: suitable applications', there are included architectural and constructional details; examples of common mistakes in design and work; and references. Instructions for work and maintenance embrace this subject, site testing and instructions for cleaning and maintenance. Other headings are economics; specification of distribution; sales organization; contract work; technical service. "Because it is intended to be comprehensive, not every property in the list will apply to every product, and the Working Commission (W 31) is going to produce separate lists, based on this master list, that will relate to various classes of materials or products and to materials or products used for particular purposes." This is a most useful summary of data essential to modern building practice and should be in the hands of all concerned with building construction where only the best will satisfy.



## National Soil Survey of Ireland

A SYSTEMATIC soil survey of Ireland has been undertaken by the Agricultural Institute, and Bulletin No. 1 is an account of the *Soils of Co. Wexford*, by M. J. Gardiner and Pierce Ryan (Pp. 171+xiii. 31 Plates. 3 Maps. Foras Taluntais: 33 Merrion Road, Dublin 4, 1964. 30s.). Apart from the Leinster Mountains, which rise to 2,610 ft. in the north-west, most of the county consists of gently undulating country below 750 ft. with a relatively favourable maritime climate, the average rainfall varying from 34 in. on the coast to 48 in. near the mountains, the average monthly temperature from 40° to 60° F and the relative humidity from 70 to 80 per cent. The solid geology consists mainly of granite and mica-schist in the higher ground, Ordovician shale associated with volcanic rocks in the central region, and Cambrian shale with a belt of Carboniferous limestone in the low-lying area. But most of the County is covered by glacial deposits of different ages and most of the soils have been derived from the Saale glaciation and form a complex pattern. A chapter of 50 pages is given to a full description of the soil associations and series, including regosols, brown earths, podzols, gleys, lithosols and organic soils, and their classification into the Great Soil Groups. There are discussions of analytical data in relation to the main soil associations and of the suitability of the various soils for the usual range of crops, including pasture and forest, concluding with a suitability classification. A chapter on the agricultural pattern is contributed by M. T. Connolly. The three maps, on the scale 2 miles to 1 in., describe the soils, the soil drainage and the soil suitability.

## Measuring Techniques in Coastal Waters

A PAPER in the *South African Journal of Science* (60, No. 9; September 1964), by C. C. Stacopoulos, describes the classical methods of measuring temperature, salinity and ocean currents, and discusses their limitations in coastal waters. Preference is given to techniques which give rapid coverage in space and time, rather than to those which give a precision which may not be justified in near-shore water. Electrical salinometry and electrical thermometry in various forms have proved the most useful. A method of measuring near-surface currents up to distances of 3 miles offshore is recorded, as well as a rapid method of measuring currents at numerous depths in profile and to greater distances offshore. Time series current measurements have been obtained from observations on a system of buoys offshore.

## Cardiac Pacemakers

VOLUME 3, Article 3 of *Annals of the New York Academy of Sciences* presents the papers and records the discussion that took place from them at a conference held under the chairmanship of Dr. W. W. L. Glenn, and sponsored jointly by the New York Academy of Sciences and the Council of Cardiovascular Surgery of the American Heart Association (*Cardiac Pacemakers*. Pp. 813-1122. New York: New York Academy of Sciences, 1964. 7 dollars). Twenty-five papers are presented. They range from reviews of the normal anatomy and clinical aspects of the conduction system of the heart in man to its abnormal behaviour in disease leading to the need for artificial cardiac pacemaking. Papers cover the full range of present knowledge on the means available for artificial pacemaking. Conventional non-synchronous electrical pacemakers now support an increasing population of patients in active life, who were previously condemned to sudden loss of consciousness and the risk of death from cardiac standstill. The techniques used for this treatment are reviewed in detail and the disadvantages of these methods are clearly stated. Numerous papers on the experimental approach to more effective methods are also presented in this volume. They give an exciting glimpse

of technical, pharmacological and physiological advances in this field which will yield both an increase in benefits to patients and new knowledge of the biochemical and physiological behaviour of the heart itself. This volume should be looked on as essential reading for the clinician and clinical research worker in this field. There is one surprising omission to be found in this very complete symposium. The cardiologist required to provide a medical and technical service for artificial electrical pacemaking of the heart would have valued an article giving special guidance on the medical and technical hazards and requirements needed to provide an efficient testing and maintenance service for these devices once they have been installed in the patient's body. The patient's life depends on the efficiency and the reliability of these devices. These in turn depend on the efficiency of the medical and technical organization for installation, maintenance, servicing and replacement of procedures within the patient's body when the need arises.

## The Paul Instrument Fund Awards

THE Paul Instrument Fund Committee has made grants as follows: £3,500 to Dr. J. E. Baldwin, assistant director of research in the Cavendish Laboratory, Cambridge, for the construction of a receiver for measuring power spectrum of a radio-frequency noise source using auto-correlation techniques in a large number of parallel channels. £7,800 to Dr. R. D. Davies, senior lecturer in radio astronomy, University of Manchester, for the construction of a spectrometer for radio astronomy using digital auto-correlation techniques with a sampling switch rate of 4 Mc/s, which it is hoped can be increased to 10 Mc/s and even to 100 Mc/s. £2,700 per annum for three years to Prof. J. D. McGee, professor of applied physics, Imperial College of Science and Technology, London, for the development of a photoelectronic image device for time resolution of rapidly changing optical images. £3,000 to Dr. D. W. Turner, lecturer in organic chemistry, Imperial College of Science and Technology, London, for the development of high-resolution electron spectrometry techniques, which are to be joined with the new molecular photoelectron spectroscopy approach to higher ionization potential determination: two spectrometers are envisaged, for the study of molecular *L* shells and *K* shell electrons, respectively. The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society, and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much-improved types of physical instruments and apparatus for investigations in pure or applied physical science".

## Nuffield Foundation Scholarships and Bursaries

THE Nuffield Foundation is offering a number of biological awards, scholarships and bursaries to scientists who wish to receive training in other fields of science. Until 1963, graduates in biology were not eligible for these awards, the purpose of which was to encourage graduates in the physical sciences to make a complete change by receiving training in biology. It was then decided that the conditions should be extended to assist graduates in biological subjects to study appropriate aspects of mathematics, physics or chemistry. The purpose of the enlarged scheme remains the same: to encourage progress in the many branches of botany and zoology, which is at present blocked for lack of workers who have an adequate grasp of the physical sciences, and to break away from the traditional descriptive form of teaching. In the case of candidates making the change to biology, awards are made for nothing less than a training in a fairly wide, selected field of biological subjects, and it must always be the candidate's intention to make his future career in biology. It is in the desire to

turn to biology and to apply in it the knowledge and methods of the physical sciences that success for the scheme and progress in biology will lie. This needs to be emphasized; experience has shown that graduates and even their sponsoring professors have sometimes failed to understand the purpose of these awards. For example, it is not uncommonly proposed that a graduate in organic chemistry should use his award to take a course in biochemistry and, at the same time, embark on some biochemical research. This would fall far short of the purpose of these awards. Recipients of awards have made their subsequent careers in teaching and in research in biological and medical departments of universities and in research institutes for biological subjects; two recipients found their way back into physical subjects. Subjects of recipients' first degree (total for scholars, first figure; for bursars, second figure) were: physics (5, 6); chemistry (4, 4); mathematics (2, 2); general sciences (1, -). Subjects studied under award were: physiology (7, 7); zoology (4, 2); botany and zoology, (2, 2); biophysics (-, 1). It is noteworthy that only once since 1952 was botany chosen for postgraduate study. Further information and application forms can be obtained from the Director, the Nuffield Foundation, Nuffield Lodge, Regent's Park, London, N.W.1.

#### Training Course in Taxidermy

THE Museums Association, through the generosity of the Carnegie United Kingdom Trust and in association with several of the major museums, is offering opportunities for, and financial assistance in, training in taxidermy to a limited number of individuals who intend to follow a career in the museum field. The training course will cover a period of three years and candidates should be between the ages of 18 and 25 years and have a sound general education. Forms of application and further information can be obtained from the Secretary, the Museums Association, 87 Charlotte Street, London, W.1. Completed applications should be submitted not later than February 15.

#### University News:

Hull

PROF. R. H. BARBACK has been appointed to the newly established second chair of economics in the Department of Economics and Commerce. The following appointments have also been announced: *Senior Lectureships*, Dr. J. Annett (psychology); G. Richardson (education); *Research Fellowship*, Dr. D. D. Singh (chemistry).

London

THE following titles have been conferred: professor of botany on Dr. Maud B. E. Godward, in respect of her post at Queen Mary College; professor of zoology on Mr. H. R. Hower, in respect of his post at the Imperial College of Science and Technology; professor of oral anatomy on Dr. H. J. J. Blackwood, in respect of his post at the Royal Dental Hospital. Dr. D. A. Mitchison has been appointed reader in bacteriology at the Postgraduate Medical School and Dr. D. A. Walker has been appointed reader in enzymology at the Imperial College of Science and Technology.

Manchester

THE following appointments have been made: *Reader-ships*, Dr. G. Allen (chemistry); Dr. C. N. W. Litting and Dr. G. R. Hoffmann (electrical engineering); D. R. Bland and Dr. S. Levine (mathematics); Dr. S. D. Silvey (statistics in the Department of Mathematics); Dr. G. M. Leak (metallurgy); Dr. A. Herzenberg (theoretical physics); Dr. E. L. Patterson (anatomy); Dr. A. H. Gowenlock (chemical pathology). *Senior Lectureships*, Dr. H. O. Pritchard (chemistry); Dr. F. H. Sumner (computer science); Dr. E. R. Bryan and F. Cheers (engineering); Dr. J. H. Gerrard (mechanics of fluids); Dr. D. St. P. Bunbury (physics); Dr. K. G. Cowling

(agricultural economics); Dr. D. Bulmer (anatomy); Dr. F. B. Beswick (experimental physiology); Dr. S. Thomas (physiology). *Lectureships*, Dr. C. A. Stace (botany); Dr. R. Bryant (chemistry); C. T. Elliott and J. M. Townsend (electrical engineering); P. J. Vermeulen, J. M. Davies, R. C. F. Dye, P. Mason, T. A. Henry, R. J. Aird and W. B. Wilkinson (engineering); Dr. J. E. Pollard (geology); G. Walker, Dr. W. A. Sutherland and Dr. C. E. M. Yates (mathematics); Dr. S. Blairs (metallurgy); G. G. Benson and N. A. Dickinson (pharmacy); Dr. J. W. Murphy and Dr. J. S. Dowker (theoretical physics); E. J. Daintree and Dr. R. J. Long (radio-astronomy); Dr. M. P. Hatton (chemical bacteriology).

#### Newcastle upon Tyne

DR. J. H. D. PRESCOTT has been appointed lecturer in animal production in the School of Agriculture as from December 1, 1964.

#### Reading

THE title of research professor has been conferred on Dr. S. K. Kon, deputy director of the National Institute for Research in Dairying and head of its Department of Nutrition, and on Dr. S. J. Folley, head of the Department of Physiology in the Institute.

#### Announcements

PROF. A. HOLMES has been awarded the Makdougall-Brisbane Prize for the period 1962-64, by the Council of the Royal Society of Edinburgh, in recognition of his contributions to the fundamental philosophy of the Earth sciences.

BRIGADIER R. C. A. EDGE has been appointed director-general of the Ordnance Survey, with the rank of major-general. Brigadier Edge will succeed Major-General A. H. Dowson, who is retiring in September.

DR. H. R. V. ARNSTEIN has been appointed committee secretary, and Prof. K. S. Dodgson meetings secretary, of the Biochemical Society, following the recent retirement of Dr. P. N. Campbell.

ON February 1, the Manchester Office of the Cement and Concrete Association moved into new premises at the following address: Cement and Concrete Association, Television House, 10 Mount Street, Manchester, 2.

VARIAN ASSOCIATES, LTD., have moved to a new address at Russell House, Molesey Road, Walton-on-Thames, Surrey.

AN ordinary meeting of the Scottish Section of the Society for Analytical Chemistry will be held in the University of Strathclyde on February 26. Dr. D. T. Lewis will present a paper entitled "Research Work at the Government Laboratory". Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

AN exhibition of the Dead Sea Scrolls, arranged by the Smithsonian Institution and sponsored by the Government of Jordan, will be held in the Institution during February 28-March 21. The exhibition will also visit Philadelphia (April 3-25), Berkeley (May 8-30), Los Angeles (June 12-July 5), Omaha (July 17-August 8), and Baltimore (August 21-September 19).

A JOINT meeting of the North of England Section of the Society for Analytical Chemistry and the Newcastle upon Tyne and North East Coast Section of the Royal Institute of Chemistry will be held in Newcastle upon Tyne on February 16. Mr. G. B. Crump will present a paper entitled "Thin-layer Chromatography". Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

## U.S. NATIONAL SCIENCE FOUNDATION SUPPORT FOR THE DISSEMINATION OF SCIENTIFIC INFORMATION

A PAMPHLET on the dissemination of scientific information, issued by the National Science Foundation\*, outlines the Foundation's programmes to discharge the responsibilities for interchange of scientific information and support of the dissemination of such knowledge placed on it by the Congress Act of 1950, and extended in 1958 by directives from the Congress and from the President. An Act of 1958 also established a Science Information Council to be an advisory group to the Foundation's Office of Science Information Service. The Foundation's programmes have two fundamental objectives: promotion of newer and better techniques for handling and disseminating scientific information, and making existing systems more effective. Its scientific information activities are carried out under five programmes organized in two Sections: the Studies and Support Section, which includes programmes of basic studies, improved processes and systems and support of communication; and the Science Information Co-ordination Section, which includes programmes of Federal science information and of the domestic and foreign science information.

Under the first section, support is provided for research development, experimental application and evaluation of systems of information retrieval, mechanical translations, libraries and publications, with the principal emphasis on improving basic understanding of general problems rather than on establishing particular systems. The Foundation continues to support long-range research on systems for the automatic processing of natural language text with the

eventual aim of mechanizing procedures for indexing, abstracting, organizing and storing information. Research in mechanical translation is a part of this broader field, while, in the work on publication systems, projects have been undertaken to analyse the part played by computers in scientific publication and to investigate machine recording of textual information during the publication of scientific periodicals.

As part of the Foundation's general programme for strengthening the science library network of the country, the information systems programme is seeking ways to improve the effectiveness of those libraries which provide substantial science information services. The publication support programme provides support for journals publishing results of original research, as well as temporary financial assistance for the cover-to-cover and selective translation of research published in Russian, Japanese and Chinese. Any publication considered favourably for support must be making, or showing good promise of making, a significant contribution to the scientific research literature; moreover, its proposed mechanics of publication must be efficient and economically sound.

The principal interests of the programmes for science information co-ordination are to promote non-Federal science information activities in the United States and to co-ordinate these with developments in foreign countries. Two major federal information centres are supported and administered. The first is the Science Information Exchange, which acts as a clearing house for information on current research, while the second is the National Referral Center for Science and Technology, located at the Library of Congress.

\* National Science Foundation. *Programs for Improving the Dissemination of Scientific Information*. (NSF-64-22.) Pp. 15. (Washington, D.C.: National Science Foundation, 1964.)

## RESEARCH ON SHELTER IN AGRICULTURE AND HORTICULTURE

THE effects of exposure to wind are of direct concern to agriculture, horticulture and forestry, and the practice of using shelterbelts and non-living windbreaks to reduce local levels of wind is of long standing. The broad picture of reduced windiness, altered microclimatic conditions and improved production of crops in the vicinity of windbreaks is generally established; the detailed analysis of these influences is, however, still incomplete. Research into the effects of shelter on microclimate and crop yields began in the early years of this century in a few countries, gained momentum in the United States and U.S.S.R. during the 1930's and, after 1945, attracted much wider interest and support. Early research was concentrated on determining the most suitable type of windbreak for a particular situation and providing a local measure of the benefit likely to be derived from shelter. More recently, it has become necessary for a more fundamental approach to the understanding of the nature and value of shelter: better definition of the physical environment near the ground arising as a result of shelter, and of the relationships between environment and biological processes in both crops and animals. Consequently, it is appreciated that further developments in the field of shelter research must be reviewed periodically in the light of developments in other environmental studies such as agronomy, ecology, micro-meteorology, plant and animal physiology and nutrition.

In order to provide for closer communication between research workers in the various fields relevant to the ques-

tion of shelter, the Ministry of Agriculture, Fisheries and Food, through its Shelter Research Planning Group (now Shelter Research Committee), arranged a first symposium on shelter research at Aberystwyth in 1962 (*Nature*, 194, 1130; 1962). The success of this venture led to a second symposium arranged in co-operation with the Department of Agriculture and Fisheries for Scotland and held at Edinburgh on September 15-16, 1964.

In the second symposium, sessions were again divided between horticultural and field crop aspects of shelter, meteorological developments associated with shelter, and shelter in relation to livestock. In a paper on wind speed and plant growth, Dr. R. M. Wadsworth (University of Reading) dealt with the effect of wind on plant environment and on plant growth as measured by such parameters as relative growth rate, net assimilation rate and leaf-area ratio. Laboratory experiments under controlled wind conditions have suggested that the optimum wind speed for plant growth varies according to other environmental conditions, being between 0.5 and 3 m/sec for very favourable conditions and lower for less favourable conditions. Since wind affects growth to a greater extent when plants are isolated, windbreaks may be more essential in the early life of a crop than later. Low light intensities may also suggest a greater need for wind reduction, whereas light winds are likely to prove increasingly beneficial as light intensity increases in order to prevent reduction in CO<sub>2</sub> availability.



Mr. W. H. Hogg (Meteorological Office, Bristol) reviewed recent horticultural investigations at Rosewarne and Stockbridge House Experimental Horticultural Stations. At Rosewarne, variations in the local climate between shelter hedges have been observed over a 3-year period. Although higher rates of wind speed and evaporation than at the control station were found with certain wind directions, air temperatures and soil temperatures at 2-, 4- and 8-in. depths were consistently higher than in the controls. At Stockbridge House changes in the degree of shelter in relation to the height growth of a young shelterbelt are being examined.

On the subject of shelter effect on crops, Prof. J. N. Black (Department of Forestry and Natural Resources, University of Edinburgh) pointed out that the Agricultural Research Council was financing a new research programme at Edinburgh, which it was hoped would reveal the role of wind in limiting crop production in the exposed arable and pastoral areas. Reviewing previous work in this field from the point of view of future investigations on crop aspects, Dr. J. M. Caborn (University of Edinburgh) suggested that it was necessary to explore the manner in which environmental changes near windbreaks are translated into crop growth and development. A suitable approach might be a detailed study of the heat and water balance of a sheltered area in conjunction with growth analysis of the protected crop.

In the livestock shelter session, papers were presented on present research projects at the Hannah Dairy Research Institute, the Hill Farming Research Organization and the Rowett Research Institute. Drs. K. L. Blaxter, J. P. Joyce and A. J. F. Webster (Hannah) discussed four aspects of their investigations of environmental stress in sheep and cattle: the effect of wind and rain on metabolism; radiation heat loads on sheep as related to incident radiation on a horizontal surface; the time taken by sheep to attain metabolic equilibrium on exposure to cold; and the derivation of a general equation for estimating the thermal demand of an environment. The effects of heavy rain and of wind are approximately similar so far as heat losses are concerned, and the immediate effect of heavy rain has been found to increase heat production by about 30 per cent, provided the ambient temperature is below the animal's critical temperature. The heat derived from total solar radiation at 55° North under clear

skies in mid-winter appears to be about a quarter of that produced by metabolism, whereas in mid-summer it is 10 times as great. Part of the cold stress in Scotland may stem from overcast conditions and consequent diminution of the heating effect of solar radiation. Heavily fleeced sheep also take a longer time than shorn animals to adjust to cold. It is encouraging to note that a rational formula has now been derived which enables heat losses of sheep to be predicted from fleece length, solar radiation, air temperature and wind velocity.

Turning to management aspects, Dr. J. M. Doney (Hill Farming Research Organization) felt that the response to climatic exposure, both physiological and in terms of economic production, was complicated by the nature of the annual cycle of nutrient intake and by the animal genotype involved. At levels of production provided by prevailing hill farm management systems, centred around the present well-adapted hill breeds, the provision of shelter could be quite uneconomic in terms of production increase; on the other hand, changes in management affecting the annual nutrient cycle might demand a change of breed structure and, hence, of the need for shelter. Dr. E. Cresswell (Rowett) reported that energy conservation, in the form of better gains and/or less food consumption, in fattening sheep provided with shelter had not been found so far in their studies. In the discussion which followed it was suggested that, although much of the research in this field so far has concentrated on shelter for the animal, more attention might be given to improving the herbage on hill land, and in this context shelter might be important.

Dr. R. W. Gloyne (Meteorological Office, Edinburgh) surveyed recent developments in instrumentation available for field experiments on shelter and the problems of analysis of data from long-term investigations of the influence of a series of shelterbelts on the climate of a previously exposed hill in North Wales.

In a closing address, the chairman of the Shelter Research Committee, Mr. R. G. A. Lofthouse, referred to the value of these meetings in co-ordinating research related directly or indirectly to shelter, in exchanging information and ideas and, particularly, in reviewing from time to time the present state of research and future needs.

J. M. CABORN

## WEIGHTS AND MEASURES TWELFTH GENERAL CONFERENCE

THE twelfth General Conference of Weights and Measures was held at the Centre International des Conférences in Paris during October 6-13, 1964, under the chairmanship of M. Poivilliers, President of the Paris Academy of Sciences, and was opened by M. Louis Joxe, Minister of State, deputizing for the Minister of Foreign Affairs of the French Republic. Delegations from 37 of the 40 States signatory to the Metric Convention took part in the conference, the United Kingdom being represented by the Director of the National Physical Laboratory and the Superintendent of the Standards Division, National Physical Laboratory. The business of the conference is best summarized by reference to the ten resolutions which were adopted; six were concerned with units and standards of measurement and the remainder with administrative and financial matters.

In the first category the most important was resolution 5 (standard of time-interval) which expressed the need to adopt an atomic or molecular frequency standard for accurate measurements of time-interval but stated that, despite the advances made with the caesium frequency standard, the moment had not yet come to discard the astronomical definition of the second\*. This was because

the hydrogen atom (and possibly the thallium atom) might furnish a standard more precise even than the caesium atom, which already provides a frequency standard reproducible in different laboratories to the order of 1 part in  $10^{11}$  (equivalent to 1 sec in 3,000 years). Nevertheless, as recognition of the use of atomic and molecular frequency standards in physical measurements of time could not be further delayed, the resolution authorized the International Committee of Weights and Measures to designate atomic or molecular standards for temporary usage for this purpose; it also invited those organizations and laboratories which are expert in this field to continue their investigations so that a final recommendation for an atomic or molecular definition of the second may be prepared in due course.

In the declaration made later by the International Committee the first standard to be so designated is the transition between the hyperfine energy levels  $F =$

\* The second, adopted by the International Committee of Weights and Measures in 1956 and ratified by the Eleventh General Conference in 1960, is equal to the fraction  $1/81\,558\,925\,974\,7$  of the tropical year for 1900, January 0 at 12 h ephemeris time. This ephemeris second is made available in practice with the aid of atomic clocks, but only retrospectively as an average value over several years, by means of observations of lunar position; it is reproducible at present to the order of 2 parts in  $10^9$ .

4,  $M = 0$  and  $F = 3$ ,  $M = 0$  of the fundamental state  $^2S_{1/2}$  of the atom of caesium-133 unperturbed by external fields; the value assigned to the frequency of this transition is 9 192 631 770 hertz (c/s) (ref. 1). The resolution represents a major step towards the adoption of an atomic constant as the universal basis of time measurement. Effectively it means that for all purposes in physics, and some in astronomy, the caesium transition is recognized as the present basic standard, but the resolution is so drafted that it will be easy to change from the caesium transition to the hydrogen transition if, for instance, the latter should prove in due course to be superior.

Resolution 6 (the litre) abolished the definition of the litre established in 1901 by the third General Conference, declared that the word 'litre' can be used as a special name given to the cubic decimetre and recommended that the name 'litre' shall not be used to express the results of volume measurements of high precision.

The litre was the name given to the cubic decimetre when the metric system was established during the last decade of the eighteenth century and it now reverts to its original meaning. The intention is that this litre shall only be used for ordinary transactions in trade and not for scientific purposes. The experimental evidence is that the litre, re-defined in 1901 as the volume occupied by 1-kg mass of water at its maximum density, is 28 parts in a million larger than the cubic decimetre (1 litre = 1.000 028 dm<sup>3</sup>). The litre of 1901 is, moreover, a unit which is non-coherent with the International System (SI) of Units (see British Standard 3763: 1964), in which the cubic metre, its multiples and sub-multiples, constitute the reference for volume measurements.

Resolution 7 defined the curie (symbol Ci) as having the value  $3.7 \times 10^{10}$  s<sup>-1</sup>. The curie, although non-coherent with SI units—the SI unit of activity is the reciprocal second (s<sup>-1</sup>)—has been recognized because of its universal use for practical measurements of the activity of radionuclides.

Resolution 8 (unit prefixes) added 'femto' and 'atto' to the list of recognized prefixes forming submultiples of the SI units. Both words are of Danish origin; femto represents the factor  $10^{-15}$ , symbol *f*, and atto the factor  $10^{-18}$ , symbol *a*.

Resolution 9 (gyromagnetic ratio of the proton) invited the national laboratories and international specialists in the determination of atomic constants to continue studies of the gyromagnetic ratio of the proton with the object of establishing the precise value of this constant for application to improving the reproducibility of the ampere, a basic SI unit.

One method of measuring the proton gyromagnetic ratio is to observe the frequency of free precession of protons (in water) in the calculable magnetic field of a coil carrying a known current. The interest thus lies in improving the method, establishing the value of the constant accurate to 1 part in a million or better, and then using the method to maintain the ampere. The ampere is realized absolutely in terms of the metre, the kilogramme and the second by weighing the forces exerted between current-carrying coils of measured dimensions and disposition; maintenance by this method is laborious and inconvenient, and in the best circumstances the reproducibility obtained in different laboratories is not better than a few parts in a million.

Resolution 10 (International Practical Scale of Temperature, IPST) directed attention to the pressing need to revise the IPST of 1948 (as amended in 1960) and to extend the scale below the present limit at the boiling-point of oxygen ( $-182.97^\circ$  C) down to the boiling-points of hydrogen and helium. It recognized that much work has yet to be done in order that the International Committee may recommend the adoption of a new definition of the scale at the next ordinary General Conference, expected to be convened in 1968. The resolution invited the national

laboratories and international specialists in this field to concentrate especially on the following projects: (1) gas thermometry throughout the whole range applicable to the method, including studies of the expansion coefficient of bulb materials especially at high temperatures; (2) measurements of black-body radiation between  $630^\circ$  C and  $1,063^\circ$  C; (3) formulation of a platinum resistance thermometer scale between  $630^\circ$  C and  $1,063^\circ$  C to replace the thermocouple scale specified in the existing scale; (4) verification of the table of values of 'reduced' resistance of platinum below  $0^\circ$  C, including a study of the calibration procedure for platinum resistance thermometers; and (5) new determinations of the boiling-point of oxygen.

Turning now to the administrative and financial resolutions, the first approved the actions taken by the International Committee to establish laboratories and a section at the Bureau International des Poids et Mesures (BIPM) for work on the basic metrology of the ionizing radiations<sup>a</sup>, and invited the committee to continue the work at the BIPM. Resolution 2 recognized the need to complete the constructional work on the new laboratories and to purchase the basic equipment still required for the scientific programme of the section. Provision was made to meet this need by granting a third special contribution of 850,000 gold francs (£100,000) to be paid to the BIPM by Governments in accordance with the same rules as apply to the repatriation of the annual subvention for maintenance of the BIPM.

Resolutions 3 and 4 (annual subvention to the BIPM) referred to the conference convocation document, issued to Governments by the International Committee in December 1963, which described the modern functions of the BIPM and gave an assessment of the basic monetary requirements for proper maintenance and development of its scientific activities. This document also announced that a proposal would be submitted by the International Committee to the General Conference for an increase of the total annual subvention from the present 900,000 gold francs to 1,750,000 gold francs (£204,000) for the 4-year period 1965–68. When the resolution containing this proposal was submitted for discussion at the conference, two delegations announced that their Governments could not agree to such an increase in the annual subvention on the case put forward by the International Committee. Eventually a compromise resolution (No. 4), proposing a graded increase in the annual subvention rising to 1,750,000 gold francs during 1967 and 1968, received qualified approval (16 votes in favour, 20 abstentions and no contrary vote). The Governments whose delegations abstained from voting were committed to deciding one way or the other by December 31, 1964. If only a single contrary vote is received, the resolution becomes void and an extraordinary General Conference must be convened as soon as possible to consider the finances of the BIPM, which temporarily finds itself in a most unsatisfactory and uncertain position for planning its future programme.

The International Committee, which is responsible for management of the BIPM and for the organization of the General Conference, held meetings during October 1–13. The Committee decided to establish a new advisory committee (comité consultatif) to deal with units of measurement. This step has become essential to ensure that the best advice is received on all questions relating to units of measurement from other specially interested organizations, which will be invited to appoint representatives on the committee. The main task is to consider the improvement and development of the SI units and to make recommendations for their extension to cover all fields of measurement of physical quantities. There are six other advisory committees dealing respectively with length (definition of the metre), time (definition of the second), electricity, temperature, photometry and standards of measurement for the ionizing radiations.

Under the auspices of the International Committee international journal of metrology, *Metrologia*, commenced quarterly publication by Springer in January 1965. The general editor is Dr. L. E. Howlett (National Research Council, Ottawa), chairman of the International

Committee, who is assisted by an international editorial board.  
H. BARRELL

<sup>1</sup> Markowitz, W., Glenn Hall, R., Essen, L., and Parry, J. V. L., *Phys. Rev. Lett.*, **1**, 105 (1958).

<sup>2</sup> Barrell, H., *Nature*, **189**, 195 (1961).

## NON-CUTTING USES OF INDUSTRIAL DIAMONDS

**M**OST industrial diamonds are used for cutting purposes where the objective is removal or displacement of relatively hard materials such as metal or rock. There are, however, other important applications of these diamonds which are not so well known, in particular 'non-cutting' uses. In this category are recognized mechanical and non-mechanical applications; the former include processes where the diamond is employed as an integral working part of a machine; the latter implies those instances where it is performing other independent functions. A recently issued publication entitled *Non-Cutting Uses of Industrial Diamonds*\* briefly describes and illustrates some of the more important non-cutting applications of these diamonds.

In the category of mechanical uses are styli, what are known as 'feeler' styli, diamond distance stops, gramophone styli and bearings. All these uses depend on the factor of extreme hardness of diamond and the facility with which it can be shaped and polished to a very high degree of accuracy and tolerance for the particular purpose in view, with the assurance of retention of shape and high surface finish for a far longer time than any other material. Feeler styli are employed as sensitive feelers in a wide variety of surface measuring instruments; they are ground

into cone shape and have an accurate radius polished on the tip; for example, high-resolution types of feeler styli have a tip radius of 0.00005 in. Diamond distance stops are used in distance pieces to ensure accuracy in lapping and honing operations in cases where the finished work-piece is to be of pre-determined thickness. With gramophone styli the diamonds are ground to cone shape and the tip polished to a radius varying according to the type of record to be produced or played; styli for microgroove- and stereo-records require far finer tips than those used to play the old standard 78-r.p.m. records.

As an example of their use as bearings, precision watch-making may be cited, where in such fine mechanisms diamonds replace natural or synthetic ruby, especially in cases demanding accuracy, long life and frictionless bearings. In the category of non-mechanical applications there are optical uses, electronic functions, and diamond-tipped indenters, such as are used on some hardness testing machines. In the optical field, the diamonds must be of gem quality, clear as optical glass. Electronic applications chiefly concern the use of semiconducting diamonds which have proved extremely efficient as sensitive detector heads in thermistor equipment; they can also be used as radiation counters; for this purpose only very small diamonds are used, and since they are chemically inert they make eminently suitable implant detectors in the living body, apparently without any harmful effects.

\* *Industrial Diamond Information Bureau. Non-Cutting Uses of Industrial Diamonds*. Pp. 12. (London: Industrial Diamond Information Bureau, 1964.)

## ANGULAR SIZES OF THE X-RAY SOURCES IN SCORPIO AND SAGITTARIUS

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AND

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**T**HE angular diameters of the X-ray source regions in the constellations Scorpio and Sagittarius respectively have been investigated in two rocket experiments conducted on August 28, 1964 (flight I), and October 24, 1964 (flight II), at the White Sands Missile Range. These sources were first observed by Giacconi *et al.*<sup>1</sup> as a single unresolved source of cosmic X-rays with wave-lengths around 3 Å and located near the galactic centre. Subsequent observations by Friedman *et al.*<sup>2</sup> and by Giacconi *et al.*<sup>3</sup> established the existence of two separate sources, in this part of the sky, one located near right ascension 16<sup>h</sup> 15<sup>m</sup>, declination - 15°, and the other near or coincident with the radio centre of the galaxy. In the present experiments we observed these two sources with a detector using a special collimator which has a high angular resolving power and at the same time a large field of view.

Flight I was launched at a sidereal time of 20 h 56 min, and flight II at a sidereal time of 20 h 20 min. In both flights, the rocket motion consisted of a rapid spin about

its long axis and a slow precession of the long axis around a yaw cone of small opening angle. The detectors themselves were banks of Geiger-Müller tubes with beryllium windows 0.002 in. thick. Each bank had a sensitive area of approximately 100 cm<sup>2</sup>. The collimators, which have been described elsewhere<sup>4</sup>, consist of two grids of parallel wires. Each wire is separated from the adjacent wires by slightly less than one wire diameter, and the two grids are mounted one in the back of the other and separated by a distance of 1.5 in. (as dictated by the available space in the instrument section of the rocket). Parallel radiation falling on the collimator casts a shadow of the wires forming the front grid on to the back grid. The transmission of the incident radiation passing through the back grid and into the detectors is determined by whether the shadow coincides with the back wires or with the openings between. As the apparatus is rotated around an axis parallel to the wires, the intensity transmitted by the collimator is modulated in a way that is determined by the dimensions of the wire grids and the rate of rotation. On the other

\* On leave from the Institute for Nuclear Study, University of Tokyo.

† On leave from the Institute of Physical and Chemical Research, Tokyo.



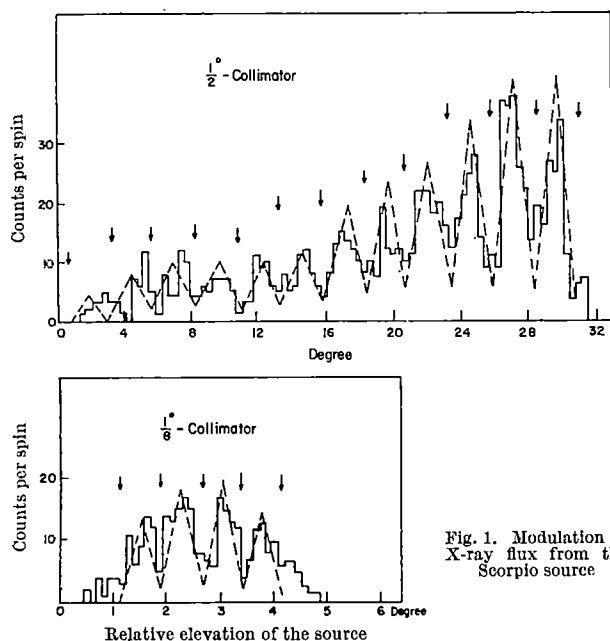


Fig. 1. Modulation of X-ray flux from the Scorpio source

hand, if the incident radiation is not parallel, as in the case of radiation from an extended source, then the shadow is diffuse, and the degree of modulation observed as the collimator is rotated is correspondingly reduced. It is apparent that the modulation is large or small depending on whether the angular size of the source is large or small compared with the effective angular resolution which is the angle subtended by a wire diameter at the distance of separation between the two grids in the collimator. In this experiment the rotation of the collimator was provided by the motion of the rocket and the angular resolutions of the two collimators were  $1/2^\circ$  and  $1/8^\circ$ , respectively, for flight I, and  $1/2^\circ$  for flight II.

The collimators for flight I were provided with baffles parallel to the spin axis of the rocket defining a field of view with a  $30^\circ$  full width and a triangular response. The wires of the collimator were tilted at an angle to the spin axis in such a way as to produce three modulation cycles as a source crossed the  $30^\circ$  field of view of the baffles. The orientation of the collimator was such that it measured the size of a source in the direction parallel to the spin axis of the rocket.

Because the Scorpio source provided only about five counts per spin and the rocket's motion was complicated by the precession of the spin axis, it was necessary to analyse the aspect of the rocket in detail before it was possible to combine data from each spin. Fig. 1 shows the superposition of about 200 sec of flight after corrections have been made for the shift in phase of the modulation pattern caused by the precession of the spin axis. It is clear that there is a modulation of the X-ray flux due to the

change of relative elevation of the Scorpio source with respect to the detector. The arrows indicate the expected positions of the minima in the modulation pattern as determined from the design parameters of the collimator. The dashed lines represent the expected modulation for a source of less than  $1/2^\circ$  and  $1/8^\circ$  angular diameter respectively, including the smearing effect of the background radiation and the partial transmission of the  $1/8^\circ$  collimator for a point source.

In flight II the collimator had an angular resolution of  $1/2^\circ$  and was provided with a narrower field of view with a  $15^\circ$  full width in order that the two source regions would be well separated. The wires this time were made perpendicular to the direction of the spin axis of the rocket. The modulation was thus expected to occur not as the result of the rotation of the rocket, but rather as the result of the variation in the elevation of the source with respect to the collimator axis caused by the precession of the rocket's spin axis. The source was seen 5 times during one yaw cycle. It is estimated that at least 90 per cent of the flux at the maximum is obscured at the minimum.

Similar analyses were made for the Sagittarius source region. The modulation is not so clear as in the case of the Scorpio source. A test analysis of artificial data generated by Monte Carlo simulation, with the isotropic background taken into consideration, showed that, in spite of the smearing effect of the background, a true modulation should have been clearly seen. The failure to observe a clear modulation, therefore, leads to the conclusion that the Sagittarius source is an extended source, or several sources distributed within a region of angular diameter larger than the resolution of the collimator.

We conclude from the foregoing analysis that the extension of the Scorpio source along the direction approximately parallel with the galactic plane is definitely less than  $1/2^\circ$  and probably smaller than  $1/8^\circ$ . Although we cannot rule out the possibility that the source extends in the direction perpendicular to the galactic plane, the fact that the modulation was seen throughout all phases of the yaw cycle while the axis of the rocket varied by  $25^\circ$  sets an upper limit of about  $1^\circ$  along the perpendicular direction to the galactic plane. We also conclude that the flux from Sagittarius does not all come from a single point source. Rather, it is spread over a region more than  $30$  min of arc in diameter and is either an extended source or is composed of more than a single source.

We are grateful to Prof. Bruno Rossi for many helpful discussions in connexion with this experiment.

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## GEOCHEMICAL CONSTANTS FOR RUBIDIUM AND STRONTIUM IN BASIC ROCKS

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WE have developed a new approach<sup>1-3</sup> for deriving some geochemical constants from variation of abundance of elements in silicate material. Factors essential to this theoretical treatment are initial concentration, partition coefficient, and liquid fraction value. For two or three

elements, if data are available on their abundances it is sometimes possible to deduce their initial abundances and their partition coefficients. Actually, this line of investigation led us to some success in a theoretical consideration<sup>2-4</sup> of the distribution of thorium, uranium, and lead within

the Earth. The present attempt is aimed at applying the same logic to the problem of abundance of rubidium and strontium in the Earth.

As shown elsewhere<sup>1-5</sup> there are two conjugate types of silicate materials within the Earth, namely, liquid and solid. (The genesis of the Earth's mantle is assumed to have proceeded in such a way that the concentration ratio of any minor element between solid and liquid phases in equilibrium was constant.) The following general formulae hold for primary liquid and solid types, respectively:

$$\log C = \log C^0 - (1 - k) \log f_w \quad (1)$$

$$\log C' = \log C^0 + \log k - (1 - k) \log f_w \quad (2)$$

where  $C^0$  is the initial concentration,  $C$  the concentration in liquid-type material with liquid fraction value of  $f_w$ ,  $C'$  the concentration in the corresponding solid-type material, and  $k$  the partition coefficient (which is equal to  $C'/C$  in the equilibrium system). Application of these general formulae to a combination of rubidium and strontium yields:

$$\log C_{Sr} = b \log C_{Rb} + \log C^0_{Sr} - b \log C^0_{Rb} \quad (3)$$

$$\log C'_{Sr} = b \log C'_{Rb} + \log (k_{Sr} C^0_{Sr}) - b \log (k_{Rb} C^0_{Rb}) \quad (4)$$

where:

$$b = (1 - k_{Sr}) / (1 - k_{Rb}) \quad (5)$$

Therefore, when we deal with the materials which separate perfectly into liquid and solid phases and when abundances of two elements, for example, rubidium and strontium, in such materials are plotted on a diagram of logarithmic scales, we gain two straight lines, 'liquid' and 'solid', running parallel to each other. If the separation of the phases is imperfect, some points may be found in the zone between these two lines. In addition, secondary partial melting or repetition of melting and crystallization may complicate or obscure the simple features which should otherwise be found in such a diagram.

With these in mind, let us direct our attention to a relation (see Fig. 1) of the abundances of rubidium and strontium as determined by Faure and Hurley<sup>6</sup> for basic rocks by the method of isotope dilution. Here we shall assume as a working hypothesis that Nos. 1, 2, 3 and 4 belong to a typical solid type, while the specimens such as Nos. 5, 13, 10, etc., are of a typical liquid type. (As to such deviations as seen for Nos. 11 and 12, an interpretation will be given later on.) To make the calculation easy, it is assumed that the solid-type line passes through No. 1, while the liquid-type line passes through No. 5. The value of the slope of such a correlating line is of fundamental significance to any estimate of basic constants, because the value of the slope corresponds to  $b$  as defined in equation (5). In Fig. 1 it appears that two slopes are possible; the slope of the line joining No. 1 and No. 3 is 0.735 and that of the line joining No. 5 and No. 7 is 0.865. Since it is believed that the true value of  $b$  is related to the range of these values of slope, or very closely associated with it, two series of theoretical calculation will be undertaken based on these two values. If  $b$  is taken to be 0.735 (Series I), the solid line passing through No. 1 can be expressed as:

$$\log C'_{Sr} = 0.735 \log C'_{Rb} + 1.871 \quad (6)$$

and the corresponding liquid line which passes No. 5 becomes:

$$\log C_{Sr} = 0.735 \log C_{Rb} + 1.564 \quad (7)$$

As evidenced by equations (3) and (4) and from equations (6) and (7):

$$1.871 - 1.564 = 0.307 = \log k_{Sr} - 0.735 \log k_{Rb} \quad (8)$$

$$b = 0.735 = (1 - k_{Sr}) / (1 - k_{Rb}) \quad (9)$$

Solving equations (8) and (9) diagrammatically gives values for  $k_{Rb}$  of 0.084 and  $k_{Sr}$  of 0.33. If a similar calculation is made using a value of 0.865 for  $b$  (Series II), we obtain values for  $k_{Rb}$  of 0.066 and  $k_{Sr}$  of 0.19.

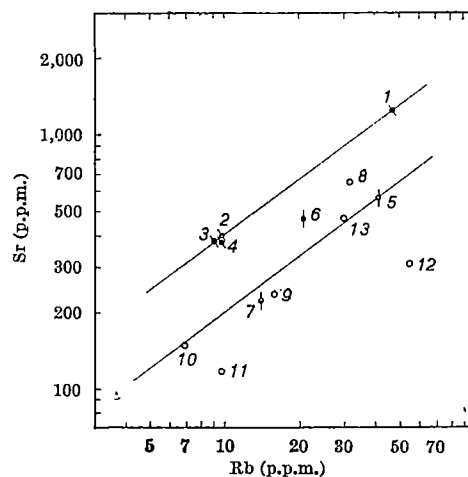


Fig. 1. The abundance relationship between strontium and rubidium in basic rocks. Nos. 1-4 are from Hawaiian Islands and Nos. 5-7 from Samoan Islands<sup>6</sup>. The slope drawn in this diagram is 0.735, that is, Series I.

Now let us estimate the initial abundances of rubidium and strontium. For this purpose, it is convenient to deal with the average abundances in basic rocks, because the liquid fraction value for the average basic rock has been estimated to be about 1/80 (ref. 2). As seen in Fig. 1, the point representing the abundances (rubidium 30 p.p.m., and strontium 465 p.p.m.) given by Turekian and Wedepohl<sup>7</sup> for the average basic rock (No. 13) falls almost on the liquid-type line. In this article we shall use abundance values for rubidium of 31 p.p.m. and for strontium of 460 p.p.m. for the average basic rock, for the corresponding point falls just on the liquid line of Series I, even though this modification of abundances has a very slight effect on a result of the calculation. For a set of partition coefficients for  $k_{Rb}$  of 0.084 and  $k_{Sr}$  of 0.33 according to  $b = 0.735$ , we gain the equations (cf. equation (1)):

$$\log 31 = \log C^0_{Rb} - (1 - 0.084) \log (1/80) \quad (10)$$

$$\log 460 = \log C^0_{Sr} - (1 - 0.33) \log (1/80) \quad (11)$$

From these equations, we obtain values for  $C^0_{Rb}$  of 0.56 p.p.m. and  $C^0_{Sr}$  of 24 p.p.m., respectively. For Series II, values for  $C^0_{Rb}$  of 0.51 p.p.m. and  $C^0_{Sr}$  of 13 p.p.m. are obtained. (The corresponding values of  $\bar{R}$  are shown in Table 1, where  $\bar{R}$  refers to the abundance ratio of average basic rock to initial matter.) It is of considerable interest to compare these resulting values with the abundances in chondrites. The chondritic abundance of rubidium is known to be 3 p.p.m. (ref. 8). This suggests that the effective initial concentration of rubidium for the Earth's 'oxide sphere' was much less than for the chondrite. This result is also in good agreement with the conclusion gained from a combined treatment<sup>9</sup> of strontium isotopes and lanthanides. Although Gast<sup>10</sup> did not take into account the partition coefficient and hence his deduction is not quite exact, he first pointed out the possibility of depletion of rubidium relative to strontium in the Earth when making comparisons with chondrites. As to strontium, on the other hand, its initial concentration derived from Series II is almost equal to the chondritic concentration (11 p.p.m.) determined by Pinson *et al.*<sup>11</sup>, while the corresponding value from Series I is almost equal to the concentrations of strontium in meteorites adopted by Goldschmidt<sup>12</sup>, Urey<sup>13</sup>, and Levin *et al.*<sup>14</sup>. These authors

Table 1. PARTITION COEFFICIENT, INITIAL CONCENTRATION, AND THE ENRICHMENT FACTOR FOR AVERAGE BASIC ROCK

	Series I		Series II	
	Rb	Sr	Rb	Sr
$k$	0.084	0.33	0.066	0.19
$C^0$ (p.p.m.)	0.56	24.4	0.51	13.2
$\bar{R}$	55.3	18.8	60.8	34.8

adopted, respectively, 20, 23 and 22 p.p.m. (As the chondrite might contain on an average 16.8 per cent non-oxide components<sup>18</sup>, the effective concentration of oxyphile element in the oxide phase of the chondrite is considered to be 1.20 times the apparent concentration in the whole chondrite.) So far as such a comparison is concerned, it is difficult to decide which value of initial strontium concentration is preferable or which value of  $b$  is appropriate. For reasons which will be mentioned later, however, the value of Series I seems to be advantageous—not decisively but slightly.

It was fortunate that the rocks analysed by Faure and Hurley for rubidium and strontium involved the eclogite (No. 10) from the Robert Victor Mine, South Africa, for the concentrations of lanthanides in this rock were determined by Schmitt *et al.*<sup>18</sup>, and the liquid fraction value for it has been estimated by us from the analysis of the lanthanide pattern. According to our estimation<sup>17</sup>,  $\log f_w$  for this eclogite has a value of  $-1.204$ . (Note that the value of liquid fraction is a value assigned to the rock, and not dependent on the species of chemical elements contained therein.) Since the initial concentration and partition coefficient necessary for equation (1) have already been obtained for each series of calculation, we can evaluate the liquid fraction value for the eclogite in question by introducing the observed abundances (rubidium 6.90 p.p.m., and strontium 149 p.p.m., ref. 6) into equation (1). Table 2 shows the result of the calculation. (Disagreement between the values obtained from rubidium and strontium is due to the fact that the point representing the observed abundances does not fall strictly on the representative liquid-type line passing No. 5.) It is seen in Table 2 that Series I gives the liquid fraction value closer to that obtained from lanthanides than Series II. Hence it is thought that  $b = 0.735$  (Series I) is closer to the true value than  $b = 0.865$  (Series II).

It is of interest to plot the obtained value of  $\bar{R}$  in a diagram<sup>15</sup> where the abundance ratio of average basic rock to average oxide phase of chondrite has been plotted against the reciprocal of the ionic radius (see

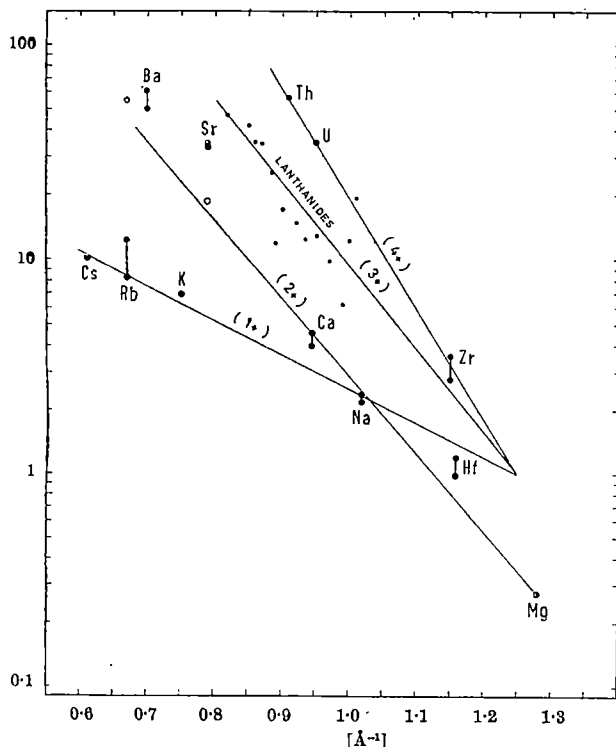


Fig. 2. Enrichment factor (ordinate) between average basic rock and average oxide phase of chondrite as a function of reciprocal (abscissa) of ionic radius<sup>15,16</sup>. However, the abundances of lanthanides are for shales analysed by Minami<sup>18</sup>.

Table 2. LOGARITHM OF LIQUID FRACTION VALUE FOR ECOLOGITE, ROBERT VICTOR MINE, SOUTH AFRICA

Series I		Series II	
Rb	Sr	Rb	Sr
-1.180	-1.185	-1.207	-1.314

(The corresponding value from lanthanides is  $-1.204$ )

Fig. 2). (In this diagram the points for  $\bar{R}$  of Series I are shown as open circles.) It can be seen in Fig. 1 that the deviation of an open circle for strontium from the extrapolation of the join calcium-magnesium is small in comparison with the solid circle. This may also suggest that the value for  $C_{Sr}^0$  of 24 p.p.m. (Series I) is preferable to  $C_{Sr}^0$  of 13 p.p.m. (Series II). Meanwhile, it is a remarkable fact that the value of  $\bar{R}$  obtained here for rubidium falls close to the main trend of the polyvalent elements. It is felt that the calculated value of  $\bar{R}$  for rubidium reflects the actual effect, and the alkali metals are thought to be exceptional in that their abundances in chondrites are not necessarily equal to their initial effective concentrations in relation to the process of formation of Earth's mantle, as suggested by Gast<sup>10</sup>.

According to the data presented by Faure and Hurley<sup>6</sup>, the rubidium/strontium ratios calculated from  $^{87}\text{Sr}/^{86}\text{Sr}$  are in accordance with the directly measured rubidium/strontium ratios (see Table 3). However, the discordance is exceptionally large for basic rocks from the Mid-Atlantic Ridge and Columbia River, Oregon. However, this can be explained by assuming that these two basalts were produced by partial melting of primary liquid-type material. It should be noted that, while the rubidium/strontium ratio in the product of partial melting differs from that in the parent material, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio reflecting the rubidium/strontium ratio in the parent material is passed on to the product of partial melting. (Actually, a similar case was found with respect to Th/U ratio and to  $^{206}\text{Pb}/^{207}\text{Pb}$  ratio of alkali rock, Japan<sup>19</sup>.) Since the partition coefficients for rubidium and strontium have been evaluated here, we can estimate the rubidium/strontium ratio pertaining to the source material for the product of partial melting. In Fig. 3, a conjugate relation between solid and liquid in equilibrium is shown by the broken line; the slope of a conjugate line must be equal to  $\log k_{Sr}/\log k_{Rb}$ . This line also indicates the contemporaneous or commensal relationship for hybrids of conjugate solid and liquid. (The chain line in Fig. 3 indicates the liquid line relative to the primary liquid-type material.) Thus, the parent material for the basalt boulder from the Mid-Atlantic Ridge (No. 11) is estimated to correspond to  $P$ , while that for the basalt from Columbia River (No. 12) is estimated to be  $Q$ . According to Series I, the rubidium/strontium ratios for  $P$  and  $Q$  are 0.030 and 0.036, respectively. If the same correction is made for a rather small deviation of Deccan Plateau basalt (No. 9), we obtain 0.046 as the theoretically corrected value of rubidium/strontium ratio. Similar estimations can be made for Series II. In Table 4 the estimated ratios for parent materials are listed. It can be seen that the agreement between the isotopically estimated ratio and the theoretically corrected one is good. The agreement is especially

Table 3. COMPARISON OF ISOTOPICALLY CALCULATED Rb/Sr RATIO (A) OF THE SOURCE MATERIAL WITH MEASURED Rb/Sr RATIO (B) OF BASALT

Locality	A	B	A/B
Hawaiian Islands (Nos. 1-4)	0.034	0.0240 ~ 0.0375	0.71 ~ 1.10
Samoa Islands (Nos. 5-7)	0.038	0.0445 ~ 0.0733	1.17 ~ 1.93
Ascension Island (No. 8)	0.035	0.0496	1.42
Mid-Atlantic Ridge (No. 11)	0.030	0.0824	2.75
Deccan, India (No. 9)	0.043	0.0885	1.59
Columbia River, Oregon (No. 12)	0.032	0.176	5.50

After Faure and Hurley<sup>6</sup>.

Table 4. COMPARISON OF ISOTOPICALLY CALCULATED Rb/Sr RATIO (A) WITH THEORETICALLY CORRECTED Rb/Sr RATIO (C)

Specimen*	A	C	C/A	Ser. I	Ser. II
No. 11	0.030	0.0300	1.00	1.59	1.59
No. 12	0.032	0.0360	1.12	1.45	1.45
No. 9	0.043	0.0465	1.08	1.44	1.44

\* Compare with Table 3.



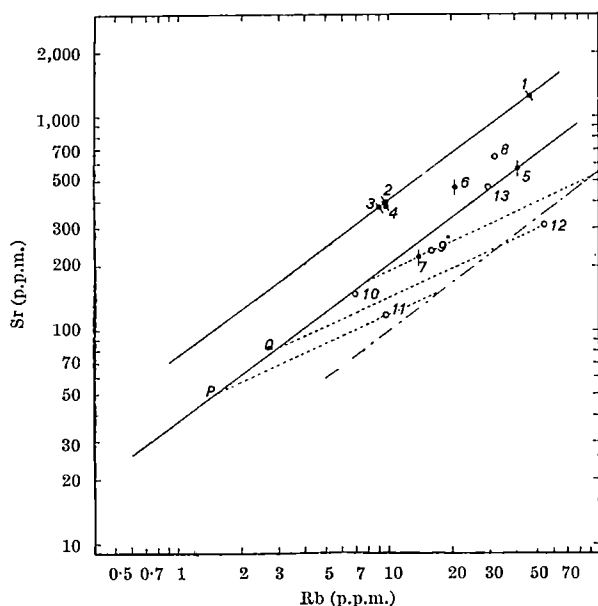


Fig. 3. The strontium versus rubidium abundance relation, with conjugate or commensal relationship pertinent to Series I

good for the result of Series I. Thus, in this respect, Series I based on  $b = 0.735$  also gains a slight advantage over Series II (based on  $b = 0.865$ ).

The small difference between calculated and measured (or corrected) rubidium/strontium ratios may be attributed in part to an uncertainty of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio for the stage of rise of heterogeneity in the Earth. The depletion of rubidium might have occurred in the Earth's primitive material prior to its main differentiation. If so, it is likely that the primeval  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio for the Earth was somewhat lower than for chondrites.

According to the foregoing correction, the corrected abundances (Series I) of rubidium and strontium for Deccan Plateau basalt are 7.9 and 170 p.p.m., respectively. A calculation of liquid fraction value for these abundances results in  $\log f_w$  equal to  $-1.256$ . On the other hand, the previous calculation<sup>4</sup> relative to isotopic composition of lead indicated that the logarithm of liquid fraction value for the source material of lead in ores averages  $-1.233$ . The mutual closeness of these values is noteworthy, because the common lead of ores is deduced as having originated from continental basalts and because the Deccan Plateau basalt is a representative continental basalt. Conformity between the results from three independent approaches (rubidium-strontium, lanthanides, and lead-uranium-thorium), though starting from one and the same line of thought, can be regarded as a corroboration of our theory.

I thank Dr. Yoshito Matsui for his advice.

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## MEASUREMENTS OF NOISE EMISSION FROM MERCURY BALLS

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THIS article describes a procedure and shows the results of radio frequency noise emission measurements from two kinds of mercury balls.

A mercury ball<sup>1</sup> is a device consisting of a glass sphere approximately 3 in. in diameter containing a small amount (about 5 cm<sup>3</sup>) of mercury and a gas under low pressure. Of the two balls described, one contained dry air and the other neon. When the neon ball is shaken in the dark it exhibits the characteristic glow of an ionized neon discharge confined to the vicinity of the mercury globule. Simultaneously there is an emission of radio frequency energy which is measurable up through the tens of megacycles when using sensitive equipment in a radio frequency quiet environment.

It is reported<sup>2</sup> that the mercury ball produces electromagnetic oscillations with remarkable regularity at a frequency of 3.4 kc/s. The emitted work was found by Von Halla and Nowotny to be  $3 \times 10^{-11}$  W when the ball was shaken with a terminal velocity of 50 cm/sec.

Inasmuch as the noise emission from mercury balls is relatively small in amplitude, it is desirable to undertake the measurements in an environment as free from extraneous noise as possible. This environment was provided by a 15 ft  $\times$  30 ft. double-shielded room which has an attenuation to electric fields in excess of 120 dB over the frequency range of interest.

Fig. 1 shows a close-up view of the mercury ball mounting arrangement. The ball is attached to a flexible phenolic strip which is clamped in a vice. The phenolic strip has adjustable-length aluminium stiffeners which limit the amplitude of excursion the ball may attain. Compressed air is supplied through the hose and nozzle-valve to a vibrating flapper which amplitude modulates the air column striking the phenolic strip holding the ball. Once the flapper is set in motion it continues to vibrate at a self-adjusted rate dependent on such parameters as air velocity, flapper mass, its lever arm and stiffness. In this particular arrangement it was possible to obtain approximately 0.5 in peak-to-peak ball excursions at a rate of 5–10 c/s. The entire assembly shown in Fig. 1 was placed on a copper-topped work-bench oriented lengthwise in the shielded room.

Fig. 2 shows the placement of the low-frequency noise meter and antenna. The rod antenna is located 3 ft. directly in front of the mercury ball. The noise meter is a Stoddard NM10A which covers the frequency range 14–250 kc/s. This meter measures peak noise values (time constant of detector approximately 600 msec) and is calibrated in terms of dB above 1  $\mu$ V. Ground bonding is accomplished only at the noise meter to avoid multiple loops. This is visible in Fig. 2 at the left side of the meter.

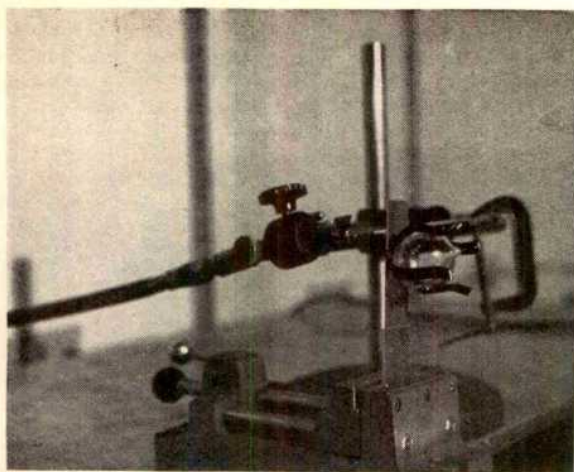


Fig. 1. Mercury ball mounting

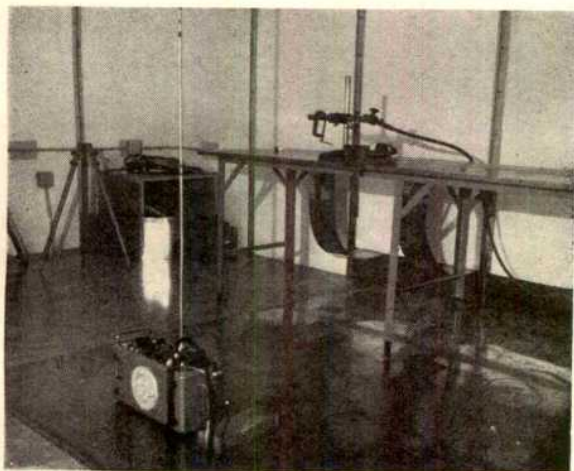


Fig. 2. Low-frequency test apparatus

In Fig. 3 the set-up for higher frequency noise measurements is shown. Here the equipment consists of an Empire Devices *NF105* noise meter and a 41-in. vertical antenna. The range of this instrument with the antenna shown is 150 kc/s–30 mc/s. This meter also measures peak noise values and is calibrated by means of a built-in impulse noise generator to read dB above 1  $\mu$ V per megacycle of receiver band-width. The 41-in. vertical antenna with its matching transformer is mounted on a tripod which is attached with a wide copper strap to the front edge of the test bench. This strap provides the only ground point for the measuring instrument. The antenna-to-ball distance was 36 in. at the lower frequencies and 12 in. at the higher frequencies. During the actual measurements the cart containing the noise meter was located some distance behind the set-up to minimize operator interaction.

The test procedure consisted of scanning the frequency spectrum, beginning at 14 kc/s, and recording the noise intensity at a minimum of three points per octave. A variable time was spent at each frequency to record the maximum pulse, since pulse height was not uniform.

The *NM10A* was placed in the 'Peak' detector function with a short charge-long discharge time constant and the operator used the aural slide-back method of matching the unknown pulse amplitude. With this procedure, the readings are in terms of  $\mu$ V (or dB above 1  $\mu$ V) existing at the antenna terminals. The reading may be expressed in terms of band-width by multiplying by the instrument's 'impulse factor' which accounts for the impulse

band-width of the *NM10A* receiver. Expressing the readings in terms of band-width facilitates instrument comparison and allows the data to be plotted as though only one instrument was used.

The *NF105* was also operated in the 'Peak' detector function and in this case the operator chose the visual substitution method of matching the unknown pulse amplitude. In this method the output from a built-in impulse generator is adjusted to match the unknown pulse. The impulse generator was previously calibrated in terms of dB above 1  $\mu$ V/Mc/s instrument band-width.

Since the impulse noise band-width of the *NF105* is automatically taken into account when the impulse generator is used, the readings are in terms of dB above 1  $\mu$ V/Mc/s at the input terminals of the measuring instrument.

A knowledge of the transfer characteristics of the antennae will allow the input voltage to be specified in terms of field intensity,  $\mu$ V/mc/s. The particular transfer characteristic of interest is known as 'effective height'. The determination of a given antenna's effective height while located inside a shielded enclosure and near large metallic objects is difficult and subject to errors of unknown magnitude. In addition, all the measurements took place while in the 'near' field, which increases the error in specifying the results in terms of 'far' field units. Generally, the procedure consists of producing an electromagnetic field of known intensity which is uniform throughout an area containing the unknown antenna. By measuring the open circuit voltage induced in the unknown antenna, the effective height is found from the relation:

$$h = \frac{V}{E} \quad (1)$$

$E$  = electric field (V/m),  $V$  = induced voltage,  $h$  = effective height (metres).

If one ignores the implications of being in the near field, a satisfactory solution for the determination of effective height can be obtained through the following considerations<sup>3,4</sup>:

$$R_{in} = 2.5 (\beta L)^2 \quad (2)$$

With:

$$\beta = \frac{2\pi}{\lambda} \text{ and } L = 2l$$

where  $R_{in}$  is input resistance of a short vertical antenna in ohms,  $\lambda$  is wave-length in meters,  $l$  is physical length in meters:

$$R_{in} = \frac{40\pi^2 l^2}{\lambda^2} \quad (3)$$

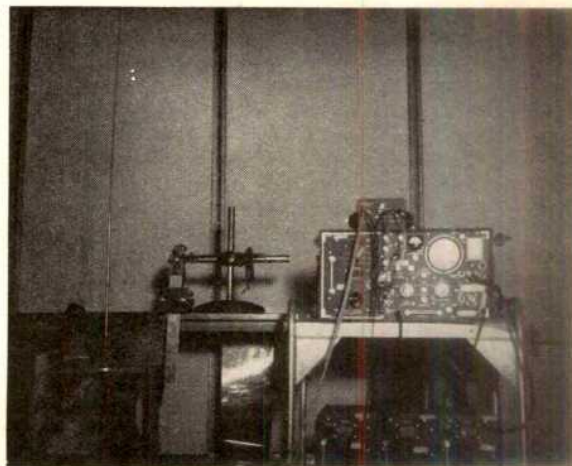


Fig. 3. High-frequency test apparatus

for a short vertical antenna above a perfect ground plane. Further:

$$h = \frac{\lambda \sqrt{R_{in}}}{\pi \sqrt{160}} \quad (4)$$

and, on combining, one finds:

$$h = \frac{l}{2} \quad (5)$$

The antenna used with the NM10A was 2.2 m long, making its effective height equal to unity, for practical purposes. Readings obtained when using this antenna can be expressed directly in  $\mu\text{V/m}$  (ref. 5).

The situation, however, is not as clear-cut in the case of the NF 105. The VA 105 antenna has an effective height of 0.5 meter when used with an adequate ground plane. In addition, the antenna contains a matching network which transforms the antenna impedance to 50 ohms. Thus, assuming the match is perfect, the antenna factor would amount to 27 dB (6 dB for effective height + 6 dB for open circuit voltage conversion + 15 dB for transformation ratio). Therefore, 27 dB has been added to the data in order to convert the readings into field intensity.

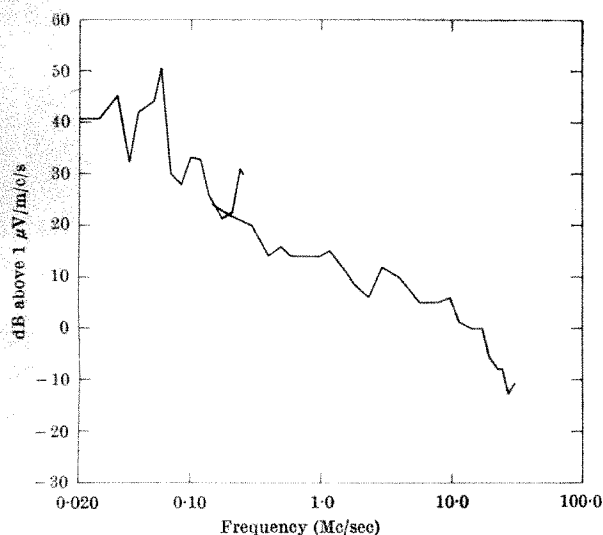


Fig. 4. Neon ball test results

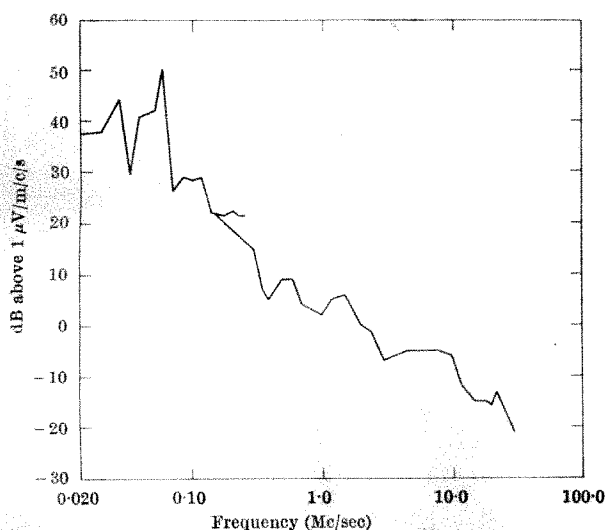


Fig. 5. Air ball test results

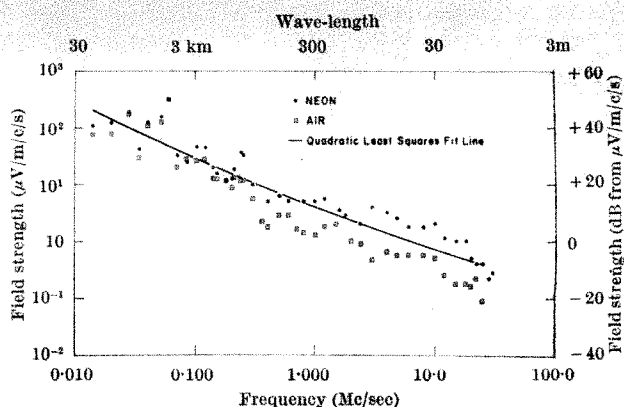


Fig. 6. Joint data results

The results for the mercury ball containing neon are shown in Fig. 4 and those of the mercury ball containing air are shown in Fig. 5. It can be seen that the noise values are not particularly large and have descended into instrument ambient levels near 25 Mc/s. There was no evidence of noise beyond 30 Mc/s from either ball, even though the neon ball had not quite reached instrument ambient.

In order to facilitate analysis of the results, it was decided to fit a smooth curve to the calibrated data. Least-squares logarithmic fit lines were calculated with a CDC 3600 computer using a programme from the NCAR computer-programme library. Linear, quadratic, and cubic fits were called for. Using the logarithmic residuals as criteria of goodness of fit, it was decided that the quadratic curve was the best fit. The cubic fit was only slightly better than the quadratic. The data and the quadratic curve for the averaged data are shown in Fig. 6.

The data were submitted to a non-parametric approximate correlation test similar to the more common sign test<sup>6</sup>. The data were divided into four sections at the points where changes in measuring equipment were made. The first two sections contained three-quarters of the data points. The point-to-point variations in the neon measurements were compared with those in the air measurements. The third and fourth sections were not tested individually since they contained so few points each. No correlation was found in the first or the second sections, but a correlation was found in the first two sections combined. Of the four possible combinations of three sections taken together, correlations were found in the combinations of the first, second and third sections and of the first, second and fourth sections. The other combinations contained either sections one or two, but not both, and no correlations were found in these combinations. All the measurement variations were found to be correlated.

In addition to any sectional or combinational correlation, there is a downward trend in the data, so that one might expect that, as more sections are brought into analysis, a better correlation would be found. It cannot definitely be concluded from this analysis that the measurements are or are not random ones superimposed on a downward trending noise-strength versus frequency line. The variations may be artefacts introduced by the measuring equipment, or they may be real variations in the nature of emission spectrum lines. The resolution of the experimental equipment did not permit a definite decision on this point. The correlation test, however, would appear to indicate that the measurement variations are random ones scattered about a downward trending line.

The balls do not have identical frequency dependencies. This can be attributed to differences in physical construction such as amount of mercury, gas pressure, etc. The variation of noise amplitude with ball parameters is



not known; it forms the basis of investigations which are being continued.

In general, mercury balls, whether containing neon or air, emit electromagnetic radiation of a broadband nature the intensity of which is an inverse function of frequency.

Much of this work was performed while one of us (G. H. S.) was employed by Colorado Research Division of ITT, Broomfield, Colorado.

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## RELATIONSHIP BETWEEN GLUTATHIONE STABILITY AND *IN VIVO* SURVIVAL OF AUTOLOGOUS, DEGLYCEROLIZED, RESUSPENDED RED BLOOD CELLS

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FELGER<sup>1</sup> has reported that marked increase in spontaneous haemolysis occurred *in vitro* when reduced glutathione was 40 per cent or less of its initial value. Benesch *et al.*<sup>2</sup> have shown a relationship between erythrocyte integrity and sulphhydryl groups, in that haemolysis occurs on incubation of red blood cells with various mercurial compounds. Jacob *et al.*<sup>3,4</sup> have reported that the loss of viability of erythrocytes is related to the inhibition of membrane sulphhydryl groups and is unrelated to intracellular sulphhydryl levels *per se* or the overall rate of cellular glycolysis.

Beutler and co-workers<sup>5,6</sup> have reported that erythrocytes susceptible to drug-induced haemolysis *in vivo* are deficient in reduced glutathione (GSH), and manifest glutathione instability as demonstrated by a marked decrease in content of glutathione after incubation with acetylphenylhydrazine. These abnormalities of glutathione metabolism have been attributed to the reduction of glucose-6-phosphate dehydrogenase activity as demonstrated by Carson *et al.*<sup>7</sup>

This paper reports on the relationship of reduced glutathione level, glutathione stability, glucose-6-phosphate dehydrogenase level, and MCV to 24-h and 7-day chromium survival of autologous deglycerolized resuspended erythrocytes.

Blood (approximately 450 ml.) was collected in plastic bags containing acid citrate dextrose (ACD; NIH A) solution, from 28 white and two negro healthy male donors, ages 18–35. The samples were glycerolized and frozen on the day of collection or after 1–12 days of storage at 4° C. The red blood cell mass and the plasma of each sample were separated and the intracellular preservative, glycerol, was added to the red blood cells by the closed, continuous, sterile centrifugation process using the Cohn fractionator. This process has been reported by Haynes *et al.*<sup>8</sup> The glycerolized red blood cells were frozen slowly to –80° C and stored for 1–2 months. On the day of reconstitution, the preserved red blood mass of each blood sample was thawed for approximately 15–20 min in a water bath maintained at 37° C, and deglycerolized. Sixteen of them were resuspended in autologous plasma, and 14 were resuspended in an artificial 5 per cent albumin resuspension medium (Hyland Laboratories, Los Angeles, California) which had been stored at –20° C. The reconstituted blood was stored at 4° C for 1–17 days prior to transfusion or washing. Five lots resuspended in autologous plasma and four resuspended in the 5 per cent albumin medium were washed in the Cohn fractionator after a period of storage at 4° C (3–13 days) with 2,000 ml. of buffered isotonic saline (pH 7.2). The washed red blood cells were resuspended in the 5 per cent albumin resuspension medium and stored for either 2 h or 24 h at 4° C before transfusion. All transfusions were autologous. On

the day of transfusion, a 40–50 c.c. aliquot of each unit was transferred aseptically to a transfer pack and was incubated for 20 min at 37° C with 50–60  $\mu$ c. of radioactive disodium chromate (Squibb 'Chromotope'; Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub>).

The recipient's red blood cell volume was estimated by measuring plasma volume (determined with Evans blue) and the total body haematocrit (peripheral venous haematocrit multiplied by 0.93). The red cell survival of 10 ml. of the labelled, preserved, reconstituted blood was measured. The 100 per cent survival and the apparent survival were calculated by applying Strumia's formulae<sup>9</sup>.

100 per cent survival was calculated as the total activity of the red blood cells injected (c.p.m.) divided by the total red cell volume (ml.). Apparent percentage survival was calculated as the c.p.m./ml. red blood cells in the peripheral blood divided by the 100 per cent survival value (c.p.m./ml. red blood cells) multiplied by 100.

24-h and 7-day survival values are reported. Intravascular liberation of haemoglobin associated with the removal of non-viable infused erythrocytes was evaluated by measuring plasma radioactivity 10 min after the transfusion to detect radioactivity in excess of the level estimated from the amount of radioactivity in the resuspension medium infused into the recipient's plasma volume<sup>10</sup>.

Blood from the remainder of each sample was used for the following determinations: reduced glutathione level, by Beutler's modified method using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)<sup>11</sup>; glutathione stability as described by Beutler<sup>6</sup> with incubation of blood at 37° for 2 h with 10 mg of acetylphenylhydrazine; glucose-6-phosphate dehydrogenase level as described by Zinkham<sup>12,13</sup>; the MCV determined by micro-haematocrits<sup>14</sup> and red blood cell counts using the Coulter counter; and the total haemoglobin measured by the cyanmethaemoglobin method<sup>15</sup>.

All samples of blood had normal glucose-6-phosphate dehydrogenase levels (mean 6.7 units/min/g Hgb, *S.D.*  $\pm$  1.5, range 4.7–12.3), reduced glutathione levels (mean 2.3 mg/g Hgb, *S.D.*  $\pm$  0.30, range 1.9–3.3), glutathione stabilities (mean 68 per cent, *S.D.*  $\pm$  10.0, range 55–85 per cent) and MCV's (mean 101  $\mu$ <sup>3</sup>, *S.D.*  $\pm$  8.0, range 83–113) on the day of collection.

Table 1 shows the MCV, reduced glutathione level, glucose-6-phosphate dehydrogenase level, and glutathione stability (all measured on the day of transfusion) and 24-h and 7-day survival of deglycerolized red blood cells resuspended initially in 5 per cent albumin medium. The length of storage of whole blood in ACD at 4° C before glycerolization, the length of storage after resuspension at 4° C prior to transfusion or washing, and the length of storage at 4° C after the washing, are shown. Reduced glutathione level, glucose-6-phosphate dehydrogenase level, and MCV were not related to the *in vivo* survival of the preserved erythrocytes.

Table 1. DEGLYCEROLIZED ERYTHROCYTES RESUSPENDED INITIALLY IN 5 PER CENT HYLAND ALBUMIN MEDIUM, EVALUATED ON DAY OF TRANSFUSION

Sample	Age when glycerolized (days)	Days stored at 4° C after reconstitution	Period between wash and transfusion	MCV ( $\mu^2$ )	G-6-P d-ase. activity (units/min/g Hgb)	GSH (mg/g Hgb)	GSH stability (%)	Chromium survival	
								24-h	7-day
1	12	1	Not washed	—	3.3	2.6	65	69	52
2	5	1	—	93	3.7	3.0	69	87	72
3	7	1	—	106	2.9	2.4	82	78	68
4	5	1	—	95	4.4	2.5	62	85	68
5	4	2	—	101	4.6	2.2	69	81	67
6	0	3	—	94	4.3	2.4	54	80	67
7	2	5	—	92	4.1	2.2	49	85	—
8	3	7	—	102	4.6	2.0	62	84	67
9	1	8	—	101	3.2	2.1	44	78	63
10	0	17	—	102	4.3	2.8	25	62	50
11	0	7	2 h	110	5.2	2.1	45	66	50
12	1	8	2 h	96	3.7	2.5	54	80	62
13	0	12	24 h	79	3.1	1.9	0	44*	33
14	0	13	24 h	105	4.4	2.2	0	59*	44
			Mean	98	4.0	2.4			
			S.D.	$\pm 7.9$	$\pm 0.7$	$\pm 0.3$			
			Range	79-110	2.9-5.2	1.9-3.0			

\* *In vivo* haemolysis.

Table 2. DEGLYCEROLIZED ERYTHROCYTES RESUSPENDED INITIALLY IN AUTOLOGOUS PLASMA, EVALUATED ON DAY OF TRANSFUSION

Sample	Age when glycerolized (days)	Days stored at 4° C after reconstitution	Period between wash and transfusion	MCV ( $\mu^2$ )	G-6-P d-ase. activity (units/min/g Hgb)	GSH (mg/g Hgb)	GSH stability (%)	Chromium survival	
								24-h	7-day
1	6	1	Not washed	86	2.7	2.0	80	85	72
2	11	1	—	—	2.7	1.9	61	64	54
3	5	1	"	97	2.9	2.5	68	90	75
4	0	1	"	—	3.8	2.0	56	76	—
5	5	1	"	—	5.0	2.3	66	89	72
6	4	2	"	94	2.7	2.7	42	84	73
7	0	3	"	86	2.4	2.0	71	89	78
8	3	6	"	83	3.4	2.7	79	84	70
9	0	11	"	91	3.6	2.3	42	84	64
10	0	13	"	110	2.8	2.1	74	80	73
11	0	14	"	95	3.2	2.3	65	80	69
12	4	3	2 h	98	4.1	2.2	46	79	69
13	0	7	2 h	96	2.8	2.4	67	93	78
14	0	7	2 h	89	4.4	2.4	45	82	70
15	1	8	2 h	107	2.3	2.2	53	87	74
16	0	13	24 h	83	3.4	1.9	43	65	54
			Mean	93	3.3	2.3			
			S.D.	$\pm 8.5$	$\pm 0.8$	$\pm 0.3$			
			Range	83-110	2.3-5.0	1.9-2.7			

Fig. 1 shows that glutathione stability of approximately 40 per cent or greater is related to 24-h survival of 65 per cent or greater. Two samples of blood resuspended initially in 5 per cent albumin medium, washed after storage at 4° C for 12 or 13 days, resuspended again in 5 per cent albumin medium, and stored at 4° C for 24 h before transfusion, had 24-h chromium survival of 44 per cent and 59 per cent respectively. Both samples had nil glutathione stability. *In vivo* haemolysis (excessive plasma radioactivity 10 min after transfusion) was observed only with these two samples. Another sample in this group had glutathione stability of 25 per cent associated with a 24-h survival of 62 per cent.

Table 2 shows the same measurements for deglycerolized erythrocytes initially resuspended in autologous

plasma. All samples in this group had glutathione stability of 40 per cent or greater and 24-h survival of approximately 65 per cent or greater (Fig. 2). No excessive plasma radioactivity was noted after infusion of any of the samples in this group. Reduced glutathione level, glucose-6-phosphate dehydrogenase, and MCV were not related to *in vivo* survival in this group.

In this investigation the *in vivo* survival of deglycerolized erythrocytes was related to glutathione stability. It was noted that with a glutathione stability of approximately 40 per cent or greater, the *in vivo* survival was approximately 65 per cent or greater. The arbitrary selection of 65 per cent 24-h survival and 40 per cent glutathione stability is extremely useful in reporting the

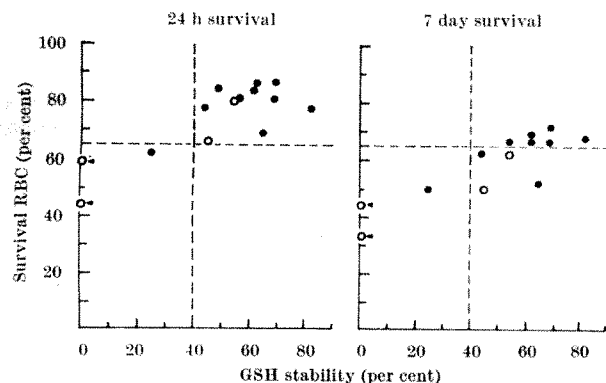


Fig. 1. Deglycerolized erythrocytes resuspended initially in 5 per cent Hyland albumin medium; GSH stability determined on day of transfusion. ○, erythrocytes washed before transfusion; ●, erythrocytes not washed before transfusion; ▲, *in-vivo* haemolysis

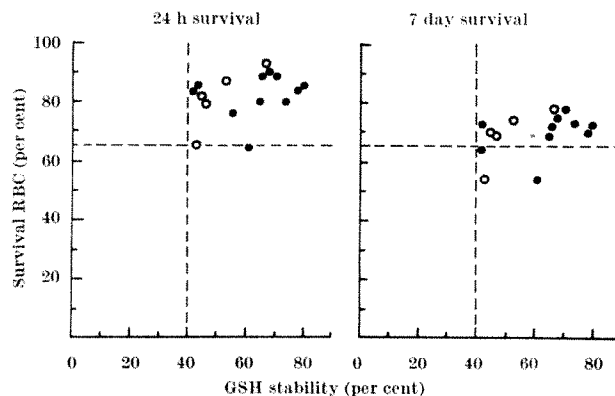


Fig. 2. Deglycerolized erythrocytes resuspended initially in autologous plasma. GSH stability determined on day of transfusion. ○, Erythrocytes washed before transfusion; ●, erythrocytes not washed before transfusion

data. *In vivo* survival was unrelated to reduced glutathione level *per se*, glucose-phosphate-6-dehydrogenase level, or MCV. Two of the 30 blood samples examined showed intravascular haemolysis, and the glutathione stability in these two was nil. Both were initially reconstituted with 5 per cent albumin medium, washed after storage for 12 and 13 days at 4° C, resuspended again in 5 per cent albumin medium, and stored for approximately 24 h before transfusion. Autologous plasma as an initial resuspension medium maintained the viability and glutathione stability of the deglycerolized erythrocytes better than the 5 per cent albumin medium.

The relationship between glutathione stability and *in vivo* survival may be related to the enzyme glutathione reductase. Ebaugh *et al.*<sup>16</sup> have reported that there is a slight but definite decrease in survival of human red cells the glutathione reductase of which has been reduced to 40 per cent, and there is marked shortening of survival to 50 per cent of normal if the glutathione reductase is further reduced to levels of 10 per cent by chromate inhibition.

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The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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## SOME CHARACTERISTICS OF THE RESPIRATORY METABOLISM OF SUSPENSIONS OF RAT INTESTINAL EPITHELIAL CELLS

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A SIGNIFICANT gap has existed in knowledge of the function of the small intestine, because the isolated, intact, functioning, intestinal, epithelial cell had not been available for examination. This article presents the first biochemical investigation on suspensions of isolated small intestinal epithelial cells. Harrer, Stern and Reilly<sup>1</sup> recently reported a trypsin-pancreatin digest method for isolating mucosal cells which largely overcomes the problem of contamination with mesenchymal elements and microbial flora. They represent a mixture of epithelial cells from the entire rat small intestine and are suitable for *in vitro* biochemical investigations. We shall describe some characteristics of the respiration and glycolysis of suspensions of rat intestinal epithelial cells obtained by this method. In addition, measurements have been made of the invertase, lactic dehydrogenase and fructose-1,6-diphosphate aldolase activities of these cells.

Mucosal epithelial cells from the small intestine of male Sprague-Dawley rats (125–175 g) were isolated according to the procedure of Harrer *et al.*<sup>1</sup> with the following modifications. The preliminary incubation in calcium-magnesium-free Tyrode's solution was omitted. All procedures were carried out at 5°–10° C except for the 15-min incubation in trypsin-pancreatin solution (at 35° C). Following trypsinization, the enzyme solution was removed and a solution composed either of 0.2 M sucrose, 0.05 M potassium phosphate, pH 7.4, or 0.2 M sucrose, 0.05 M potassium glycylglycine, pH 7.4, was introduced into the lumen of the intestine. In some experiments various proportions of sodium ions were substituted for potassium ions. The cells were readily released into the medium by gently pressing the filled intestine with one's fingers and were collected in a syringe at the opposite end. This suspension was adjusted to give a ratio of suspending medium to trypsin-pancreatin of 7:1. The cells were collected by centrifugation at 500g for 5 min and washed twice in the suspending medium. Alternatively, the trypsin-pancreatin solution was discarded and cells were collected in the

suspending medium by centrifugation and washed once. They were re-suspended each time in the media by repeated flushing through a 14-gauge needle. Oxygen uptake was measured at 30° C, in 100 per cent oxygen unless otherwise indicated. Warburg vessels, in which the usual centre well was replaced by a trough fused to the wall of the main compartment and connected with one side-arm<sup>2</sup>, were used. The trough contained 0.5 ml. of 20 per cent potassium hydroxide. Equal amounts of the cell suspension were added to the Warburg vessels for each experiment. The vessels were equilibrated for 10 min. The amount of cell protein added varied from 3 to 19 mg per vessel in different experiments. The rate of respiration and glycolysis was independent of cell concentration in this range. It took 80–90 min from the time the experiment was initiated until the cells were placed in Warburg vessels. The cell metabolism is expressed according to the following quotients:  $Q_{O_2}$  =  $\mu$ l. O<sub>2</sub> uptake/mg protein/h in oxygen;  $Q_L$  =  $\mu$ l. lactic acid produced/mg protein/h in oxygen;  $Q_L^A$  =  $\mu$ l./mg protein/h in argon. Protein was determined by the method of Lowry *et al.*<sup>3</sup> on aliquots of cell suspensions. Lactic acid was determined by the method of Barker and Summerson<sup>4</sup>. The values given in the table were corrected for both the initial lactic acid content of the cells and that produced during the 10-min temperature equilibration in the Warburg vessel. Lactic dehydrogenase was determined on aliquots of the cell suspension by the method of Kornberg<sup>5</sup>. Glucose formed by the action of invertase on sucrose was determined with a special glucostat reagent (Worthington Biochemicals Corporation). Values were always corrected for the initial glucose content of the cell suspension. Fructose-1,6-diphosphate aldolase was determined by the method of Peansky and Lardy<sup>6</sup> on aliquots of cells suspended in 0.2 M sucrose, 0.05 M potassium glycylglycine, pH 7.4, with appropriate blanks.

Cell counts were taken for every experiment and the viability determined by the Nigrosin staining technique<sup>7</sup>. The viability of the cells immediately before respiration

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measurements ranged from 55 to 90 per cent, while at the end of 1 h at 30° the viability of the cells ranged from 45 to 60 per cent. The cells were morphologically normal when stained with haematoxylin and eosin. Two types of epithelial cells were distinguished: a large columnar or slightly rounded cell with brush border, and a smaller rounded form seen individually or within isolated crypts. Differential counts revealed that about 81 per cent of the cells were intact epithelial cells, 13 per cent of the cells were lymphocytes and granulocytes and 6 per cent were naked nuclei. Since the lymphocytes and granulocytes were about 1/5 the size of the epithelial cells, their contribution to the experiments reported here would not be significant. Cells which were suspended in sucrose phosphate medium were examined under the electron microscope by Dr. Charles Johnson and appeared morphologically intact. The mitochondria appeared normal, but the cytoplasm contained some fat droplets. No bacteria were visible and bacterial colony counts showed negligible contamination.

Table 1. EFFECT OF VARIOUS SUBSTRATES ON THE RESPIRATION OF INTESTINAL EPITHELIAL CELLS WASHED AND SUSPENDED IN A BUFFERED SUCROSE MEDIUM

Exp. No.	Additions	Q <sub>O<sub>2</sub></sub>
1	None	-5.0
	Pyruvate $9.5 \times 10^{-3}$ M	-6.1
	Succinate $9.5 \times 10^{-3}$ M	-7.4
2	None	-4.4
	Lactate $9.5 \times 10^{-3}$ M	-5.3
	Fumarate $9.5 \times 10^{-3}$ M	-6.2
	Citrate $9.5 \times 10^{-3}$ M	-4.9
3	None	-5.9
	Succinate $9.5 \times 10^{-3}$ M	-10.0

Exps. 1 and 2. Warburg vessels contained cells (equivalent to 19 mg of cell protein in Exp. 1 and 10.5 mg of cell protein in Exp. 2) suspended in 0.2 M sucrose, 0.05 M potassium phosphate, pH 7.4 (final potassium concentration = 0.095 M) and 142 units each, of penicillin and streptomycin in a final volume of 1.05 ml. The substrates were added as their potassium salts, pH 7.0, and a comparable amount of KCl was added to the control vessel.

Exp. 3. Warburg vessels contained cells (equivalent to 11.3 mg of cell protein) suspended in 0.2 M sucrose, 0.05 M glycylglycine, 0.047 M potassium ion, pH 7.4 and 142 units each, of penicillin and streptomycin in a final volume of 1.05 ml.

Results presented in Table 1 indicated that intestinal epithelial cells from the rat respired after being washed and suspended in a 0.2 M sucrose medium buffered with either potassium phosphate or potassium glycylglycine. Enzymatic hydrolysis of sucrose by epithelial cell invertase provided glucose as an energy source. Oxygen consumption was usually linear for 40 min and in some experiments for 60 min. Respiration was stimulated when succinate, pyruvate, citrate, fumarate and lactate were added. The addition of 0.002 M DPN, ADP and MgCl<sub>2</sub> alone or in combination had no effect, indicating that these co-factors were not rate-limiting or lost during cell isolation. Added glucose, glutamate, acetate or CaCl<sub>2</sub> did not affect respiration.

It should be emphasized that the 0.2 M sucrose, 0.05 M potassium phosphate solution was employed for isolation and washing of cells because, in our experience, this medium proved to be best for obtaining intact, viable cells. However, we were interested in examining the effects of lower sucrose concentrations on respiration and glycolysis. It was necessary to reduce the sucrose concentration after the cells were isolated. This was accomplished by diluting the cells out in the Warburg vessel to a final concentration of 0.05 M sucrose, 0.05 M potassium phosphate, pH 7.4, producing a cell lysate. These cells were fragmented when observed under the microscope. The results are not given, but this cell lysate respired as well and produced as much lactic acid as intact cells. Berry<sup>8</sup> found that suspensions of cells from mouse liver isolated in 0.3 M or 0.4 M sucrose respired in a simple buffered medium containing 0.06–0.2 M sucrose. Liver cells oxidized citric acid cycle intermediates, but carbohydrate substrates did not stimulate oxygen uptake owing to the loss of glycolytic enzymes from the liver cells during isolation. Results will be presented later to show that intestinal epithelial cells retain their glycolytic enzymes. Berry<sup>8</sup>, Zimmerman *et al.*<sup>9</sup>, Kalant and Young<sup>10</sup> and Laws and Stickland<sup>11</sup>

Table 2. EFFECT OF THE SODIUM AND POTASSIUM CONCENTRATION OF THE SUCROSE PHOSPHATE MEDIUM ON OXYGEN CONSUMPTION AND GLYCOLYSIS OF INTESTINAL EPITHELIAL CELLS

Exp. No.	Potassium conc. (M)	Sodium conc. (M)	Q <sub>O<sub>2</sub></sub>	Q <sub>L</sub>
1		0.095	-1.5	2.7
2		0.095	-1.2	
3		0.095	-1.1	
4	0.01	0.085	-3.1	4.2
5	0.01	0.085	-3.0	4.2
6	0.019	0.076	-7.7	6.6
7	0.019	0.076	-7.7	7.1
8	0.05	0.045	-4.8	
9	0.05	0.045	-6.3	
10	0.095		-3.8	5.4
11	0.095		-4.9	7.9
12	0.095		-6.1	8.5

Cells were isolated and suspended in 0.2 M sucrose, 0.05 M phosphate, pH 7.4, adjusted to the potassium and sodium ion concentration as shown in the table. All solutions contained penicillin and streptomycin as indicated in Table 1. Samples of cell suspensions containing 9–16 mg of cell protein were added to Warburg vessels.

have reported that suspensions of isolated liver and kidney cells do not respire in Krebs's phosphate-saline with or without glucose, but will respire if succinate is present. The respiration of intestinal epithelial cells in Krebs's phosphate-saline-glucose varied considerably from experiment to experiment. In some experiments rates as high as 15  $\mu$ l./oxygen uptake/mg protein/h were obtained, whereas in other experiments there was little or no oxygen consumption. Since consistent results could be obtained in the buffered sucrose medium, experiments reported here were all carried out with cells isolated and suspended in sucrose.

The effect of substituting sodium ions for potassium ions in the sucrose-phosphate isolation and suspending medium was investigated and the results are shown in Table 2. Cells isolated in sucrose sodium phosphate were morphologically normal and the viability was as high as cells isolated in sucrose potassium phosphate. However, cellular respiration and glycolysis were very low when potassium ions were replaced by sodium ions and could not be augmented by adding potassium ions to the reaction mixture in the Warburg vessel. If potassium ions were present in the isolation medium, the rate of oxygen consumption and lactic acid formation increased. The data show that oxygen consumption rose as the potassium ion concentration was increased to 0.019 M, and then, at higher potassium ion concentrations, declined somewhat. Added sodium ions were not necessary for respiration or glycolysis. The results are not given, but the cells responded in a similar manner when sucrose sodium glycylglycine instead of sucrose potassium glycylglycine were used for cell isolation and suspension. The presence of potassium ions in the isolation and suspending medium is essential for the maintenance of cellular function as measured by respiration and glycolysis.

One of the most striking features of the carbohydrate metabolism of intestinal mucosa is its high aerobic and anaerobic glycolysis. The relationship between respiration and glycolysis of intestinal epithelial cell suspensions is given in Table 3. The rate of aerobic and anaerobic glycolysis is very high. There was an increase in lactic acid production in the absence of oxygen (Pasteur effect). Wilson and Wiseman<sup>12</sup>, using everted sacs of rat intestine, found an appreciable Pasteur effect only in the lower half of the rat intestine. Inasmuch as cell suspensions used in experiments reported here represent a mixture of cells from various levels of the rat intestine, no conclusions can be drawn in regard to a possible Pasteur effect in the jejunum.

Table 3. OXYGEN CONSUMPTION AND AEROBIC AND ANAEROBIC LACTIC ACID PRODUCTION OF INTESTINAL EPITHELIAL CELLS IN SUCROSE PHOSPHATE MEDIUM

Exp. No.	Q <sub>O<sub>2</sub></sub>	Q <sub>L</sub>	Q <sub>L'</sub>
1	-4.7	7.5	10.2
2	-6.1	8.5	14.1
3	-4.0	6.7	9.9

Warburg vessels contained cells (equivalent to 3.0, 14.8 and 15.3 mg of cell protein in Exps. 1, 2 and 3 respectively) suspended in sucrose phosphate medium, pH 7.4. The composition of the medium was identical with that used in Exps. 1 and 2 of Table 1.

**Table 4. EFFECT OF IODOACETATE ON OXYGEN CONSUMPTION AND LACTIC ACID PRODUCTION BY INTESTINAL EPITHELIAL CELLS**

Exp. No.	Experimental conditions	QO <sub>2</sub>	QL
1	No additions	-3.9	6.0
	Iodoacetate $5 \times 10^{-5}$ M	-2.6	3.0
	Iodoacetate $5 \times 10^{-4}$ M	-1.9	0.4
	Iodoacetate $5 \times 10^{-3}$ M	-1.5	0.3
2	No additions	-3.8	5.4
	Iodoacetate $5 \times 10^{-4}$ M	-1.8	1.5
	Iodoacetate $5 \times 10^{-3}$ M	-1.9	0

Warburg vessels contained cells (equivalent to 11.5 mg of cell protein in Exp. 1 and 16.0 mg of cell protein in Exp. 2) suspended in sucrose potassium phosphate buffer, pH 7.4. The composition of the medium was identical with that used in Exps. 1 and 2 of Table 1.

The effect of iodoacetate on respiration and glycolysis of intestinal epithelial cells is shown in Table 4. Lactic acid production was virtually abolished in the presence of  $5 \times 10^{-3}$  M iodoacetate. Oxygen consumption was decreased to about 50 per cent of the control value, indicating that either the cells accumulated enough substrates to sustain respiration or that they utilized pathways other than the Embden-Meyerhof for channeling metabolites into the tricarboxylic acid cycle and electron transport chain.

**TABLE 5. INVERTASE, LACTIC DEHYDROGENASE AND ALDOLASE ACTIVITIES OF INTESTINAL EPITHELIAL CELLS**

Invertase ( $\mu$ moles of sucrose hydrolysed/mg protein/h)	3.2, 3.7, 4.1, 7.5
Lactic dehydrogenase ( $\mu$ moles lactate formed/mg protein/min)	0.5, 0.7, 1.0, 1.5
Fructose-1,6-diphosphate aldolase ( $\mu$ moles of fructose-1,6-diphosphate split/mg protein/5 min)	0.13, 0.14, 0.23, 0.35

Each of the above figures represents an enzyme determination on an aliquot of cell suspension obtained from a different rat. Assays were carried out as described in the text.

In Table 5 the activities of invertase, lactic dehydrogenase, and aldolase in isolated suspensions of intestinal epithelial cells are shown. Each value represents a determination on cells from a different rat. There was a considerable range in the specific activity of these enzymes. This is as expected, since there are gradations in enzyme activities along the intestine and in separate experiments various proportions of cells from different regions of the small intestine are obtained. Invertase was measured as the amount of sucrose hydrolysed in 1 h under the same experimental conditions as oxygen uptake measurements. Iodoacetate was added to prevent glucose utilization by the Embden-Meyerhof pathway. The presence of invertase in the isolated epithelial cells confirms the work of Miller and Crane<sup>13</sup>. The amount of cell protein present in the respiration experiments with sucrose phosphate was 3-19 mg/200  $\mu$ moles sucrose. If one takes an average value of 4.6  $\mu$ moles of sucrose hydrolysed/h/mg protein, then 13.8-87  $\mu$ moles of glucose were produced during

1 h at 30°. The concomitant decrease in the sucrose concentration over a 1-h period did not affect respiration or lactic acid production as indicated earlier in the experiments where a cell lysate was prepared by diluting out the cells in the Warburg vessel to a final concentration of 0.05 M sucrose 0.05 M potassium phosphate. Aliquots of cell suspensions were sonated before measuring lactic dehydrogenase and aldolase. The activities of both fructose-1,6-diphosphate aldolase and lactic dehydrogenase indicate that these enzymes were present in sufficient concentration to account for the amount of lactic acid produced by these cells. Lactic dehydrogenase was assayed by measuring the oxidation of DPNH by pyruvate in the presence of the epithelial cell homogenates. There was no DPNH oxidase activity in the absence of pyruvate. This confirms the work of others<sup>14</sup> that mitochondria from mammalian tissues, when undamaged by preparative techniques, are unable to oxidize extramitochondrial DPNH, presumably because of the impermeability of the mitochondrial membrane to the reduced pyridine nucleotide. Berry<sup>5</sup> has reported that liver cells lose most of their aldolase and lactic dehydrogenase during isolation and washing. Though no attempt was made to measure the amounts of enzymes lost in washing, it is clear that intestinal epithelial cells which have been washed extensively possess a sufficient complement of at least two enzymes of the Embden-Meyerhof pathway to carry out a vigorous glycolysis.

Data reported here represent the first biochemical investigations of suspensions of isolated, intestinal epithelial cells and support the concept of the dissociated intestinal epithelial cell as an intact functioning unit which is appropriate for investigation of problems peculiar to the cellular level of intestinal function.

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## IMMUNOSEDIMENTATION: A BI-DIMENSIONAL AGAR-GEL PLATE TECHNIQUE FOR ANTIGEN ANALYSIS

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THE adaptation of sucrose gradients for examining sedimentation behaviour of enzymes, developed by Martin and Ames<sup>1</sup>, can be used advantageously for the separation of antigens in a mixture as in whole human serum. The distribution concentration of antigens down the centrifuge tube can be obtained by protein measurements of the separated fractions, but the overlap of the proteins excludes the possibility of attaining sedimentation coefficients from serum components, with the exception of albumin normally present in sufficient amount to produce a distinct peak.

Because centrifugation of protein mixtures in gradients with subsequent analysis of human serum fractions by a series of agar double diffusion gel tests against rabbit anti-human serum did not improve the resolution of antigens, we decided to combine electrophoretic separation, across the sedimentation axis in the agar plate, as a second step before developing the antigens with the antiserum.

As at present used, the technique consists of overlaying 0.1-0.2 ml. of undiluted human serum, or another protein solution or mixture for which there is available a potent precipitating antiserum, on top of a sucrose gradient



established in a cellulose nitrate centrifuge tube (12 × 50 mm) for the swinging bucket rotor, *SW 39 L*, of the Spinco model *L* preparative ultracentrifuge. A useful gradient for normal human serum, with a calibrated scale of 0–13S (Svedberg units), was obtained with 9–24 per cent sucrose solutions centrifuged for 18 h at 100,000*g* and 4° C. In serum analyses 0.9 per cent sodium chloride was used for preparing the sucrose gradients.

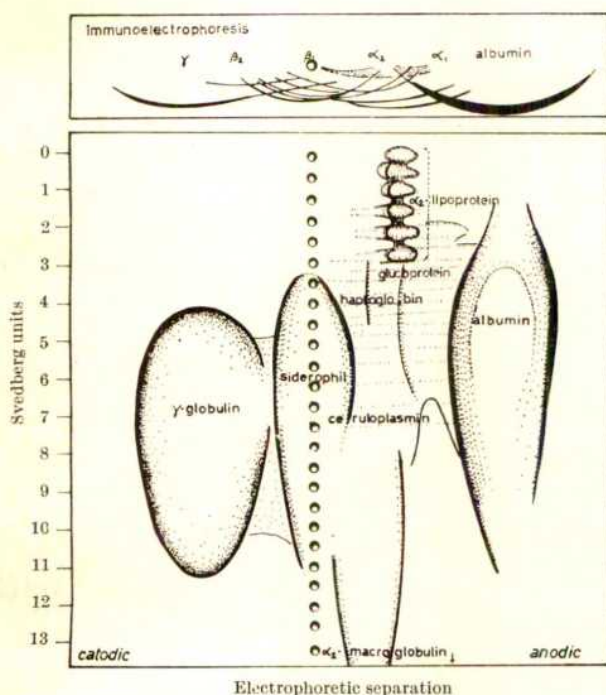
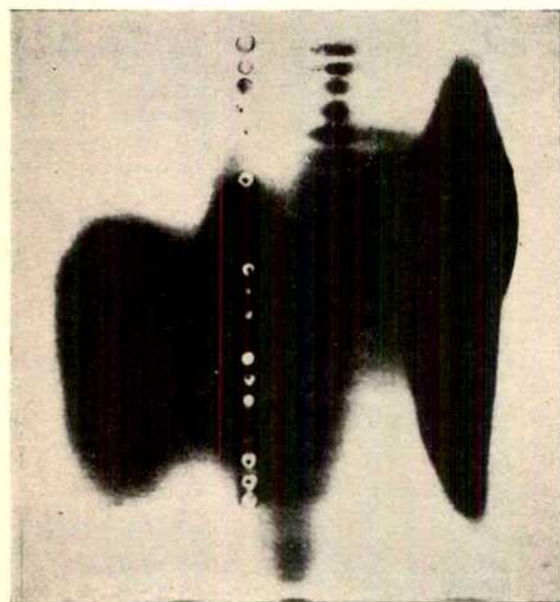


Fig. 1. Photograph of a normal human serum sample analysed by immunosedimentation with rabbit anti-human serum. Precipitates were stained with amido black<sup>3</sup>. Positive side is to the right. The elongated spot to the anodic side is serum albumin specific precipitate. The round spot to the left is due to 7S  $\gamma$ -globulins precipitate. Outermost edge of the spot corresponds to 7S value in the vertical co-ordinate. The diagram below was drawn directly from the agar plate, under a photographic amplifier. Albumin and  $\gamma$ -globulin spots were identified in control runs with the pure proteins,  $\alpha_2$ -lipoproteins by lipid staining with oil red<sup>2</sup>. The names of the rest of the antigens shown in the diagram were placed in the expected position, for these antigens, in immunoelectrophoresis. Note the relative place and shape of the spots in the 0–13S sucrose gradient. The diagram at the top is from the immunoelectrophoretic analysis of the same normal human serum sample developed with the same rabbit antiserum.

At the end of the run the centrifuge tubes were removed from the rotor, fixed to a stand, and pierced at the bottom with a hypodermic needle to collect, into 25 small test-tubes, consecutive volumes of the spun-down solution from the centrifuge tube. Each of the 25 aliquots contained mixtures, in various proportions, of the antigens in human serum separated in accordance with sedimentation coefficients<sup>1</sup>.

The electrophoresis step was performed on an agar plate filled with 18 ml. of 1.5 per cent agar Difco 'Noble' in Aronson's buffer<sup>2</sup>, of 60.5 g *tris*, 6.0 g EDTA and 4.6 g boric acid per litre, at pH 8.7. The melted agar was poured into trays constructed with 7 cm × 7 cm glass plates with glass rods glued to the edges. At the centre of the plate 25 small round holes were implemented with punches fixed, at 1-mm intervals, to an acrylic square-shape block. These perforations were filled with about 20  $\mu$ l. of each of the solutions of antigens in the 25 test-tubes. The electrical connexions were secured to the plates with thick, wet paper wicks, and a potential of 120 V was supplemented, from a regulated power supply, for 2 h at room temperature. When the electrophoretic run was terminated the current was turned off and the agar plates were overflowed with rabbit antiserum, prepared against pooled human serum, and placed inside a humid chamber awaiting the development of the 'spots' of specific precipitate to appear. Albumin spot was visible in a few hours and the rest of the antigens of human serum were clear in 48 h. The excess antiserum was poured off and the plates washed exhaustively with pH 8.6 borate buffer 0.1 M for at least 3 days and with several rinses of running tap-water afterwards. Finally, they were dried, as immunoelectrophoresis plates, with filter paper, and stained with amido black for proteins and oil red in 60 per cent ethanolic solution for lipids<sup>3</sup>.

Fig. 1 shows a photograph of a sample of normal human serum developed by immunosedimentation. The accompanying diagram was outlined after the original plate. A diagram of a control immunoelectrophoresis run, of the same human serum sample under the same experimental conditions and developed with the same antiserum, is shown on top. The presence of a particular spot is due to the precipitation of antibody by the antigen in the agar plate, exactly as in the method of 'direct immunoelectrophoresis' recently described<sup>4</sup>.

In the plate (Fig. 1), the horizontal axis corresponds to electrophoretic mobilities with the anodic side to the right. The vertical axis, along the sequence of the 25 symmetrically dispersed perforations containing the various antigen mixtures, can be visualized as a sedimentation constant axis (Svedberg units). The calibration of this parameter was established, in the same experiment, with a centrifuge tube containing 4 mg of twice crystallized human albumin and analysis of the protein by Folin's reagent<sup>5</sup>, in each of the solutions in the 25 test-tubes.

There are several features which distinguish this technique from previous ones which in our view are of some relevance. First, the possibility of appraising approximate sedimentation coefficients of some antigens, that is, albumin, 7S  $\gamma$ -globulin, macroglobulin, in serum samples, simultaneously with their electrophoretic localization and serological reactivity with a particular antiserum, seems interesting; and, secondly, the visual overall picture of a family of 'spots' of immunosedimentation runs, with human sera, can be of practical application.

Another interesting feature worth mentioning is that some antigens, albumin for example, appear in the plate as a regular elongated spot and not as a number of discrete spots that one would expect from multiple circular sources. This coalescence is due to the diffusion rate of albumin and other proteins, like 7S  $\gamma$ -globulins, during the lapse of the electrophoretic migration.  $\alpha_2$ -Lipoproteins that were identified with oil red and are shown in the diagram produce, due to their much slower diffusion



rate, a number of spots corresponding each to a place in the gradient in which they are located at the end of the run.

The outermost edge of each spot should correspond to maximum antigen concentration, to the peak, and to the sedimentation coefficient when this point is moved, horizontally, to the Svedberg units axis<sup>1</sup>. The estimation of this point is feasible for some antigens; the outer edge of the  $\gamma$ -globulin spot is at the 7S mark and the one of albumin close to the 4.5S, both values in close agreement to the ones in the literature<sup>6</sup>. It is evident that this simple procedure cannot be employed, with accuracy, with most of the revealed antigens because they are too close together to give sharp contours. A few other antigens, nevertheless, are present in their expected positions;  $\alpha_2$ -macroglobulin comes to the bottom of the tube, off the scale of 13S of this particular gradient, and is easily

distinguished in the photograph. Other proteins shown in the diagram were named, in spite of the fact that they have not been unquestionably identified, as a reference for the approximate site of these antigens in immunosedimentation.

A protein in a mixture, analysed bi-dimensionally as already described here, can be detected by different means instead of antiserum. Thus, an enzyme in a crude extract can be detected by adding the appropriate substrates and couplers to give a non-diffusible coloured spot.

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## HAEMOGLOBIN SYNTHESIS AND POLYSOMES IN INTACT RETICULOCYTES

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THE active units in protein synthesis are the polysomes<sup>1-3</sup>—structures consisting of clusters of ribosomes held together by a single-stranded messenger RNA molecule<sup>4</sup>. As shown by electron microscopy, the principal component in reticulocytes is the penta-ribosome<sup>5,6</sup>. Investigations with isolated polysomes have indicated that probably the attachment of monosomes at one end of the mRNA initiates peptide formation<sup>7,8</sup>. In the present concept of polysome function<sup>2,9</sup> the polypeptide chain grows as the ribosome moves along the mRNA, and becomes detached at the other end with the completion of the chain. This orderly breakdown of polysomes *in vitro* and its dependence on protein synthesis have been demonstrated in reticulocytes<sup>10</sup>, rat liver<sup>11</sup>, and HeLa cells<sup>7</sup>. While in the cell-free system the incorporation of amino-acids into ribosomes normally ceases after 30 min<sup>12</sup>, intact reticulocytes synthesize haemoglobin for several hours at a nearly linear rate<sup>13</sup>. Examining the *in vitro* maturation of reticulocytes, Marks *et al.*<sup>14</sup> observed that as reticulocytes mature the activity of the polysomes declines while their concentration in the cell decreases, but to a lesser extent. This suggests that polysomes may become partially inactive while remaining polysomes. Glowacki and Millette<sup>15</sup> came to a similar conclusion examining reticulocytes separated according to their state of maturation on a bovine serum albumin gradient. Evidence is presented here that under certain conditions the activity of polysomes in reticulocytes decreases drastically while there is actually no decrease in the ribosomes sedimenting as polysomes.

Reticulocytosis was induced in female rabbits, weighing between 3.0 and 3.5 kg, by six daily subcutaneous injections of 2.5 per cent neutralized phenylhydrazine solution (0.25 ml./kg). Approximately 80 ml. blood was obtained on the 7th day from each animal by heart puncture and heparin was added immediately to give a final concentration of 0.08 per cent. The reticulocyte count was between 65 and 85 per cent. The cells were collected by centrifugation at 4,000 r.p.m. for 5 min and washed with NKM (0.153 M NaCl, 0.005 M KCl, 0.005 M MgCl<sub>2</sub>). Ten ml. of packed cells were incubated with 20 ml. of anaemic plasma and 20 ml. of a reagent mixture, containing in a total volume of 500 ml.: 50 ml. Earle's solution, Spinner, 10×; 100 ml. of an amino-acid mixture; 5-ml. vitamin solution (Eagle), 100×; 400 units penicillin G and 2 mg streptomycin. The concentration in the reagent mixture

of MgSO<sub>4</sub> was brought to 1.2 mM and NaHCO<sub>3</sub> (40 mM) was added. The pH of the incubation mixture was 7.5 at 37° C under 95 per cent oxygen: 5 per cent carbon dioxide. Two milligrams of ferrous ammonium sulphate and 12.75  $\mu$ moles L-leucine-<sup>14</sup>C (uniformly labelled), spec. activity 2.5 to 3.0 mc./mmole, were added. The amino-acid mixture contained (mmoles/l.): L-alanine, 1; L-arginine, 2; L-asparagine, 1; glycine, 2; L-histidine, 5; L-isoleucine, 2; L-lysine, 5; L-methionine, 1; L-phenylalanine, 4; L-proline, 2; L-serine, 2; L-threonine, 2; L-tryptophan, 1; L-tyrosine, 2.5; L-valine, 8; L-cysteine, 1.0; L-glutamine was added separately corresponding to 4.0 mM in the reagent mixture. All incubations were made under 95 per cent oxygen: 5 per cent carbon dioxide at 37° in a water-bath shaker.

At the end of the incubation the cells were collected by centrifugation at 4,000 r.p.m. and washed twice with NKM. They were then 'shock-lysed' according to Marks *et al.*<sup>14</sup>. Ribosomes were prepared by layering the haemolysate in Spinco rotor 30 tubes on 3 ml. of STM (0.5 M sucrose, 0.01 M tris pH 7.1, 0.0015 M MgCl<sub>2</sub>, 0.04 M KCl) and centrifugation at 78,000g for 3 h. The ribosomal pellet was suspended in 1/8 original volume of Solution F<sub>2</sub> (0.01 M tris pH 7.1, 0.0015 M MgCl<sub>2</sub>, 0.04 M KCl) using a loose-fitting homogenizer, and centrifuged in rotor 40 tubes through 5 ml. of STM for 5 h. The concentration of ribosomes was determined using an extinction coefficient at 260 m $\mu$  (25° C) of 11.2/mg/ml. (ref. 17) and as molecular weight  $4.1 \times 10^6$  (ref. 18). 0.5 mg samples were precipitated with 10 per cent cold TCA. After 1 h the precipitate was collected on 'Millipore' filters (0.45  $\mu$  pore size) which were dried, glued on aluminium planchets, and counted in a Nuclear Chicago low-background gas-flow counter.

For sucrose gradient analyses ribosomes centrifuged once through STM were gently suspended in Solution F<sub>2</sub> using a glass rod instead of a homogenizer. One millilitre of this suspension was layered on to a linear sucrose gradient of 30–15 per cent sucrose w/v in Solution F<sub>2</sub> and centrifuged in a SW 25 rotor at 4° C. Usually fractions of 0.60–0.75 ml. were collected and analysed as described elsewhere<sup>19</sup>.

L-Leucine-<sup>14</sup>C was obtained from New England Nuclear. The constituents for the reagent mixture were from Hyland Laboratories, Los Angeles. All preparations were made at 4° C.

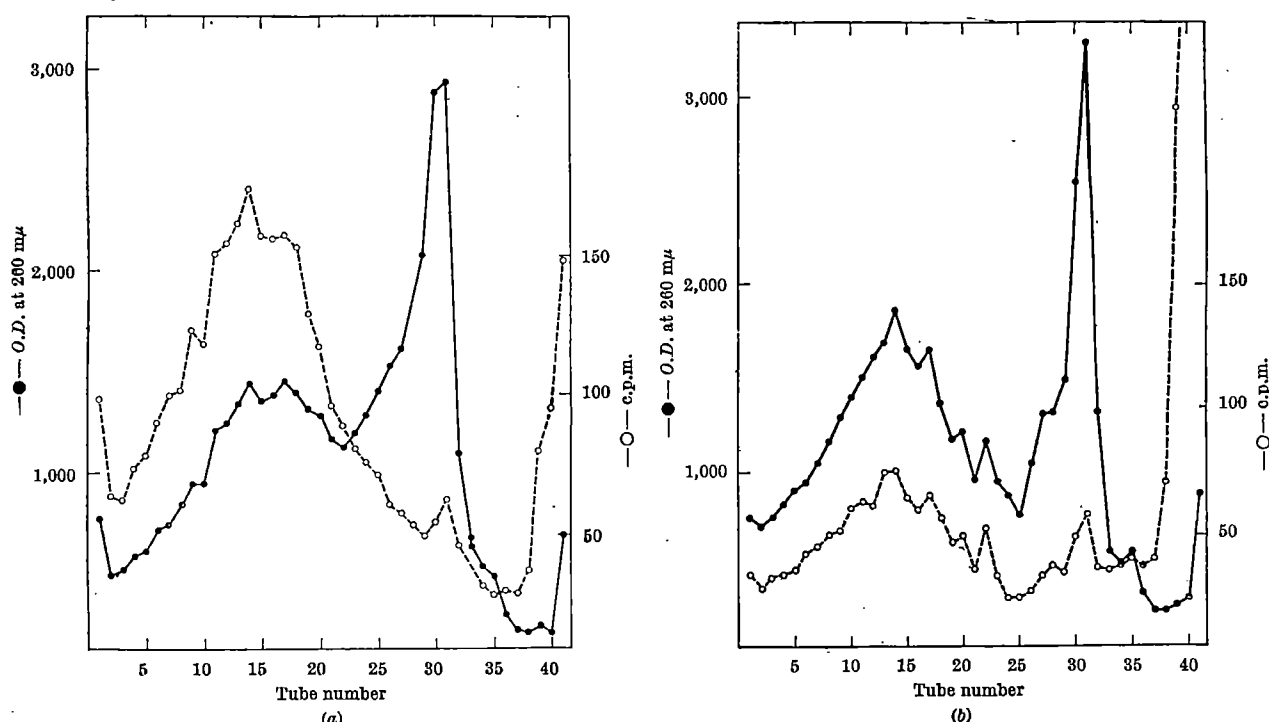


Fig. 1. Sucrose gradients of polysomes after whole cell incubation with L-leucine- $^{14}\text{C}$  in the presence of 11.2 mM glucose (Exp. 4, Table 2). 1 ml. of ribosome suspension was layered on 26 ml. of a 30–15 per cent sucrose gradient and centrifuged for 3 h in a *SV* 25 rotor (—). (a) Polysomes obtained after 15 min incubation; (b) after 120 min incubation

These investigations were aimed at establishing an optimal system for *in vitro* incubation of reticulocytes, which would permit the cells to synthesize proteins at an even rate for several hours. As a criterion for the activity of the cells the radioactivity incorporated into ribosomes was taken. Ribosomes, prepared as already described here, do not retain any haemoglobin, as shown by spectrophotometry. No absorbancy at 418 mμ was found if the ribosomes were centrifuged twice through *STM*, while some absorbancy was still present if the ribosomes were centrifuged twice for the same length of time but using *STM* only in the second centrifugation. This, and the fact that ribosomal protein is not synthesized in reticulocytes<sup>19</sup>, enabled us to associate all label on the ribosomes with the growing peptide chain. The incorporation of amino-acids into polysomes and, for comparison, into soluble proteins was followed for various times of incubation. Using an incubation medium similar to that described previously<sup>20</sup>, it was found that ribosomes lose some of their initial activity during a 90-min incubation period. Sucrose gradient analyses of ribosomes obtained after 15 min and 90 min (120 min) of incubation indicated no breakdown of polysomes but rather a decrease in their specific activity.

To establish if the state of reticulocytosis reflects in any way the ribosomal activity in these cells, rabbits were injected with phenylhydrazine for various lengths of time. As can be seen in Table 1, the reticulocyte count

does not reflect the 'average activity' of the ribosomes. If the rabbits were rested for one day after the last injection, the specific activity of the ribosomes was usually slightly higher after 15 min but decreased faster during the 2-h incubation period. Since it appears that two classes of reticulocytes exist, one with a life-span of 17 days, the other with a much shorter life-span of 2 days<sup>21</sup>, this has to be taken into account in interpreting my results. The differences are small, however. In those animals which were injected for a longer period of time the peripheral blood contains more reticulocytes, as well as cells which are more mature. In these experiments, plasma and cells were obtained from the same animal. I examined the possibility that a factor in the plasma might be responsible for the decrease in cell activity during the incubation. The substitution of anaemic plasma obtained from normal animals had very little effect on the ribosomal activity. Treatment of plasma with bentonite, known to absorb RNases, did not increase the capacity of the cells to synthesize proteins, either during 15 min or 120 min of incubation. It seems unlikely, therefore, that RNases present in the plasma enter the cells under conditions adopted here.

The general requirements for *in vitro* incubation of reticulocytes had been examined by Borsook *et al.*<sup>13</sup>, using the incorporation of label into soluble proteins as the criterion of cell activity. Approximately 90 per cent of all soluble proteins in reticulocytes represent haemoglobin, while the remainder constitute a variety of non-haem proteins. At least part of these are synthesized at a much faster rate (Philipps, G. R., unpublished results). However, since the incorporation of amino-acids into nascent protein reflects more accurately the activity of the cells, several aspects of the system were reinvestigated. A high concentration of plasma in the incubation mixture retained the activity of the cells longer. The concentration of electrolytes in the reagent mixture is approximately the same as in the plasma. A high concentration of amino-acids and iron, as compared with the plasma concentration<sup>23</sup>, is necessary to guarantee a continuous protein synthesis for 2 h (Table 2, Exps. 1–3). By increasing the glucose concentration the activity of the cells increased

Table 1. HAEMOGLOBIN SYNTHESIS AND THE STATE OF RETICULOCYTOSIS

No. of days injected	Per cent reticulo-cytes	15-min incubation		120-min incubation	
		Specific activity: (c.p.m./O.D. <sub>260</sub> )		Specific activity: (c.p.m./O.D. <sub>260</sub> )	
		Ribosomes	Soluble proteins	Ribosomes	Soluble proteins
7*	77	43.0	257	25.4	604
7	90	41.2	280	28.6	799
6*	76	45.6	323	30.5	914
6	89	43.3	424	34.5	1,068
5*	85	43.4	232	26.7	647
5	74	46.9	413	30.7	974

Rabbits were injected for the indicated number of days; an asterisk means that these animals rested one day after the last injection before the blood was collected. 20 ml. of cells were incubated with 40 ml. of reagent mixture<sup>20</sup> containing 11.2 mM glucose and 5 ml. of normal plasma. 0.04 mc. algal protein hydrolysate, diluted with the amino-acid mixture, was added to 20 ml. of cells. Ribosomes were prepared as described in the text; the soluble proteins were obtained from the supernatant of the last ultracentrifugation and dialysed for 24 h. Each value represents the mean of two experiments.

Table 2. INCORPORATION OF LEUCINE INTO RIBOSOMES AND SOLUBLE PROTEINS

Exp.	Concentration in the reagent mixture*				$\frac{\mu\text{moles leucine}^\dagger}{\mu\text{moles ribosomes}}$		Decrease in ribosomal activity (%)	$\frac{10^{-4} \mu\text{moles leucine}}{\text{O.D.}_{260} \text{ soluble proteins}}$	
	L-leucine (mM)	Amino-acids (mL/100)	Glucose (mM)	Iron (mM)	15 min	120 min		15 min	120 min
1	0.54	10	5.6	0.06	4.85	3.42	29	4.24	24.95
2	1.04	20	5.6	0.06	5.09	4.20	17	8.18	38.97
3	1.04	20	5.6	0.25	6.86	6.67	3	15.52	52.05
4	0.54	10	11.2	0.06	5.06	2.81	44	8.45	37.90
5	0.54	20	11.2	0.25	6.24	3.51	56	13.20	41.38

\* The reagent mixture was diluted 2/5 in the incubation mixture. Due to this dilution, the actual concentration of amino-acids and iron is smaller; the glucose concentration is approximately the same in the incubation mixture.

† These values were corrected for the decrease of the specific activity of leucine due to the intracellular pool in reticulocytes ( $0.25 \text{ mM}$ )<sup>22</sup> and the amount present in plasma ( $0.4 \text{ mg per cent}$ )<sup>23</sup>. The actual values were 18 and 22 per cent smaller, respectively.

when used in a medium which is sub-optimal with respect to the concentration of amino-acids and iron (Table 2, Exps. 2 and 4), otherwise the glucose effect is negligible. The glucose concentration in the whole blood is approximately 5 mM, which corresponds to the concentration used in Exps. 1-3. At a high glucose concentration (Exps. 4 and 5) the activity of ribosomes decreases rapidly as compared with the two incubation periods.

This 'glucose effect' was examined in more detail using sucrose gradient analyses of the polysomes (Fig. 1). The distribution of a 15-min incubation is plotted in Fig. 1a. As expected, the radioactivity overlaps with the absorption peak characteristic for polysomes, while a small number of counts found in the 80S ribosome peak are probably due to polysomes disrupted during the prepara-

tion. Fig. 1b depicts the situation after a 120-min incubation. The major part of the activity is still found associated with the polysomes; however, the specific activity is much less than after 15 min of incubation. This is more clearly illustrated in Fig. 2. The specific activity of the polysomes and monosomes is compared for a 15-min and a 120-min incubation. Using a glucose concentration of 5.6 mM, the specific activity is highest in the region of the heaviest polysomes, reaching a minimum at the 80S ribosomes. After 2 h of incubation, the slope of the curve has not changed essentially, but a small shift of the activity towards the lighter polysomes is apparent (Fig. 2a). The situation is very different if a glucose concentration of 11.2 mM in the incubation medium is used (Fig. 2b). A much greater loss of specific activity is found after 2 h. The decrease is most in the heavier polysomes and somewhat less in those which sediment in the middle of the gradient. At either glucose concentration a minimum of activity is associated with the monomer fraction. The relative amount of polysomes, compared to the monosome fraction, did not change greatly, but a shift towards the fractions containing the heavier polysomes appeared (Fig. 3). This increase of the amount of polysomes sedimenting with a high *S* value after prolonged incubation, though not great, was found in several experiments. As I shall describe elsewhere<sup>19</sup>, ribosomes do not easily aggregate under these experimental conditions. In a similar experiment, I compared the total amount of ribosomes after 15 min and 120 min of incubation. Starting with exactly 5 ml. cells, I recovered in a duplicate experiment 5.6 and 5.5 mg ribosomes after 15 min and 5.0 mg after 120 min. A probable explanation of these results is a gradual degradation of polysomes to ribosomes and the formation of ribosomal sub-units and smaller degradation products. The heavier polysomes, most active in protein synthesis, would be more stable than the lighter ones.

It has been shown here that by using the proper conditions for the incubation of reticulocytes, the polysomes preserve their activity throughout a 2-h incubation.

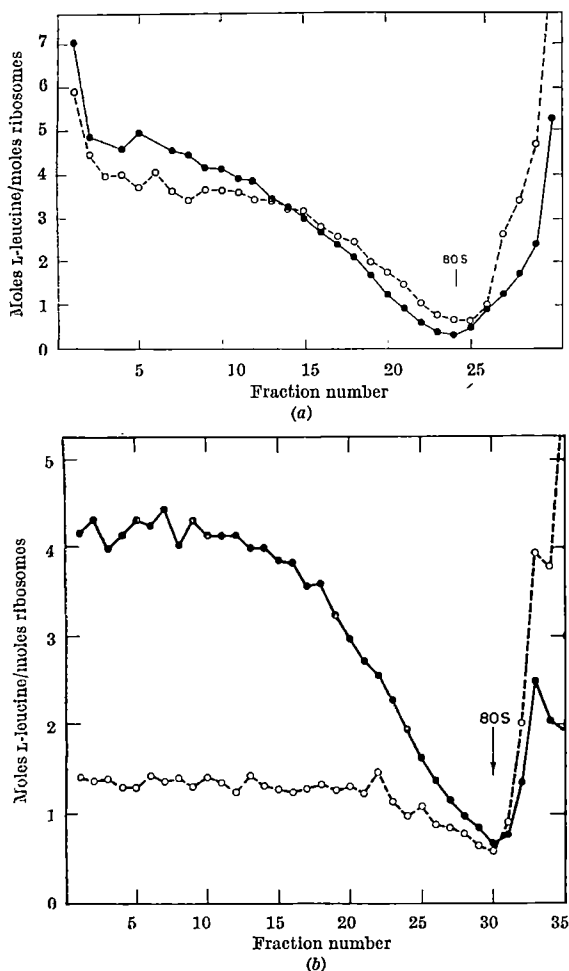


Fig. 2. Specific activity of ribosomes centrifuged through a sucrose gradient. Polysomes were analysed as described in Fig. 1 and the incorporation of moles leucine per mole ribosome plotted against the fraction number. (a) Incubation in 5.6 mM glucose (Exp. 2, Table 2); (b) incubation in 11.2 mM glucose (Exp. 4, Table 2). —●—, 15-min incubation; —○—, 120-min incubation. (The total fractions collected in (a) and (b) were somewhat different, which explains the different position of the 80S peak in both graphs)

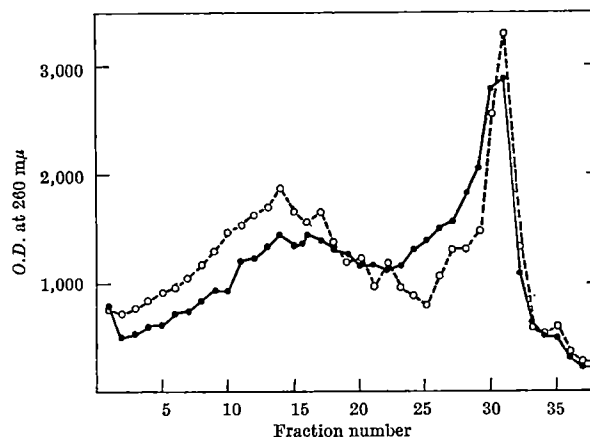


Fig. 3. Centrifugation of ribosomes in a sucrose gradient. Polysomes were analysed as described in Fig. 1 and the absorbance at 260 mμ plotted against the fraction number. Experimental conditions as described in Exp. 4, Table 2. —●—, 15-min incubation; —○—, 120-min incubation



Disregarding the fact that a small percentage of the newly synthesized proteins are not haemoglobin, it can be calculated that approximately 80 per cent of all polysomes are active in the synthesis, if there are 17.5 leucine residues per globin chain. These numbers are somewhat smaller than those published by Rich *et al.*<sup>22</sup>. The stimulating effect of iron and glucose has been described<sup>13</sup>. However, taking the labelling of the polysomes as a criterion for the activity of the cells, the stimulation at a high glucose concentration is only transitory and the activity of the polysomes decreases during two hours of incubation. The results suggest that this glucose effect is different from the stimulation by other substances. At the present time I cannot offer an explanation for this observation. The fact, however, that it is possible to depress the synthesis of proteins while preserving the polysomal structure raises some questions.

It has been shown in cell-free experiments that polysomes break down during protein synthesis and that as incubation proceeds the activity in the polysome peak gradually disappears and shifts to smaller units. Simultaneously the activity of the 80S ribosomes increases<sup>7,10</sup>. The limiting factor for the synthesis of proteins is, under these conditions, the re-attachment of the monosomes to the polysome cluster. My results indicate that during protein synthesis in whole cells no breakdown of polysomes takes place. A slight increase in polysomes sedimenting with a higher *S* value was found not only under conditions of reduced protein synthesis (Fig. 3) but also when the synthesis proceeds at a nearly equal rate. The relative activity of the polysomes after 2 h of incubation was somewhat displaced towards smaller structures, but the activity of the 80S fraction always showed a minimum.

The present theory of polysomal function in a monocistronic system, as assumed for the haemoglobin synthesizing apparatus, correlates the length of the prospective peptide chain and the length of the mRNA strand. At least 60 per cent of all polysomes appear in clusters of five or four ribosomes in electron micrographs<sup>8,24</sup>, and the length of the expected mRNA strand coincides with the spatial arrangement of these polysomes<sup>6</sup>. Gradient analyses of polysomes obtained by most investigators, however, show an equal distribution of various classes of polysomes. Since, due to the manipulations involved in the preparation of gradients, the contamination by RNases cannot be excluded, the degradation of pentamers to smaller units would be expected. The extreme sensitivity of polysomes to RNases was established by several groups. Mathias *et al.*<sup>24</sup> have demonstrated that even after sucrose gradient fractionation, all sizes of polysomes were found in electron micrographs taken from the fast sedimenting fractions. The omnipresence of RNases is a major disadvantage in all nucleic acid work. (Recently, E. Glowacki has shown that two major brands of creatine phosphokinase contain RNase activity, which is not destroyed by heating at 80° C (personal communication).) By disrupting the cells the balanced equilibrium inside the cell is disturbed and the probability of RNase action increases greatly. The occurrence of labelled monosomes with the continuation of the synthesis in cell-free systems, which is absent during whole-cell incubation, could be explained by RNase action on the mRNA strand. This process might be facilitated by the exposure of mRNA during the movement of the ribosomes. If, however, the detachment of monosomes with the completion of the growing peptide chain does not occur in whole cells, it is reasonable to assume that the limiting factor or factors for the protein synthesis have to intervene either before the arrangement of the amino-acids on the ribosomes or after the polypeptide chain is released from the polysomes. Our results clearly demonstrate that in whole cells the decrease of protein synthesis is independent of the polysome structure; they do not offer an explanation for the mechanism involved in the declining haemoglobin

synthesis in reticulocytes. Further experiments are necessary to investigate this point.

According to present-day concepts the breakdown of polysomes and the reattachment of monosomes to the ribosomal cluster are pre-requisites of protein synthesis<sup>2,7,9,11</sup>. Experiments with whole cells could not demonstrate the appearance of labelled monosomes in the cell at any time. Unless the release of the polypeptide chain in whole cells is much faster than in cell-free systems, the mechanism is different under the two experimental conditions. Differences with respect to the system were found in the replication of DNA: *in vivo* only one strand of the template is copied<sup>25</sup>, while in a purified system RNA is transcribed on both strands of the DNA duplex<sup>26</sup>. The reattachment of ribosomes to the polysome cluster *in vitro* was demonstrated by two groups<sup>7,8</sup>, however, in both cases, the monosomes attached preferably to the lighter polysomes instead of the expected equal distribution throughout the polysome peak.

In view of these observations, an alternative hypothesis for polysome function will be presented here. If the polysomes are arranged in closed circle configuration, as they actually appear in electron micrographs<sup>8,25</sup>, ribosomes, carrying the growing peptide chain, could move along the circular mRNA without being released. The information for the release of the completed chain and for the beginning of a new one would be imprinted somewhere on the mRNA molecule. The polysomes in this model would probably act more efficiently than if they were arranged along an extended mRNA strand with single ribosomes moving from one end to the other, detaching there and attaching on the other end of the strand again. The observation that the limitation of the synthesis is independent of the breakdown of polysomes in whole cells favours such a model.

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## A BIOACTIVE SUBSTANCE IN THE CAECUM OF GERM-FREE ANIMALS

## Demonstration of a Bioactive Substance in Caecal Contents of Germ-free Animals

IT was recently reported<sup>1</sup> that the supernatant of caecal contents from germ-free animals contains a substance (or substances) which is (a) toxic on parenteral administration and which (b) affects smooth muscle organs *in vitro* and cardiovascular function *in vivo*. Caecal contents from comparable conventional control animals showed such activities only in substantially reduced form.

**Preparations.** Germ-free mice (Swiss Webster) and rats (Wistar), reared under standard conditions<sup>2,3</sup>, fed uniformly steam-sterilized diet (L-462) (ref. 4) and killed by a blow on the head were used as donors. Caecal contents were diluted with saline to 15 per cent dry matter and centrifuged at 97,000*g* for 30 min. The caecal supernatant (CS) of germ-free (gf) and conventional (conv) animals obtained in this manner was essentially isosmotic with blood; pH was maintained at 6.8.

**Toxic effects.** Toxicity of mouse CS was tested by injecting it intraperitoneally into young, 20 g conventional mice. The results of these observations, shown in Fig. 1, indicate for CSgf an  $LD_{50}$  of approx. 0.5 ml.; CSconv in comparable doses was relatively non-toxic. The germ-free preparation, when lethal, killed the mice within 30 min to 6 h. The main symptoms were: skeletal muscle paralysis, cardiovascular distress and, terminally, respiratory failure. On autopsy, intensive vasodilatation was apparent in the abdominal viscera. In laparotomized

germ-free mice under pentobarbital sodium anaesthesia (35 mg/kg body-weight), extrusion of small amounts (about 0.5 ml.) of their own caecal contents into the peritoneal cavity was lethal in less than 1 h after successive wound repair. It is calculated that a germ-free mouse contains in its caecum 5- to 8-fold of its own lethal dose. Preliminary observations carried out with rat CS indicate the same trend. Toxicity of mouse and rat CSgf appears to be fairly similar, qualitatively and quantitatively.

**Effects on smooth muscle and on the cardiovascular system.** CS preparations cause contraction of the isolated rat uterus and guinea-pig ileum *in vitro*. A summary of these observations, in comparison with the effects of other musculo-active substances, is given in Table 1a. As the actions of CSgf aligned best with those of bradykinin, this peptide (synthetic bradykinin, Sandoz) was used as standard in a 'four-point assay' test<sup>5</sup>. A representative sample of mouse CSgf indicated an activity per ml. which was equivalent to  $0.64 \pm 0.13 \mu\text{g}$  bradykinin ( $\pm$  is S.E.). The slope of regression lines for bradykinin and CS was not significantly different ( $P < 0.05$ ). In this work Krebs-Henseleit solution was used with a 10 per cent aliquot of the calcium chloride added. The rat uterus was in oestrus; temperature was 37°C; the data refer to a 15-ml. muscle bath.

Table 1. ACTIONS OF GERM-FREE MOUSE (RAT) CAECAL SUPERNATANT

	CSgf	BK	ACH	5HT	HIST
(a) Isolated uterus (rat)					
No pre-treatment	+	+	+	+	0
Atropine sulphate 0.1 mg, D-lysergic acid diethylamide tartrate 1 mg/ml. bath	+	+	0	0	
Isolated ileum (guinea-pig)					
Atropine sulphate 0.1 mg, D-lysergic acid diethylamide tartrate 1 mg, triptenamine hydrochloride 1 mg/ml. bath	+	+	0		0
(b) Blood pressure (rat)					
No pre-treatment	↓	↓	↓		↓
Atropine sulphate 5 mg, triptenamine hydrochloride 6 mg/kg body-wt.	↓	↓	0		0
Blood pressure (dog)					
No pre-treatment	↓	↓	↓		↓
Cardiac output (rat)	↑	↑	↑		↑
Blood flow, intestines, liver (rat)	↑	↑			

CSgf, germ-free caecal supernatant; BK, synthetic bradykinin (Sandoz); ACH, acetylcholine chloride; 5HT, 5-hydroxytryptamine; HIST, histamine diphosphate.

+, contraction or positive effect; ↑, increase; ↓, decrease; 0, no effect.

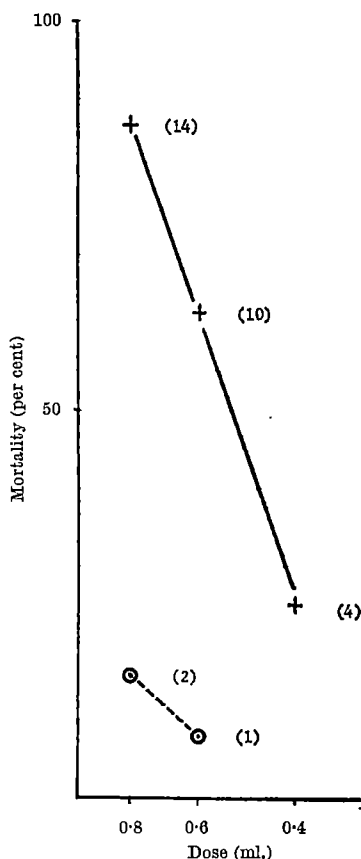


Fig. 1. Mortality of mice receiving intraperitoneal injection of caecal supernatant. Donors, gf and conv mice, young adults, ♂ ♀, L-462 steril. diet; recipients, conv mice, 6 weeks old, ♂, approx. 20 g body-wt., practical ration; +, germ-free caecal supernatant, 16 recipient mice per group; O, conventional caecal supernatant, 12 recipient mice per group. No. of deaths in brackets

CSgf containing activity which is comparable to the hypotensive doses of bradykinin causes pronounced fall of blood pressure in rats and dogs and an increase in cardiac output and regional blood flow in rats (mainly in the intestines and in the liver) on intravenous administration. These effects are temporary, lasting a few minutes. A summary of these observations along with results obtained with other hypotensive drugs are given in Table 1b. A parallelism between the actions of CSgf and bradykinin was also indicated in these instances. Blood pressure was recorded with a mercury manometer in the cannulated carotid (rat), or femoral artery (dog) under pentobarbital sodium anaesthesia (35 mg/kg body-weight). Cardiac output and regional blood flow were determined in rats (same anaesthesia) by the method of Sapirstein<sup>6</sup>. The time lapse between the intravenous injections of the drug or CSgf and of the indicator used (about 1  $\mu\text{C}$ . of  $^{86}\text{RbCl}$ ) was 75 sec.

**Occurrence of active substance in reference to species, diet and GI segment origin.** So far the occurrence of the active substance was ascertained only by its effects on smooth muscle. The substance was found present in caecal contents of young adult germ-free mice in comparable quantities, irrespective of the diet used. The diets were: steam-sterilized L-462, Purina 5010 C (closed

formula diet of Ralston Purina, Inc., St. Louis, Missouri), or the liquid diet of Greenstein *et al.*<sup>7</sup> sterilized by filtration (supplied by courtesy of General Biologicals, Chagrin Falls, Ohio). Germ-free mice and rats contain the substance in 6- to 8-fold concentration in comparison to their conventional controls. Among animals fed the same diet (steril. L-462), rats showed substantially higher levels of active substance than mice, irrespective of their microbiological status. Increased amounts of the substance in the GI tract of germ-free mice over conventional controls were clearly demonstrable in the ileum. However, the greatest discrepancy in this respect was attained in the caecum. In the colon and in the voided faeces the difference was waning.

**Physico-chemical and chemical properties of substance.** As yet these properties have been investigated only in reference to the effects of CS on smooth muscle.

(a) **Storage.** When stored at  $-18^{\circ}\text{C}$  for a period of 6 months, rat and mouse CSgf were found to retain at least 60 per cent of their original activity. Freezing and thawing (up to 10 times) were well resisted.

(b) **pH and temperature.** The substance (tested in mouse CSgf) appeared to be stable between pH 1 and 8 at room temperature over a period of at least 1 h. At temperatures higher than  $70^{\circ}\text{C}$  (pH 6-8, exposure 10 min and over) the activity was rapidly lost.

(c) **Dialysis.** On initial qualitative tests, the substance (from mouse CSgf) was found to pass a cellulose dialysis membrane (Fischer Scientific Co., Pittsburgh, Pennsylvania). In succession, quantitatively run observations indicated that during a period of 24 h at  $1^{\circ}\text{C}$  only a very small fraction of the substance was capable of passing the membrane.

(d) **Precipitation.** The active substance (tested in mouse CSgf) could be 'salted out' by saturation with ammonium sulphate. On successive dilution with saline, the precipitate was redissolved and a considerable proportion of the activity recovered.

(e) **Digestion.** Chymotrypsin, in conditions which were found to degrade bradykinin, did not alter appreciably the activity of mouse CSgf.

(f) **Adsorption.** It was found that various cationic exchange resins ('Dowex 50 X-4', 'Rexyn RG 51 H', 'Amberlite CG 50', 'Biorex 70') absorb the active substance and that, on successive elution with weak alkali, considerable proportions of the activity can be recovered. This method is at present used in column chromatography for initial purification of the active principle.

**Comments.** The toxic and musculoactive principles in caecal contents possibly represent two groups of substances. This is suggested by the observation that mouse and rat CSgf proved equipotent in terms of toxicity, while they are greatly different in terms of concentration of the musculoactive substance.

The close similarity in pharmacological effects between the musculoactive substance and bradykinin, the resistance of these effects to acetylcholine-, histamine- and serotonin-blocking drugs, as well as common adsorbability to cationic exchange of resins, indicate that the musculoactive substance belongs to the group of hypotensive peptides. Dependence on temperature and pH, the characteristics shown in the dialysis, precipitation and digestion experiments, suggests that we deal with a compound of higher molecular weight than bradykinin. In this context it should be noted that in the concentration of pancreatic and salivary kallikrein<sup>8</sup> and in that of caecal musculoactive substance the same species dependence seems to exist. In both instances the values observed in rats are 10-15 times higher than those in mice.

In normal life the intestinal flora appears to be directly or indirectly engaged in the inactivation of the toxic and musculoactive agents. In germ-free animals, that is, in absence of the flora, these substances accumulate in the gut lumen and may participate in the development of the anomalies which are observed in the lower bowel of

these animals (greatly distended and enlarged caecum, more liquid contents of the lower bowel).

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### Reduced Levels of a Bioactive Substance in the Caecal Content of Gnotobiotic Rats Mono-associated with *Salmonella typhimurium*

THE presence of elevated levels of a bioactive substance in the caecal content of germ-free rodents<sup>1</sup> has led us to speculate that this material may contribute to the enlargement of the caeca in these animals. The enlarged caecum, with its more liquid contents, is the most pronounced anomaly in the germ-free rodent. The reduction in the volume of the caecal contents and weight of the caecum is brought about by the introduction of the 'normal flora'<sup>2</sup> and occasionally by a single bacterial species<sup>3</sup>. The investigation recorded here was designed to determine whether the presence of *Salmonella typhimurium* (Lobund strain 750A), which was previously shown by the Lobund group to cause transient caecal reduction in the germ-free rat<sup>4</sup>, was also involved in the release or inactivation of the bioactive substance.

Six male and six female young adult germ-free rats (Wistar strain) were infected *per os* with a saline suspension of *Salmonella typhimurium* 750A. The animals were maintained in flexible plastic isolators<sup>5</sup> and fed autoclaved diet (L-462)<sup>6</sup>. At 2, 6, 17 and 45 days, groups of three rats were removed from the isolator, weighed, decapitated and the blood collected for immunological examination. Comparable untreated germ-free and conventional rats served as controls. After the caeca were removed and weighed, their contents were collected and the empty caeca reweighed. In the pooled caecal contents of various groups, bacterial counts were taken. In succession, the contents were treated and assayed for the musculoactive substance as described in the preceding communication.

Table 1 shows that in the untreated germ-free rats removed prior to infection (zero day) the caeca were enlarged and the level of the musculoactive substance was elevated. After 2 days of exposure to *S. typhimurium* the reduction of the size of the caecum was evident and the amount of bioactive substance had dropped below the quantity usually found in conventional caecal content. At this time the number of *S. typhimurium* averaged  $1 \times 10^9/\text{g}$  wet content. On the 6th and 17th days, the size of the caecum was still decreasing, but the amount of active substance showed some increase at these sampling periods. After 45 days of exposure, the size of the caecum

Table 1. CERTAIN CHARACTERISTICS OF CAECAL CONTENTS IN GNOTOBIOTIC AND CONVENTIONAL RATS

	Germ-free	Ex-germ-free ( <i>S. typhimurium</i> , oral inoc.) Days of exposure				Conventional
		2	6	17	45	
Weight of caecal contents g/100 g body-weight	11.8	4.4	3.5	1.8	7.1	0.7
Musculoactive substance in caecal supernatant, $\mu\text{g}$ BK equiv./ml.	18.6	0.6	1.3	2.8	10.3	2.5

Count of *S. typhimurium* was about  $1 \times 10^9/\text{g}$  wet caecal contents in all monocontaminated rats. 3-10 rats per group.



and the uteroactive substance had returned to about two-thirds of that found in the germ-free animal, yet the numbers of *S. typhimurium* in the caeca remained elevated. Circulating antibodies were not detected on the 6th day, but an agglutinin titre of 1:512 was obtained with serum from the rats killed on the 45th day.

When another group of 6 germ-free rats was contaminated orally with caecal content from the 45-day mono-associated rats, caecal reduction was evident within 48 h. One of the germ-free rats receiving the *Salmonella*-containing caecal content died 1 day after exposure. This suggests that the virulence and caecal-reducing potential of the bacterium was not altered during its 45-day association with the gnotobiotic rats.

The results of this work were similar to those obtained in a previous experiment in which *S. typhimurium* was superimposed *per os* in gnotobiotic rats monoassociated with an innocuous intestinal bacteroid species<sup>7</sup>.

The apparent parallelism which exists between the fall and rise of the musculoactive substance in caecal contents and the transient nature of the caecal reduction on monocontamination permit the speculation that there was causal relationship between these two phenomena. The appearance of circulating antibodies suggests that

the host overcomes the infection by responding immunologically to the infection. This response, while protecting the host, places the animal in a carrier state—a state which allows the persistence of high numbers of *S. typhimurium* in the intestinal tract, but permits the animal to re-assume the characteristics of germ-freeness.

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## MITOTIC HOMEOSTASIS

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IT has long been obvious that each of the tissues of an adult mammal has its own characteristic average mitotic rate which is in balance with its average rate of cell death. From time to time the point of balance may alter, but the balance itself remains. It is also well known that after a period of cell damage or destruction most tissues are able to react by developing an abnormally high mitotic rate which persists during the period of repair and, further, that certain tissues possess a capacity for increasing their mitotic rate as a response to some special physiological demand. A knowledge of the homeostatic mechanisms that underlie these various mitotic phenomena is obviously of fundamental biological importance, and it is equally obvious that no real understanding of the problem of cancer, which must involve a partial or complete breakdown of these mechanisms, is likely to be achieved until this knowledge is obtained.

The problem of mitotic control has often been discussed<sup>1</sup> and it has commonly been suggested that humoral substances may be involved acting as inhibitors in a negative feedback mechanism, as stimulants in a positive feedback mechanism, or as both. It is sometimes implied that such substances may have a generalized action within the body<sup>2</sup>, but the mitotic independence of the various tissues indicates clearly that each must possess its own specific mitotic control mechanism.

In the past few years considerable new information has been obtained, especially regarding mitotic control by negative feedback in epidermis<sup>3</sup> and by both negative and positive feedback in the granulocytic and erythrocytic systems<sup>4,5</sup>. Although this and other information now available relates only to a few tissues or organs, it is becoming increasingly obvious that all mitotic control mechanisms have certain common themes and that the various situations found in different tissues are merely variations on these themes. This article represents a new assessment of the evidence and it indicates some of the essential features of mitotic homeostasis in the whole body.

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### Mitotic Control by Chalones

In recent years much information has been obtained to indicate that certain tissues contain, and presumably produce, substances which depress mitotic activity<sup>3</sup>. Such substances have been termed 'chalones', and each appears to be tissue-specific in that it acts only on its tissue of origin. The first chalones to be extracted were those of the liver and kidney, and they were found to be water-soluble, non-dialysable and relatively unstable<sup>6</sup>.

More recently an epidermal chalone has been extracted and has been found to depress mitosis in normal epidermis and in the corneal and oesophageal epithelia<sup>3</sup>. It has similar physical characteristics to the liver and kidney chalones. In normal epidermis the mitotic rate appears to be inversely proportional to the chalone concentration within the cells, and this, together with the inherent life expectancy of the cells, evidently determines the size of the cell population. When epidermal cells are damaged there appears to be a decrease in the rate of chalone production, an increase in the rate of chalone loss, or both, and the consequence is a local increase in the mitotic rate. The mitotic rate returns to normal only after a sufficient number of new and normal cells have been produced to establish once again the normal chalone concentration in the affected area.

Still more recently a granulocytic chalone has been extracted from serum containing mature granulocytes, and its characteristics also resemble those of the other chalones<sup>4</sup>. The mitotic activity that leads to the production of granulocytes occurs in precursor cells in the bone marrow as part of the process of granulopoiesis. The mature granulocytes are finally released through the blood to the tissues, where their ultimate fate is death<sup>7</sup>. It is these mature cells which evidently contain the chalone that inhibits mitosis in the precursor cells.

There is also strong evidence for the existence of an erythrocytic chalone. It is known that erythrocyte production may be suppressed after an adequate erythrocyte transfusion, and it has recently been found that the serum

from rats containing extra transfused erythrocytes depresses the rate of erythrocyte production in bone marrow *in vitro*<sup>4</sup>.

It is important to note that, at least in the epidermis, the chalone itself does not appear to be fully functional and that the effective mitotic inhibitor may be an unstable chalone-adrenalin complex<sup>3</sup>. This observation provides an explanation for the well-known diurnal mitotic rhythm in the epidermis: in sleeping mice, with low output of adrenalin, the complex breaks down and the mitotic rate rises; when the mice wake up the output of adrenalin rises, the complex re-forms and the mitotic rate falls. It is important to note that a recent survey has shown that a diurnal mitotic rhythm essentially similar to that in the epidermis has been described in at least 15 other tissues<sup>8</sup>, and the obvious inference is that in all of them mitotic control may be exercised through unstable chalone-adrenalin complexes. The tissues which do not show any obvious diurnal mitotic rhythm are those in which the mitotic rate is either very low or very high, or in which it is hormone-dependent.

Other evidence indicating the existence of basically similar mitotic control mechanisms in a variety of tissues comes from mitotic reactions to wound healing and regeneration. In epidermis the infliction of a wound evidently results in a reduced chalone concentration in the closely adjacent cells and a consequent local increase in the mitotic rate, which because of the lack of chalone shows little if any diurnal rhythm<sup>3</sup>. This local mitotic reaction has the form of a gradient with a length of about 1 mm and with the highest point at the wound edge. Similar local mitotic reactions have been described in all tissues which have been studied from this point of view<sup>8</sup>, and it is also important to note that the reaction to wounding is tissue-specific<sup>9</sup>.

As regards the general mitotic reaction seen during regeneration, the large literature is confusing and contradictory and much of the published work is of doubtful significance. A critical survey has recently been prepared<sup>8</sup>. However, in the case of the liver it has been clearly shown that, when the damaged tissue exceeds about 10 per cent of the whole, the local wound reaction begins to be accompanied by a general reaction whereby the mitotic rate rises throughout the whole liver<sup>10</sup>. Beyond this point the intensity of the general mitotic response is in direct proportion to the percentage of tissue destroyed. Such a quantitative relationship is, of course, exactly what would be expected when a local chalone shortage due to wounding gives place to an increasing general chalone shortage due to the decreasing amount of normal tissue within the total body space. It has indeed been shown that a damaged kidney loses all its detectable chalone<sup>6</sup>.

**Site of chalone action.** In considering the possible points of chalone action within the affected cells, it is necessary to consider the cycle of phases through which a cell passes from one mitosis to another<sup>11</sup>. These are: *dichophase*, when the cell 'decides' whether to prepare for another mitosis or whether instead to prepare for function within the tissue; *prophase*, the period of preparation for mitosis which includes the phase of DNA synthesis (or *S*) and the antephase (or *G<sub>2</sub>*); *mitosis* itself; and *apophase*<sup>8</sup>, when each daughter cell re-establishes its normal synthetic mechanisms and grows to full size.

Since the effect of the presence of a chalone is to reduce the number of cells preparing for mitosis, it is evident that its main action must be exerted prior to the prophase, probably in the dichophase. Indeed, the implication is that the 'decision' taken by a cell in the dichophase may be dictated by the intracellular concentration of the chalone, which determines the type of synthetic activity in which the cell will indulge. Thus, in the absence of sufficient chalone the cell will embark on that series of enzyme syntheses that characterizes the prophase, while in the presence of sufficient chalone it will synthesize

those enzymes needed for typical tissue function. Any such 'decision' relating to synthetic activities is most likely to involve gene action, and indeed the situation is strongly reminiscent of that in micro-organisms in which the activity or inactivity of particular regions of the genome is determined by substances called 'effectors'<sup>12</sup>. A micro-organism is capable of many alternative syntheses that can be activated or inactivated in this way, but any typical cell in an adult mammal may be capable of only two. These may be under the control of two groups of genes, which for simplicity may be termed respectively the 'mitosis operon' and the 'tissue operon'.

Chalones are evidently tissue-specific substances, and if they act at gene level this can only mean that the operon on which they act must also be tissue-specific. Although it is not yet certain whether the 'mitosis operon' can be regarded as tissue-specific, it is obvious that the 'tissue operon' must be. The possibility, therefore, arises that chalone action may be to promote the functional activity of the tissue cells and that the inhibition of mitosis may be the secondary outcome of this action.

This could explain why so many cells when released from normal chalone control, whether by incubation *in vitro*, or during local or general regeneration, or in neoplasia, merely revert to the basic function of all cells, which is to grow and to divide indefinitely. It would also indicate that the state of functional maturity of most adult tissues must be regarded as unstable in that throughout life it needs always to be supported by adequate concentrations of the appropriate chalones.

**Length of mitotic cycle.** The operon theory of chalone action could also provide an explanation for the length of time a cell remains in dichophase: with a low chalone concentration the cell should immediately pass into prophase, with a high concentration it should immediately begin synthesis for tissue function, but with an intermediate concentration it might remain poised between these two alternatives for an indefinite time. It should follow that with a decreasing chalone concentration, not only should more cells tend to enter mitosis but also they should tend to pass through the mitotic cycle more quickly, and indeed it is well known that in wounds or during regeneration this does occur. It is also well known that these shorter cell cycles are due mainly to a reduction in what has been commonly called the *G<sub>1</sub>* period. In the present terminology this comprises the apophase, dichophase, and the pre-DNA synthesis period of the prophase, and of these it is only the dichophase which is likely to be capable of any great variation in length.

However, although the main action of a chalone on the duration of the mitotic cycle may be exerted in the dichophase, it is now clear that in both the epidermis and the granulocytic system the chalone concentration also affects the speed of the whole process prophase-mitosis, although in both tissues there is a maximum speed that cannot be exceeded. In the granulocytic precursor cells of a normal animal, preparation for mitosis is already proceeding at this maximum speed, but, if through an excess of chalone the mitotic rate is reduced, so also is the speed at which the cells enter and pass through DNA synthesis<sup>4</sup>. In normal epidermis, where the mitotic rate is lower, the speed at which cells pass through antephase-mitosis is moderate: with increasing chalone concentrations fewer cells proceed at a slower rate, while with decreasing chalone concentrations more cells proceed at a faster rate until the maximum speed is reached<sup>13</sup>.

It appears that the most sensitive periods are the dichophase and the antephase, both of which may be blocked by excess chalone, while the least sensitive periods are the phases of DNA synthesis and of mitosis, both of which may be slowed but apparently not stopped. This might be taken as evidence that a chalone does exercise some direct inhibitory action on mitosis, in which case it might follow that the mechanism of mitosis may contain one or more tissue-specific components.

*Types of tissue cells.* It has been suggested that, in any typical tissue, specialization for mitosis and specialization for function are merely opposite expressions of the same homeostatic mechanism. At any given moment a tissue rests at a point of balance with a certain proportion of its cells involved in mitosis, a certain proportion involved in functional activity, and the remainder poised part-way between the two. The position of this point of balance varies widely in different tissues so that, for example, within the crypts of Lieberkühn a relatively large percentage of the cells are involved in the mitotic cycle, while in epidermis the percentage is much smaller.

Further consideration of such examples indicates that a typical tissue is composed of at least four distinct types of cells (see Fig. 1): the progenitor cells (*P*), which are involved in the mitotic cycle; the immature cells (*I*), which are preparing for tissue function; the mature cells (*M*), which are specialized for tissue function but which if the effective chalone concentration falls are capable of reversion to mitosis; and the mature cells (*D*), which are also specialized for tissue function but which are moving towards death and are unable to revert to mitosis.

In epidermis, *D* includes only the few cells of the stratum granulosum, whereas in the granulocytic system it includes the large group of mature granulocytes distributed between bone marrow, blood and tissues, and in the erythrocytic system it includes the large group of the non-nucleated erythrocytes. Cells leave *D* only to die.

It must be stressed that the borders between the four groups of cells are not necessarily sharply defined; rather, they should be regarded as broad zones across which one cell type grades into the next. It must also be stressed that the functional cells may be those of type *M* as in the liver, or those of type *D* as in the granulocytes and erythrocytes, or possibly in some tissues those of both type *M* and type *D*.

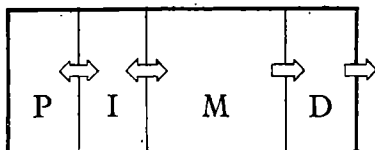


Fig. 1

The mitotic rate of the tissue illustrated in Fig. 1 is expressed partly by the size of *P* and partly by the fact that, within limits, the larger the size of *P* the faster the speed at which the cells prepare for mitosis. The rate of production of new cells determines the rate of flow of the cells from left to right, and at the point of balance the cell production rate exactly equals the cell death rate. The size of *P* is inversely proportional to the concentration of the chalone complex in the tissue, and it appears probable that the chalone may be produced mainly by the cells in *M* and *D*. Thus the chalone concentration is a function of the size of *M* and *D*, of the rate of synthesis, of the stability of the molecule, and of the rate of loss both from the tissue and from the body.

*Life expectancy of the mature cells.* In normal circumstances the chalone concentration appears to be relatively constant, but if its efficiency is increased by the addition of extra adrenalin, then mitosis is further inhibited, which means that *P* is reduced in size and that its cells also prepare for mitosis more slowly. It has been observed in epidermis and sebaceous glands that when, in stress, the mitotic rate is reduced to about a quarter of the normal, the mass of the tissue ( $P+I+M+D$ ) remains unchanged for at least a month<sup>14</sup>, which must indicate that the rate of flow through the system and the rate of cell death are both equally reduced. This new situation is expressed in Fig. 2, in which it is suggested that the chalone-adrenalin complex has pushed the *M*-*D* barrier to the right, although, of course, the rate of cell death

would equally be reduced if the chalone complex acted directly on the *D*-death barrier. In either case the reduced rate of flow from mitosis to death must mean an increase in the average life-span of the mature cells.

The opposite situation of relative chalone inefficiency follows the withdrawal of adrenalin, for example, after adrenalectomy. In these circumstances in epidermis the mitotic rate increases about three times<sup>3</sup>, but again there is no evidence of any alteration in the tissue mass. Thus the increased rate of cell production must have been offset by a shorter life-span of the functional cells. A more extreme situation can be obtained through chalone shortage induced by any form of cell damage. The resulting high mitotic activity is again accompanied by a reduced life-span of the mature cells, as has been shown both in the cirrhotic liver<sup>15</sup> and in psoriasis<sup>16</sup>. In the latter, the life of the maturing epidermal cells is reduced from a normal average of 27 days to a new average of about 4 days.

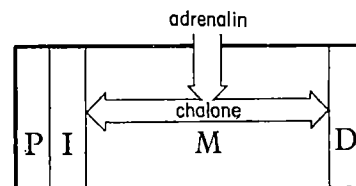


Fig. 2

It thus appears that if the primary action of a chalone is to promote those specific syntheses that are essential for tissue function, then this action has two consequences: the suppression of mitosis and the promotion of cell survival. It could be argued that longer cell survival might be the consequence of the increased efficiency of the tissue-specific syntheses, especially in *M*.

It is also important to note that the inverse relationship between the rate of cell production and the life expectancy of the mature cells only operates within limits to maintain the normal tissue mass, and that beyond these limits the tissue mass may change. Thus the high epidermal mitotic rate which results from continued irritation is accompanied by a moderate increase in epidermal thickness, and the tissue becomes stabilized at a new point of balance.

*Conclusions.* The main conclusion arising from this survey is that in most tissues mitotic homeostasis may be ensured by the effector-like action of a chalone, the concentration of which determines whether the 'mitosis operon' or the 'tissue operon' is active. However, in such tissues as nerves and striped muscle, which never show any mitotic activity, there is evidence that the 'mitosis operon' may be permanently blocked and that the synthesis of tissue proteins may be dictated at the ribosome level rather than at the DNA level. In such circumstances a chalone mechanism would be redundant.

Not only does a chalone inhibit mitotic activity but it commonly also lengthens the life expectancy of the mature cells. The consequence is that moderate changes in the mitotic rate leave the tissue mass unchanged, although large changes in the mitotic rate may result in moderate changes in the tissue mass. It also appears that the life expectancy of the mature cells is in some degree tissue-specific, and it may be suggested that the size of any tissue within the total body space and the mitotic activity of that tissue may be a function of the rate of chalone production and of the life expectancy of the mature cells. Thus, for example, a tissue of large mass and low mitotic rate may be characterized by a low rate of chalone production and a long cell life.

Adrenalin, and possibly also a glucocorticoid hormone, commonly acts to strengthen chalone function, and since the homeostatic mechanism was obviously evolved in wild animals it may only operate efficiently in conditions of moderate stress. Unstressed laboratory animals, like



tissues and organs *in vitro*, may present an abnormal situation with weakened mitotic control. The role of the adrenal glands in a stressful situation, such as starvation, is to reduce the mitotic rate and to prolong the functional cell life, and the survival value is obvious.

### Other Specific Control Mechanisms

Although it is possible that, in mammals, local or general tissue regeneration may be explicable mainly or even entirely in terms of chalone action, it is obvious that in certain tissues the mechanisms of mitotic homeostasis must be more complex. In particular, it is well known that in some tissues mitotic activity is hormone-dependent while in others the rate of cell renewal is at least partly poietin-dependent. These are tissues which, from time to time, must be able to increase the rate of cell production and also the tissue mass in response to physiological demand.

**Mitogenic hormones.** In tissues in which growth is dependent on mitogenic hormones the reaction to the presence of the hormone is two-fold: the mitotic rate increases, and the new cells so produced become functionally active. Thus, when the vaginal epithelium is stimulated to mitosis by oestrogen it also actively produces keratin, and when the thyroid is stimulated to mitosis by thyrotropin it also actively produces and secretes the thyroid hormone. An analysis of the details of this type of reaction in the vaginal lining indicates that in the absence of the mitogenic hormone the tissue rests at a point of balance characterized by a small mass, a negligible mitotic rate, and a long cell-life expectancy. All the cells appear to be of type *M* and to be non-functional. The effect of the addition of oestrogen on the point of balance in the tissue is illustrated in Fig. 3. First, many of the cells revert to type *P* and indulge in high mitotic activity, and then, as new cells are formed, these pass back through *I* to *M*. However, the new mature cells have a much shorter life expectancy, which has been estimated to be between 30 and 45 h<sup>17</sup>, and they, therefore, pass beyond *M* into *D*. It is this final type of cell which is functional and which actively produces keratin before it dies.

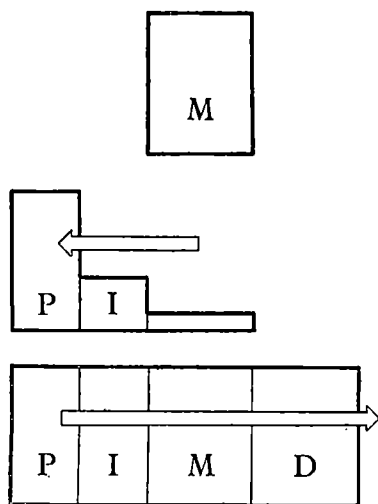


Fig. 3

Although in this type of tissue the greatly increased mitotic rate is partially offset by the reduced life expectancy of the mature cells, it nevertheless results in a moderate increase in tissue mass. The tissue then comes to rest at a new point of balance, and it remains there as long as the hormone stimulus is maintained.

Although it is only possible to speculate, the simplest hypothesis is that a mitogenic hormone, either directly or

indirectly, specifically neutralizes the chalones of its target tissues, the reaction then being basically due to chalone shortage. In support of this, a detailed analysis has indicated many close similarities between a hormone reaction and a wound reaction<sup>8</sup>, but even more important has been the recent suggestion that in mammary gland *in vitro* mitotic activity may develop when a sub-threshold hormone stimulus is added to a sub-threshold wound stimulus<sup>18</sup>.

Thus a mitogenic hormone may be regarded, at least tentatively, as part of a control mechanism which is imposed on the basic chalone mechanism. In response to some trigger stimulus, usually originating outside the animal, the hormone is produced and the hormone-dependent tissue is enabled to increase both in size and in functional activity. A typical example is the well-known effect on the ferret of an increase in day-length in spring which activates the pituitary gland to stimulate the gonadal production of oestrogens or androgens.

**Poietins.** There are at least three important tissues, the erythrocytic, granulocytic and lymphocytic systems, which appear to possess unusually complex mechanisms for the control of cell production. Unlike the hormone-dependent tissues their basic condition would seem to be one of relatively high mitotic activity which, at least in the erythrocytic and granulocytic systems, is controlled by a normal chalone mechanism<sup>4</sup>. The mature erythrocytes and granulocytes differ from such ordinary tissues as epidermis in that their life expectancy seems to be unrelated to the mitotic rate of the progenitor cells. The average life of a rat erythrocyte is about 60 days; the life of a granulocyte is unpredictable since the death of these cells is usually determined not by age but at random. Granulocytes die shortly after they have functioned as scavengers<sup>7,19</sup>.

The process of erythrocyte production, called erythropoiesis, is dependent partly on the mitotic activity of the erythrocytic precursor cells and partly on the production of new progenitor cells by the transformation of stem cells, which also evidently possess the alternative potentiality of transformation into granulocytes<sup>20,21</sup>.

In the erythrocytic system the chalone, which may perhaps be produced in the circulating erythrocytes, seems to influence only the mitotic activity of the progenitor cells in the bone marrow<sup>4</sup>, and in the usual way it may help to maintain the tissue mass. However, this is a tissue which must be able to adjust its rate of cell production not only in terms of mass but also in terms of function. Thus a reduced oxygen tension increases the functional demand for oxygen and results in the rapid production of erythrocytes in excess of the normal number. It is now known that this reaction depends on the active secretion of the substance erythropoietin<sup>5</sup>, although the secretion site is not yet known.

The mode of action of erythropoietin in stimulating the rate of cell production is quite unlike that of a mitogenic hormone. A survey of the large literature indicates that it does not change significantly either the mitotic rate of the erythrocytic progenitor cells, or the maturation rate of the developing erythrocytes<sup>22</sup>. Its primary action is to promote the conversion of stem cells into erythrocytic progenitor cells<sup>20,22</sup>.

An important question thus arises as to the nature of these stem cells, of which unknown numbers are present in bone marrow. Since it is generally agreed<sup>19-21</sup> that they may give rise to the cells of at least two distinct tissues, the erythrocytic and granulocytic systems, it is evident that they must be regarded as pluripotential cells in which final differentiation has not yet occurred. Thus the conversion of stem cells into erythrocytic progenitor cells must be regarded as an essentially embryonic process of differentiation, and erythropoietin must be regarded as an inducing substance. Little or nothing is known of the way in which the stem cell population maintains its numbers<sup>20</sup>, but it is obvious that as its cells are lost by

differentiation the remaining stem cells must recruit their numbers by mitosis. The implication is that a mitotic homeostatic mechanism must exist and it is possible that this may be controlled by a chalone mechanism. A further implication is that the cells which are recognizable as belonging to the erythrocytic tissue may not be self-maintaining. The mitotic activity of the progenitor cells appears to result more in the production of large numbers of erythrocytes than in the production of more progenitor cells.

The situation in the granulocytic tissue seems to be essentially similar to that in the erythrocytic tissue in that the cells are recruited from the stem cell population by the action of an inducing substance, called granulopoietin, and in that the progenitor cells undergo a series of mitoses which are controlled by a chalone. The probable relation of the erythrocytic and granulocytic systems to the stem cell population is illustrated in Fig. 4.

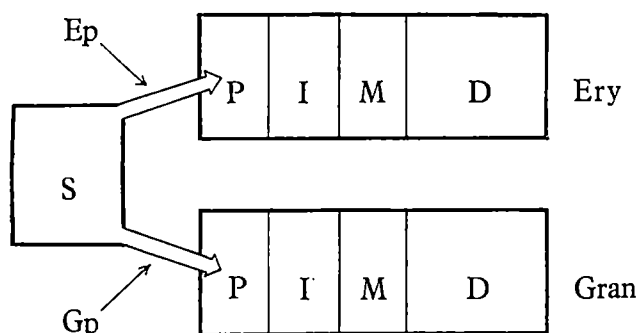


Fig. 4. Diagram of the relations of the erythrocytic (Ery) and granulocytic (Gran) systems with the stem cell population (S). Differentiation is induced either by erythropoietin (Ep) or granulopoietin (Gp).

A substance that has been called 'granulopoietin' has been obtained from rat serum and has been partly purified<sup>4</sup>, and it has been shown to increase the number of functional granulocytes produced. However, analysis has indicated that this increase may be due more to the increased mitotic activity of the progenitor cells than to the differentiation of the stem cells<sup>4</sup>. Thus the status of this substance is not yet clear, and it may be wiser to reserve the name granulopoietin for whatever factor is finally shown to induce the formation of the granulocyte progenitor cells.

**Antigens.** The only other major tissue of the adult mammal which has been clearly shown to be dependent on a stem cell population is the lymphocytic system. This appears to be in some way related to the erythrocytic and granulocytic systems, and it has even been suggested that all three may be derived from the same stem cells<sup>23</sup>. Although less is known about the manner of cell gain and cell loss in the lymphocytic system, there is sufficient evidence to indicate a basic similarity with the erythrocytic and granulocytic systems.

There is still considerable argument concerning the identity and the activity of the lymphocytic stem cells<sup>23</sup>, but it is possible that they may produce their own 'mature' type of cell, which may be the small lymphocyte. If this is true, then the normal high rate of small lymphocyte production must indicate an equally high rate of loss, either through differentiation or death, and the continuing balance of the stem cell population must indicate the existence of a mitotic homeostatic mechanism. In this connexion it is interesting to note that granulocytes can depress mitotic activity in lymphocytic stem cells<sup>24</sup>, and this may perhaps mean that the granulocytic chalone exercises some control over the multiplication of all stem cells.

The differentiation of the lymphocytic stem cells evidently occurs in response to antigen invasion, when "the

antigen, like the (embryonic) inducer . . . commits a cell to a certain pathway of differentiation . . . among several it might have taken"<sup>25</sup>. The result of such differentiation is the production of a group of progenitor cells which undergo a series of mitoses as they mature into a group of plasma cells. These then constitute a new tissue which synthesizes the appropriate antibody and possibly also a chalone.

**Conclusions.** When for physiological reasons it is necessary to increase both the mass and the functional activity of a tissue this may be achieved by a hormone which causes an increase in the mitotic rate, or by a poietin which causes new cells to be introduced into the tissue, or by an antigen which induces the creation of a new tissue. All these reactions depend on some stimulus which commonly originates externally to the animal.

The mitogenic hormones may be target-tissue-specific because of some steric affinity with the tissue chalone which they may neutralize. It has sometimes been suggested that all tissues may be subject to this type of mitotic stimulus, but the evidence is inadequate and the proposition is doubtful.

The poietins and antigens act primarily on relatively undifferentiated stem cells to induce the final step in their process of differentiation. This is a type of mechanism which must be common to all tissues, but it is one which in mammals normally operates only in the embryo. It is evident that the homeostatic mechanisms in the lymphocytic system may be the most complex that exist in the adult mammal.

### Mitotic Homeostasis and Carcinogenesis

It is reasonable to believe that carcinogenesis is the result of some breakdown in the control of cell production and cell maturation, and that such a breakdown may be the result of damage at the nuclear or the cytoplasmic level. From the evidence given above, it is clear that any failure in the chalone mechanism would be expected to result in increased mitosis, reduced functional maturity, and a reduced life-span for any cells that were able to mature. It is well known that all these are common characteristics of tumour cells. There are several possible points at which the chalone mechanism could be damaged<sup>26</sup>, but in all cases the consequence should be either a shortage of chalone or a failure to react to whatever chalone is present.

In considering the chalone theory in relation to carcinogenesis it is unfortunately necessary, in the limited space available, to over-simplify the problem and to deal very inadequately with the relevant literature. However, a more extended statement is in preparation<sup>8</sup>. It is commonly believed that the initial damage which creates the incipient tumour cell is permanent and irreversible. Since even within the one tissue this damage may lead to very different types of tumours it is clear that it must be very variable, both quantitatively and qualitatively. However, in all cases it must include damage to the mitotic and functional homeostatic mechanism. If this results in a failure to respond to the chalone that is present, then the damaged cells would be expected to lose immediately their ability to function as part of the tissue, to commence unhindered mitotic activity, and to form a rapidly growing malignant tumour. If, however, the damage results in a failure to synthesize chalone, then nothing at all may happen since the damaged cell, being embedded in a mass of normal cells, should continue to be controlled by the chalone which it receives from these normal cells. In this way it may remain relatively quiescent for a long time. Indeed, no tumour may develop at all until, as a result of slow proliferation, the damaged cell gives rise to a group of abnormal descendant cells which is so large (perhaps about 1 mm diam.) that its central region is beyond the effective range of the inward-diffusing chalone.

The length of this period of quiescence must normally be a matter of chance, but it is evident that it should be shortened in any situation, such as adrenalectomy or tissue damage, that tends to weaken the chalone mechanism in the surrounding tissue. It is now well known that both adrenalectomy<sup>27</sup> and tissue damage<sup>28</sup> do indeed have this effect and, as would be expected, so also do the mitogenic hormones and possibly the poietins. Conversely, the period of quiescence should be lengthened by any situation, such as stress, that strengthens the chalone mechanism and, indeed, one of the most potentially significant observations in the whole history of cancer research is that in mice stressed by partial starvation the period of tumour cell quiescence may be so prolonged that cancer never develops at all<sup>29</sup>. A major effort to explain this effect in terms of diet *per se* has failed, but when it is interpreted in terms of stress it falls into line with the results obtained with other forms of stress<sup>30</sup> and with the glucocorticoid hormones<sup>27</sup>.

When the quiescent period ends, the tumour which then appears is commonly still slow-growing, and it may even come to rest at some new point of balance. In such cases the cells are able to respond, at least partially, to changes in chalone concentration or efficiency. Thus, when excess granulocytic chalone is introduced by the transfusion of fresh blood, a myeloid leukaemia may be temporarily suppressed<sup>31</sup>; when the liver chalone concentration is reduced after partial hepatectomy there is a greatly increased mitotic rate in small adenomatous nodules<sup>32</sup>; and in some tumours a diurnal mitotic rhythm may even be found<sup>33</sup>.

It also seems probable that hormone-dependent tumours must continue to react to chalones. If a mitogenic hormone exerts its effect through the specific neutralization of the tissue chalone, then an especially great mitotic reaction may be expected in any incipient tumour cells which are producing less than the normal amount of chalone. When the hormone is withdrawn such cells may still be able to produce enough chalone to reduce the mitotic rate and even to cause tumour regression, but when the hormone is restored the tumour should grow again in the same position. Such reactions are well known, for example, in the mammary gland<sup>34</sup>.

A tumour does not normally remain benign and it is common for its cells to undergo progressive changes leading to greater malignancy. When this process reaches its extreme the cells show a high mitotic rate combined with a complete inability to specialize for tissue function. In such cells there may be either a failure to produce any chalone at all or a failure to react to what chalone they do produce, and there is some evidence that both these situations may occur. Regarding the first, it has been noted that malignant leukaemic granulocytes may be unable to induce a chalone-like inhibition of lymphocyte mitosis<sup>35</sup>. Regarding the second, it has been found that other malignant leukaemic granulocytes do continue to produce a chalone-like substance capable of inhibiting mitosis in normal granulocytic progenitor cells *in vitro*<sup>4</sup>, but that they themselves are unable to respond to the chalone of normal granulocytes<sup>34</sup>.

At the moment the relevance of the chalone theory to carcinogenesis is only tentative, but it is certainly reasonable to suggest that the chances of active tumour growth, whether during or just after the period of quiescence, are likely to be significantly reduced if the normal homeostatic mechanism of the tissue is strengthened. Conversely, it is also relevant to question whether the low-stress effects of domestication and of a high standard of living may tend to promote the earlier and more frequent appearance of cancer.

### General Conclusions

It is obvious that the mechanism underlying mitotic and functional homeostasis in adult mammalian tissues

will prove to be far more complex than has been suggested here, but it will be enough if the present relatively simple theory can provide some basic insight into this difficult field and if, for a time, it can act as a useful conceptual basis on which future work can be planned. Recent years have seen great advances in biology, especially in the field of genetics, and one of the most baffling of all remaining mysteries is the phenomenon of differentiation, which begins in the embryo but which continues throughout life as part of the mitotic and functional homeostatic mechanism discussed here. In pathology, one of the most important of all problems concerns the manner in which this homeostatic mechanism may be weakened to permit the development of cancer, and in a recent critical article<sup>35</sup> it has been remarked that "it is biocybernetics, the science of organismal organisation . . . which must take over from cancer research". The problems discussed here form a basic part of this branch of science, which must become increasingly prominent in years to come.

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# TEMPORARY HAIR LOSS ASSOCIATED WITH THE SLATE MUTATION OF COAT COLOUR IN THE MOUSE

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'SLATE' (*slt*), an autosomal recessive mutation in the mouse characterized by a dilution of coat colour, arose spontaneously in strain YZ57/*Ch* (ref. 1). Investigations of the effect of *slt* on coat colour indicated that it could be traced to the concentration of the melanin pigment of the hair into a reduced number of large aggregated pigment granules<sup>2</sup>. In addition to its effect on melanin pigmentation, *slt* also appeared to exert some effect on the hair growth process. The observations to be described indicated that *slt* was capable of inducing the hair follicles to lose their hairs prematurely and, in some cases, of inhibiting the initiation of the subsequent hair growth cycle.

In the mouse, as in other mammals, hair growth is a cyclic process. Hairs are produced within follicles, with periods of hair production alternating with periods of follicular quiescence. The hairs produced during a given period of follicular activity are retained within the follicles for variable periods which overlap with the appearance and growth of hairs during subsequent cycles. Older hairs, the so-called club hairs, are only gradually lost and no extensive 'moulting' process is observed. In the 'Slate' strain, which was derived from a mating between the original mutant and a normal sibling female, approximately 5 per cent of the *slt* homozygotes lost most of the dorsum hairs of the first coat during the fourth week after birth, that is, following the cessation of the first hair growth cycle. An apparently normal second coat was grown by affected animals, although the onset of the cycle appeared to be somewhat delayed in some cases. Coats of affected animals were generally normal in appearance for the remainder of the life of the animals, but in some cases the coats of older animals became somewhat sparse. Matings between animals in the original 'Slate' strain produced about the same proportion of affected progeny regardless of whether or not one or more of the parents had shown the temporary hair loss condition. That *slt* was important for the determination of the temporary hair loss condition under investigation was demonstrated by observations made when *slt* was introduced into other backgrounds.

During the course of investigations carried out in order to determine the pattern of inheritance of the coat colour dilution observed in the 'Slate' strain, as well as to test for allelism with various other pigmentation mutations, 'Slate' was mated to a number of different strains. When 'Slate' was mated to *CT/Ch*, a male which showed a rather extreme expression of the temporary hair loss condition was recovered in the  $F_2$  generation. This animal was homozygous for maltese dilution (*d*) as well as for *slt* and heterozygous for pink-eye (*p*) and for brown (*b*). Its coat appeared normal until early in the third month, when all the hairs on its dorsum were lost. The animal did not grow a new coat of hair until five months later. From the  $F_2$  of the cross between 'Slate' and *CT/Ch* four separate stocks of mice were set up. These stocks were homozygous for *p*, *pd*, *d slt* (*DSLTL*) and *p d slt* (*PDSLTL*), respectively. All stocks were continued by brother-sister matings for at least four generations, but the hair loss characteristic was observed only in two stocks, *DSLTL* and *PDSLTL*.

The *DSLTL* stock was initiated by mating the affected  $F_2$  male described here to a non-sibling  $F_2$  female. This mating produced one male which also lost the club hairs from his dorsum during the third month. When this male was bred to his normal sibling sisters some variation of the temporary hair loss trait was observed in 40 per cent of the offspring ( $N = 35$ ). Of the affected progeny, two were *d slt*, seven were *d b slt*, and five were *p d slt*. In the *d slt* animals, hair loss was observed during the third month, that is, following the end of the second hair growth-cycle. This was later found to be characteristic for affected *d slt* mice. The amount of hair lost varied, however. In some affected individuals only denuded patches were found; in others, loss of hair was virtually complete except for the head region. In all affected *d slt* animals, there was an accompanying inhibition of the succeeding hair growth cycle. Inhibition lasted for as long as a year in some cases; in others, it involved only the suppression of a single hair growth cycle. The expressions of the temporary hair loss condition in *d b slt* and in *p d slt*, to be described in the next section, were similar to one another, but differed markedly from that observed in *d slt*.

Three subsequent generations of brother-sister matings using *d slt* mice produced only *d slt* progeny, and the incidence of the hair loss condition averaged 6.5 per cent. For none of these matings were affected male and female siblings available, but all other mating possibilities were represented. No differences in incidence of the trait were observed between matings involving two normal animals, or an affected male with an unaffected female (Table 1). Matings between unaffected males and affected females produced a greater proportion of affected progeny, but the difference was not significant ( $\chi^2 = 2.75$ ,  $P = 0.10$ ). Matings between two affected non-siblings were also set up, and these produced 50 per cent affected progeny. However, of the nine affected animals only five were *d slt*, the remaining four were *d b slt*.

In all the matings listed in Table 1, a substantial number of progeny failed to survive long enough to be fully classified, that is, until the third month. Mortality was especially high in crosses involving affected males and females and, coupled with the low productivity of the matings, quickly frustrated attempts to continue the stock by mating affected animals. The majority of the deaths occurred during the third week after birth. Moribund animals were extremely emaciated in appearance. Necropsy examination showed that the caecum was distended and filled with extremely dry solid matter, suggesting the condition megacolon. A similar condition has been found to be associated with piebald-lethal<sup>3</sup>, and, although this particular allele was not known to be

Table 1. INCIDENCE OF THE TEMPORARY HAIR LOSS TRAIT IN STRAIN *DSLTL*

All animals classified at the age of 3 months				
Mating type	No. of litters	No. of progeny	No. classified	Affected animals (%)
$N\delta \times N\delta$	12	82	50	4.0
$A\delta \times N\delta$	11	81	43	4.6
$N\delta \times A\delta$	11	80	73	9.0
$A\delta \times A\delta$	5	39	18	50.0

*N*, normal; *A*, affected.

Table 2. INCIDENCE OF TEMPORARY HAIR LOSS TRAIT IN STRAIN *PDSLT*

Generation	Mating type	No. of litters	No. of progeny	No. classified	Affected animals (%)
$F_1 \times F_1$	$N \times N$	5	39	11	0.0
I	$N \times N$	7	48	31	9.7
II	$A \times A$	4	29	26	85.4
III	$A \times A$	4	23	22	77.3
IV	$A \times A$	4	33	17	94.1

N, normal; A, affected.

present, piebald (*s*) had been introduced by 'Slate' and was segregating in the crosses. However, not all the moribund animals were homozygous for *s* and, conversely, not all the animals homozygous for *s* became debilitated and died.

As already indicated here, the temporary hair loss condition was also observed in a stock homozygous for *p*, *d* and *slt* (*PDSLT*). (The brown allele (*b*) had also been introduced into the stock by *CT/Ch*, and was segregating in the stock but could not be reliably detected except by genetic test because of the extremely light pigmentation of the coat of the *p d slt* animals.) Affected animals were observed in the very first mating involving full sibs, and the proportion quickly increased to 94 per cent within a few generations of selective inbreeding of full sibs (Table 2). These results indicated that, given a certain amount of genetic heterozygosity, selection could be effective in increasing the number of animals which showed the temporary hair loss condition. The rapidity with which selection approached 100 per cent expression suggested that interaction between *slt* and only a small number of genes was responsible for the gains observed. It seemed possible that much of the gain was associated with an increase in the frequency of *b* in the stock. Most of the animals tested in generation IV were homozygous for *b*, although some affected animals carried the black (*B*) allele.

All affected animals exhibited a temporary hair loss condition during the third week. It involved a random thinning of the coat. This condition, reminiscent of that reported for the mutant gene alopecia<sup>4</sup>, was characteristic for hair loss in all *p d slt* and *d b slt* animals. It was often followed by a short lag in the initiation of the second hair growth-cycle, and it was repeated in some cases after cessation of the second cycle. It usually did not recur after this time.

As in the case of the *DSLT* stock, mortality was also rather high in *PDSLT*. In some cases the deaths occurred during the third week, and the symptoms resembled those encountered in the *DSLT* strain. In addition, a number of animals in *PDSLT* died early in the second month. These were usually affected animals which lagged behind their litter-mates in the onset of the second hair growth-cycle. In each *PDSLT* litter one or two of the affected animals lagged behind the others in growth rate and in the replacement of the coat. These animals either died or, if they survived, they always remained considerably smaller than their litter-mates.

A temporary hair loss condition resembling in some particulars the trait observed in 'Slate' has been a common observation in a small percentage of individuals in various *C57* 'Black' sub-strains. Since the *YZ57/Ch* strain in which the *slt* mutation originated was derived in part from *C57* 'Black'/*10Ch*, one possible explanation for the temporary hair loss condition was that it had a genetic basis in common with that responsible for the hair loss condition in *C57* 'Black' sub-strains. (Occasional animals which showed a temporary hair loss condition had been seen in *YZ57/Ch* prior to the origin of the *slt* mutation, although detailed data on the occurrence of the condition in the strain were not available.) Crosses between 'Slate' and *C57* 'Black'/*10Ch* failed, however, to produce any individuals showing the temporary hair loss trait in either  $F_1$ ,  $F_2$  or first back-cross generations. Affected animals from Generation IV in the *PDSLT* strain (94 per cent affected) were also crossed reciprocally with *C57* 'Black'/*10Ch*, and these crosses similarly did not produce any

affected progeny in either  $F_1$  or  $F_2$  generations (102 and 89 progeny classified, respectively). Four affected individuals were observed in back-crosses to *PDSLT*, however, even though only a relatively small number of animals (55) were classified. Three of these were *p d slt*; the other was *d b slt*. These results indicated that the temporary hair loss condition associated with the presence of *slt* could not be attributed solely to the partial derivation of the *YZ57* strain from a *C57* 'Black' sub-strain.

The foregoing observations suggest that: (1) a temporary hair loss condition may result as a second consequence of the presence of *slt* in the homozygous condition; (2) the expression and incidence of the hair loss trait are subject to modification by the presence of what is probably a small number of additional genes. It is possible that *p*, *d* and *b* are the principal interacting genes disclosed by the present observations, or that any or all of these simply serve as markers for closely linked genes which are actually operative. The relative contributions of the various modifying genes remain a question, but there is some reason to suspect that *b* and/or closely linked genes exert a greater effect than do modifying systems marked by *d* or *p*. The possibility that much of the increase in incidence of hair loss in the *PDSLT* stock could be associated with an increase in the frequency of *b* in the stock has already been mentioned. Moreover, every *d b slt* animal encountered during the course of the development of strain *DSLT* showed the temporary hair loss trait. The same could be said for every *p d b slt* animal detected in strain *PDSLT*, although not every animal in the strain was tested genetically. It should be noted, however, that a small number of matings made between *d b slt* animals did not produce only affected progeny, and that matings between 'Slate' and *C57* 'Brown' did not produce any affected progeny in  $F_1$ ,  $F_2$  or back-cross generations. Clearly interaction between *slt* and the modifying system marked by *b* can only account for a part of the observations reported here.

A number of mutations resulting in the temporary loss of the coat and at least in some cases inhibition of replacement are known. Alopecia (*Al*), a temporary but repeated hair loss condition which is inherited as a dominant character<sup>4</sup>, has already been mentioned. Others which have been reported include: hypotrichosis juvenilis<sup>5</sup>, hypotrichosis periodique<sup>6</sup>, furless<sup>7</sup> and tufted<sup>8</sup>. Hypotrichosis juvenilis is a temporary condition which involves only the first coat, and which is inherited as a simple recessive<sup>5</sup>. Hypotrichosis periodique, a condition which shows variable expressivity but may involve loss of subsequent coats as well as the first, apparently is due to an incompletely penetrant recessive mutation<sup>6</sup>. In the case of furless, the vibrissae and hairs of the first coat are shortened, and hair loss begins during the third week<sup>7</sup>. A second coat is grown which is also shed and the animals then remain furless. In the case of tufted, on the other hand, continuous cycles of hair loss and replacement are encountered<sup>8</sup>. The first coat begins to be lost during the fourth week. It is replaced by a second coat, which then falls out, and so on. The loss-replacement cycle appears to be stopped only during pregnancy and lactation. No attempt has been made to determine possible relationships between *slt* and these mutations which appear to have as their most striking aspect an effect on the hair growth-cycle.

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## LETTERS TO THE EDITOR

## RADIO ASTRONOMY

## Spectrum of the Galactic Radio Emission

A NEW series of observations of the galactic radio emission has been conducted at the frequencies of 20, 30 and 85 Mc/s. These low-resolution observations made with scaled aerials were intended to investigate the spectral index of the galactic radio emission in the frequency region 10–100 Mc/s.

The aerials used in the present experiments were in each case a folded dipole suspended above a continuous reflecting screen laid out on the ground. The height of the folded dipole above the screen was a quarter wavelength. The reflecting screen made of chicken mesh was a wave-length square in area. This disposition of the aerial allowed the observation of one declination,  $\delta = -34^\circ$ . This is quite satisfactory, since at this declination the galactic centre, regions of the galactic halo, and the southern galactic pole can be seen in the aerial beam at different times. Thus it was possible to measure sky brightness temperatures from the directions of galactic centre and different halo regions. Sky temperatures were calibrated in terms of matched temperature limited noise diodes. The noise diodes were initially calibrated against thermal loads. The receiving system was a switched radiometer with a synchronous demodulator. Narrow bandwidths were used, scaled with the frequencies. With a one second time constant a good signal-to-noise ratio was obtained at each frequency.

All the observations were taken by an observer who calibrated the receiver, adjusted matching and monitored interference. The calibration consisted of replacing the aerial by a noise diode, and under matched conditions noting the noise diode current required for the particular aerial temperature deflexion. Occasional man-made interference was encountered during the observations. This interference was most common in the daytime. At the frequency of 20 Mc/s besides man-made interference sporadic daytime absorption was observed. This seems to be associated with the sporadic *E* layer in the ionosphere. During the night observations are unhampered by ionospheric effects since the ionospheric critical frequency  $f_o F_2$  was less than 5 Mc/s during the observing period March–September 1964.

The results obtained consist of sky brightness temperatures for the declination  $\delta = -34^\circ$  at the frequencies of 20, 30 and 85 Mc/s. At the frequency of 20 Mc/s some eight hours of readings could not be obtained at present. However, most of the halo region has been measured at this frequency. The sky brightness values are accurate to  $\pm 7$  per cent. This error is made up of individual contributions due to the r.m.s. noise, residual cable losses and day-to-day variations. The results at different frequencies were compared with information obtained on the value of the temperature spectral index (the usage of the spectral index  $\beta$  follows Turtle *et al.*<sup>1</sup>, that is,  $T \propto \lambda^\beta$ ). In addition our present results can be compared with those obtained by other observers.

In the frequency range 20–85 Mc/s the spectral index is found to be  $\beta = 2.6 \pm 0.2$ . This value is found by comparing the observed sky temperatures at a number of points in the direction of the galactic halo.

In the frequency region 30–85 Mc/s the spectral index is found to be  $\beta = 2.63 \pm 0.07$ . This value of spectral index is deduced from a complete scaled aerials experiment. This experiment involves the measurement of sky temperatures at many points in the direction of galactic halo

and comparing the observed temperatures. Fig. 1 shows the results of the scaled aerial experiment. A similar comparison in the frequency range 20–30 Mc/s is not yet possible because of lack of the necessary observations.

Following the calculation of the values of the spectral index we compared our present observations with those of other observers. Great caution is required in such a comparison since the different aerial systems used by each observer may lead to erroneous conclusions. We have collected most of the recently published results in the frequency range 1–404 Mc/s. The sky temperatures reported were converted to brightness values and plotted against frequency as shown in Fig. 2. These values in each case were for observations near the direction of the southern galactic pole. The results in the frequency range 1–10 Mc/s were taken from Ellis<sup>2</sup>, and include ground-based, rocket and satellite observations. The result at 18.3 Mc/s was plotted directly from the map given by Shain and Higgins<sup>3</sup>. The four points taken from Turtle *et al.*<sup>1</sup> were averaged over ten beamwidths near the direction towards the south galactic pole. Near the frequency of 85 Mc/s a number of results was available. That of Mills<sup>4</sup> was obtained with a narrow beam aerial and thus as expected falls below the other points. The 81.5-Mc/s result obtained by Purton<sup>5</sup> was treated in the same manner as the other points of Turtle *et al.*<sup>1</sup>. Our own results at 20, 30 and 85 Mc/s are also shown in

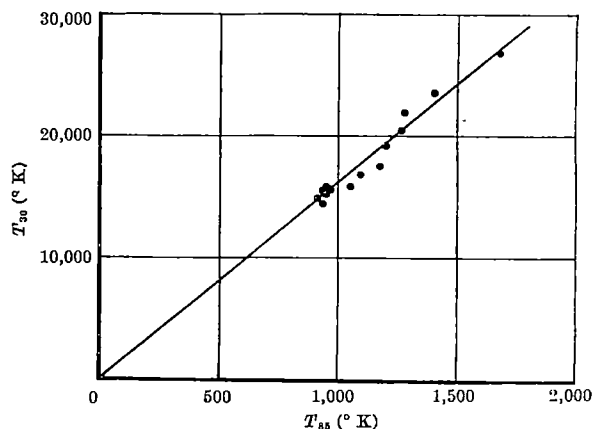


Fig. 1. Comparison of sky temperatures at 30 and 85 Mc/s

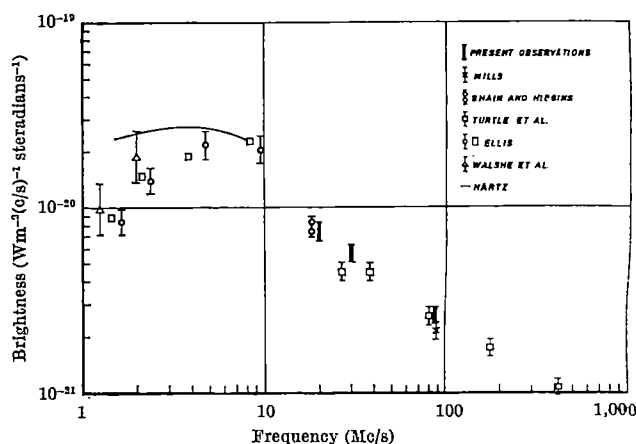


Fig. 2. Collected observations of sky brightness in the frequency range 1–404 Mc/s



Fig. 2. Rather surprisingly, results obtained with different aerial systems fit in well, with a few exceptions, in the whole frequency range of 18.3–404 Mc/s. Over this very large frequency range the spectral index is calculated,  $\beta = 2.65 \pm 0.15$ . Furthermore the shape of the spectrum can best be interpreted to be a straight line with no turnover point down to the frequency of 18.3 Mc/s.

Further observations are planned at a frequency near 10 Mc/s as well as a new scaled aerial series with improved resolution.

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## PHYSICS

### Fields of Moving Multipoles

IN free space the electromagnetic potential  $\varphi^\mu$  of Maxwell's equations is related to the sources of the field  $j^\mu$  by the equation  $\square\varphi^\mu = j^\mu$ . If the source of the field is an arbitrarily moving charge, the retarded solution for  $\varphi^\mu$  may be expressed as an integral over the world line of the charge ( $z^\mu = z^\mu(\tau)$ ):

$$\varphi_{\text{ret}}^\mu = 4\pi e \int_{-\infty}^{\infty} \dot{z}^\mu(\tau) D_{\text{ret}}(x - z(\tau)) d\tau \quad (1)$$

where  $D_{\text{ret}}$  is the four-dimensional retarded Green's function. Concise expressions for the fields  $F^{\mu\nu} = \varphi^{\mu,\nu} - \varphi^{\nu,\mu}$  can then be obtained by differentiating under the integral sign in (1). The same processes may be applied to the calculation of the radiation fields  $F_{\text{rad}}^{\mu\nu} = F_{\text{ret}}^{\mu\nu} - F_{\text{adv}}^{\mu\nu}$  (by changing the Green's function) and the method affords an effective means of evaluating the radiation field on the world line of the charge<sup>1</sup>. The latter fields have the value:

$$F_{\text{rad}}^{\mu\nu}(z(0)) = \frac{4}{3} e [\dot{z}^\mu \ddot{z}^\nu - \dot{z}^\nu \ddot{z}^\mu] \quad (2)$$

Recent work<sup>2</sup> has shown how these processes may be extended by methods of induction to arbitrarily moving electromagnetic multipoles. By integrating by parts successively until the Green's function emerges undifferentiated in the integrands and then carrying out the integration, concise and explicit expressions have been obtained for the potentials and fields of a generally moving  $2^m$ -pole. When  $m = 1$  the expressions for the fields bear full agreement with those recently found by G. N. Ward<sup>3</sup> for a dipole. By expanding the integrand as a power series in  $\tau$  the radiation fields have been calculated for a point  $x^\mu = z^\mu(0)$  on the world line of the dipole. An interesting special case has been studied when the moment vector of an electric dipole undergoes Fermi-Walker propagation along the world line of the motion. This represents in a relativistic way the notion of a non-rotating dipole. It has been found that the radiation field measured along the world line vanishes for hyperbolic motion in particular, and in general only if the dipole centre suffers constant acceleration ( $\ddot{z}^\mu \dot{z}_\mu = \text{const.}$ ). A parallel can be

drawn here with an accelerating charge. The expression (2) also vanishes for hyperbolic motion.

Although it is not known whether the derived radiation fields for a moving dipole are continuous with the values at events not lying on the world line, they are certainly continuous along it, and for moving  $2^m$ -poles in general, the world line radiation is finite only so long as  $m \leq 4$ , that is, for singlets, dipoles, quadrupoles, octupoles and  $2^4$ -poles. When  $m > 4$ , this radiation is apparently infinite. The radiation potential  $\varphi_{\text{rad}}^\mu$  satisfies the homogeneous equation  $\square\varphi^\mu = 0$  everywhere; however, the method used for evaluating the field gives rise to a singularity along the world line. This singularity in  $\square\varphi_{\text{rad}}^\mu$  is removable (finite) when  $m \leq 3$ , but when  $m > 3$  it is non-removable there (infinite). It seems worth mentioning that the situation could in some way be a reflexion on the number 3 of spatial dimensions, in the non-degenerate construction of infinitesimal multipoles by means of rigidly connected charges<sup>4</sup> according to the progression: rod, parallelogram, parallelepiped, where alternating charges occupy the positions of adjacent vertices.

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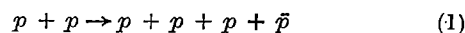
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### Upper Limits on Galactic Cosmic Ray Antiproton Intensities

KNOWLEDGE of the abundance of antiprotons in cosmic rays and in interstellar space could have important implications for cosmic ray theory,  $\gamma$ -ray astronomy and certain cosmological theories. For these reasons, attempts have been made both experimentally to measure the intensity of antiprotons in cosmic radiation and theoretically to estimate what antiproton densities might be expected in space. A recent communication<sup>1</sup> by Brooke and Wolfendale, which stimulated the calculation presented in this communication, has placed an experimental upper limit of about 5 per cent on the concentration of antiprotons ( $\bar{p}$ ) in the primary cosmic radiation near the Earth with energies of the order of 1,000 GeV.

For theoretical estimates, the sources of  $\bar{p}$  which have been proposed are: (a) primeval, (b) continuous creation, (c) cosmic ray collisions. The first two are speculative, but the third source can be estimated. In a previous calculation<sup>2,3</sup> of the production of antiprotons in the galaxy by collisions of cosmic rays with interstellar gas, Fradkin used the Fermi statistical model of high-energy nucleon-nucleon collisions, assumed a power law spectrum for the  $\bar{p}$  produced, and assumed that the antiproton lifetime in the galaxy was limited by annihilation. The calculations gave upper limits of 0.05 per cent<sup>3</sup> and 0.04 per cent<sup>2</sup> for the relative concentration of  $\bar{p}$  in cosmic radiation having total energies greater than 1.7 and 9.3 GeV respectively. More recently, without giving details, Hayakawa<sup>4</sup> estimates about  $10^{-4}$  for the ratio of antiprotons to protons in galactic cosmic rays.

In the light of more recent laboratory measurements of proton-proton collision cross-sections, and emulsion measurements of the mean amount of interstellar matter traversed by cosmic ray nuclei, it is possible now to improve the basis on which the earlier theoretical estimates of galactic antiprotons were made, although these improved estimates cannot be compared directly with the measurements of Brooke and Wolfendale. We consider here the production of antiprotons in the reaction:



due to the primary cosmic radiation incident on the interstellar or intergalactic hydrogen or helium. Other reactions generating  $\bar{p}$ , such as collisions of cosmic ray protons, antiprotons, or nuclei with interstellar hydrogen, helium, etc., and  $\bar{p}$  generation via intermediate  $\bar{n}$  production, can be neglected within the accuracy of the present calculation. The removal of antiprotons that are created in the galaxy can occur by  $\bar{p}-p$  annihilation within the galaxy and by escape from the galaxy into intergalactic space. Thus, assuming a steady state in the galaxy for the cosmic rays and antiprotons, the antiproton density in the galaxy is given approximately by:

$$\bar{n} = \bar{q} T_{\text{eff}}, \quad T_{\text{eff}}^{-1} = T_{\text{ann}}^{-1} + T_{\text{esc}}^{-1} \quad (2)$$

where  $\bar{n}$  is the number density of  $\bar{p}$   $\text{cm}^{-3}$ ,  $\bar{q}$  is the volume production rate of  $\bar{p}$   $\text{cm}^{-3} \text{sec}^{-1}$ , and  $T_{\text{eff}}$ ,  $T_{\text{ann}}$ ,  $T_{\text{esc}}$  are, respectively, the effective, annihilation and escape lifetimes of a  $\bar{p}$  in sec, corresponding to free paths of  $L_{\text{eff}}$ ,  $L_{\text{ann}}$ , and  $L_{\text{esc}}$  g  $\text{cm}^{-2}$ . For simplicity, the 'galactic model' of the origin of cosmic rays is used here<sup>5</sup>.

To find  $\bar{q}$  we use the following:

$$\bar{q} = \int_{E_T}^{\infty} n_p \sigma(E) j(E) dE \sim n_p \bar{\sigma} J(E_T) \quad (3)$$

where  $j(E)dE$  is the differential and  $J(E)$  the integral energy spectrum of primary cosmic radiation (omnidirectional values),  $n_p$  is the galactic hydrogen density,  $\sigma(E)$  is the cross-section for reaction (1) and  $E_T \approx 6$  GeV is the laboratory kinetic energy threshold for reaction (1). Experimentally, we know the cosmic ray spectrum above 6 GeV near the Earth quite well<sup>6,7</sup>, while estimates of the gas or hydrogen density in the galaxy are: an average of 0.7 protons  $\text{cm}^{-3}$  in the disk, and an upper limit of  $10^{-2} \text{ cm}^{-3}$  in the halo<sup>8</sup>.

However, information about the cross-section  $\sigma(E)$  is fragmentary. Theoretical estimates<sup>9-11</sup> give a  $9/2$  power dependence on the  $C.M.$  kinetic energy of the incident proton for  $\sigma(E)$  but are limited to the energy region near threshold, and by lack of an accurate value for the coupling constant. Experimental data bearing directly on the cross-section for reaction (1) are limited partly by the fact that it is one of many competing open channels. However, laboratory measurements at an incident proton momentum of 19 GeV/c give cross-sections for (1) of  $0.09 \pm 0.025$  and  $0.05 \pm 0.011$  mb  $\text{sr}^{-1} (\text{GeV}/c)^{-1}$  for  $\bar{p}$  recoil momenta of 3.35 and 4.6 GeV/c, respectively, at a laboratory angle of 116 mrad<sup>12</sup>. At higher energies more channels become available but the total inelastic cross-section seems to remain constant at about 20 mb, probably implying a decrease in the cross-section for any particular channel. One can place upper limits on the  $\bar{p}$  production cross-section at various energies by considering data<sup>13,14</sup> giving the total inelastic cross-sections for  $p-p$  scattering up to a few hundred GeV, and the percentages  $W_{\frac{1}{2}}^{\pm}$  of the inelastic events having charged four-pronged products.  $W_{\frac{1}{2}}^{\pm}$  is typically about 0.5 at 6 GeV, 0.3 at 27 GeV and the corresponding inelastic cross-sections,  $\sigma_{\frac{1}{2}}(E) = W_{\frac{1}{2}}^{\pm} \sigma_{\text{inel}}^{\text{tot}}(E)$  are about 11 mb and 7 mb. These values could be perhaps some orders of magnitude larger than  $\bar{\sigma}$  for  $\bar{p}$  production since many of the four charged prong channels in  $p-p$  scattering at these energies lead to pion production. In the region 6–10 GeV, where the cosmic ray beam is strong, since  $\sigma(E_T) = 0$ ,  $\bar{\sigma} < \sim 5$  mb.

Using these values in equation (3), assuming that the local cosmic ray intensity is typical of that in the galaxy, and using the estimate that the total mass of the interstellar gas in our galaxy contains about  $2 \times 10^{68}$  protons, the total  $\bar{p}$  production rate in our galaxy is then  $< \sim 2 \times 10^{68} \times (5 \times 10^{-27}) \times 1$ , that is,  $\leq 1 \times 10^{40} \text{ sec}^{-1}$ .

The quantities required to evaluate equation (2) for the  $\bar{p}$  density are found as follows: the cross-section for  $\bar{p}$

annihilation is the same as the inelastic  $\bar{p}-p$  cross-section, about 50–110 mb, for antiproton kinetic energy below 0.3 GeV (ref. 15). For  $\bar{p}$  kinetic energies of 0.5–1.2 GeV the inelastic  $\bar{p}-p$  cross-section, which is mainly annihilation, is roughly constant at about 60 mb (ref. 15), and seems to remain at this value at 2 GeV (ref. 13), falling to 37 mb at 12 GeV (ref. 16). These give  $T_{\text{ann}} \approx 1 \times 10^{18}$  sec in the disk, and  $1 \times 10^{17}$  sec in the galaxy as a whole. To find the escape time, we use the value of about 3 g  $\text{cm}^{-2}$  for the amount of matter traversed by cosmic ray nuclei from source to solar system<sup>4</sup>; this value will be assumed as the escape distance for antiprotons as well:  $L_{\text{esc}} = 3$  g  $\text{cm}^{-2}$ . Thus, the escape time is  $T_{\text{esc}} \approx 1 \times 10^{14}$  sec,  $> \sim 1 \times 10^{10}$  sec, if the  $\bar{p}$  spends most of its galaxy lifetime in the disk, or the halo, respectively. Under either of these conditions,  $T_{\text{esc}} < T_{\text{ann}}$  in the galaxy, so  $T_{\text{eff}} \approx T_{\text{esc}}$ .

From equations (2) and (3),  $\bar{n}/n_p = \bar{\sigma} J(E_T) T_{\text{eff}}$ , or:

$$\frac{\bar{p}}{p} \equiv \frac{J\bar{p}(0)}{J(0)} = \frac{\bar{\sigma} L_{\text{eff}}}{m_p} \frac{J(E_T)}{J(0)} = \frac{\bar{\sigma}}{\sigma_{\text{esc}}} \frac{J(E_T)}{J(0)} \quad (4)$$

where we have introduced an 'escape cross-section',  $\sigma_{\text{esc}} \equiv m_p/L_{\text{esc}}$ , where  $m_p$  is the proton mass, and  $L_{\text{eff}} \approx L_{\text{esc}} = 3$  g  $\text{cm}^{-2}$ , giving  $\sigma_{\text{esc}} \approx 500$  mb. With  $\bar{\sigma} < 5$  mb, equation (4) gives  $\bar{p}/p < \sim 3 \times 10^{-3}$ .

Since the effective  $\bar{p}$  production cross-section is expected to be considerably smaller than 5 mb, these calculations indicate that the  $\bar{p}$  fraction of the galactic cosmic ray beam integrated over all energies is expected to be considerably less than 0.3 per cent.

Because our estimate is for galactic  $\bar{p}$ 's of relatively low energies, while Brook and Wolfendale's measurement is for high-energy  $\bar{p}$ 's near the Earth, it is not possible to compare our theoretical limit directly with Brooke and Wolfendale's measured limit.

In the future, improved laboratory cross-section data will enable more accurate calculations of the low-energy  $\bar{p}$  intensity to be made. This, subtracted from the low-energy  $\bar{p}$  intensity measured in space, would yield an intensity difference presumably due to low-energy  $\bar{p}$ 's produced primevally or by continuous creation (Brooke and Wolfendale, private communication).

A promising observational technique for placing upper limits on  $\bar{p}/p$  is to expose nuclear emulsions to the primary cosmic rays by means of satellites. In this way, Grigorov *et al.*<sup>17</sup> find an upper limit of 0.1 per cent for the ratio of cosmic ray anti-nuclei to nuclei having  $Z > 2$  at an altitude of 300 km. Since these measurements were for stopping particles the energies involved are probably less than some few hundred MeV.

$\gamma$ -Ray measurements could also give indirect experimental information on the galactic antiproton intensity, since some antiproton annihilations eventually produce  $\gamma$ -rays, but the usefulness of this method is severely handicapped by the number and nature of the competing processes.

By using the Kraushaar and Clark<sup>18</sup> measured upper limit on the cosmic  $\gamma$ -ray intensity, astrophysical data<sup>19</sup>, and  $\bar{p}$  annihilation cross-sections<sup>20</sup>, the following approximate upper limits on  $J\bar{p}$  in  $\bar{p} \text{ cm}^{-2} \text{s}^{-1}$  can be estimated: 1–100 in interstellar space, depending on direction, 30 in the Crab Nebula, and 0.1–1 for intergalactic space. Unfortunately these limits appear to be too large to be useful.

In summary, estimates based on recent cross-section data indicate that the low-energy  $\bar{p}$  fraction of the cosmic ray beam is probably much less than 0.3 per cent. The estimates will be refined considerably when antiproton total and differential production cross-sections become available.

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### Surface Gas Eruption and Van der Waals Cohesion on Tungsten

IN some experiments on the detection of single atoms and molecules reported earlier<sup>1</sup>, it was noticed that, in the early stages of the conditioning of the point, there were occasions when, on increasing the field by some 20 per cent, an abrupt instability in the field current occurred; this invariably produced excessively large currents. The field emission pattern of the emitting facets of the point, previous to this, was, of course, badly blurred, with no definition comparable with that from clean points, but it was possible to discern 'out of focus' non-emitting areas on the fluorescent anode; as soon as the instability arose, there was complete loss of resolution, and the pattern showed a uniform streaming as if a sheet of gas was moving across the point. When the point had been fully 'conditioned', it required a much higher field to produce the effect. If, however, the whole vacuum system was let down to air at atmospheric pressure and soon after pumped out, it was possible to obtain repeated gas eruption and current instability without further increase of field.

We have in this simple observation a method of determining the Van der Waals cohesion of the topmost atoms in a multilayer gas film on tungsten. The effect of the field,  $E$ , is to polarize the adsorbed gas layer and put a strain on the film; the force on a single atom  $\alpha E dE/dz$  where  $\alpha$  is its polarizability works out at  $\sim 1/10^{12}$  newtons. This tensile force acts in the presence of a random positive ion bombardment of the point by molecular and atomic ions of oxygen, nitrogen, carbon monoxide and carbon at a rate of about 500/sec; the recovery time of the surface may have values up to and beyond 1 msec.

The random nature of the bombardment is shown in Fig. 1(a), where it is seen that the interval between successive ion impacts may lie within the recovery time of the surface; when this happens, the surface gas is violently disturbed, the field current rises abruptly, and it may take several msec for the surface to come to a stable equilibrium. This behaviour is consistently repeatable on letting the system down to air and re-pumping; it must result in the stripping off of the topmost loosely held gas layer (or layers). An excellent example of the fluctuating currents caused by three impacts within the recovery time of the surface is shown in Fig. 1(b).

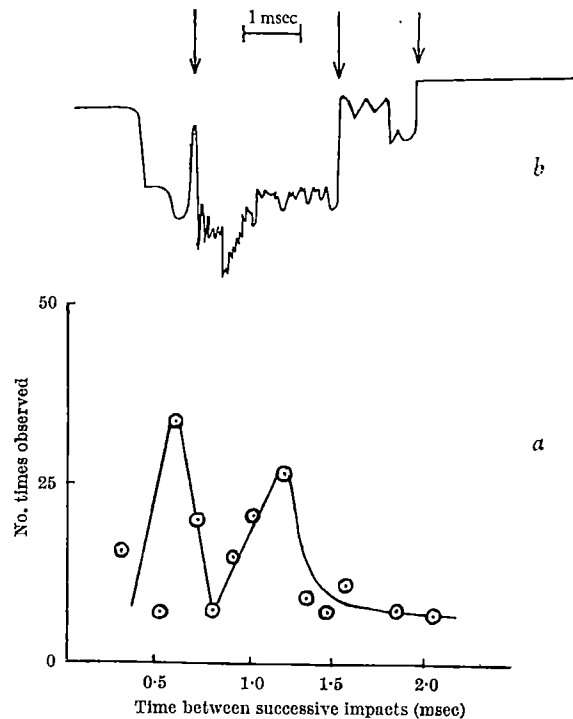


Fig. 1

A value for the cohesive energy of the gas can be derived by considering the momentum of the impacting particle to be shared between the gas and the tungsten/oxide substrate, according to their binding energies  $B_g$  and 10 eV, respectively. At 2 kV the incoming momentum is proportional to  $1.6 \times 10^7$ , so that  $1.6 \times 10^7 B_g / 10 \times 10^7$  represents the velocity taken up by the gas, as a unit, since there are approximately  $10^7$  atoms on the sensitive surface of the point. An average recovery time of 1 msec corresponds to gas moving over the linear dimensions of the point,  $10^{-5}$  cm, with a velocity of  $1/10^2$  cm/sec, hence  $B_g \sim 1/16$  eV per atom. This value is in reasonable agreement with that calculated by London for the Van der Waals energy for molecular crystals of oxygen as given in Seitz—1.69 kcal/mole.

It is interesting to note that an oxygen ion at a distance of 10 Å from the tip would have a field of  $10^8$  V/M associated with it and would act on a single surface atom with a force of the same order as that of the external field. The ensuing field emission and neutralization of the ion by an electron from the conduction band of the semiconducting tungsten/oxide contact should result in an Auger transition involving the emission of a secondary electron into the gas.

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## GEOPHYSICS

## Palaeomagnetic Dating of some Younger Dikes in Southern Norway

THE occurrence of several localities of younger basic dikes in southern Norway has aroused considerable geological interest in the past. The dikes are especially common in the coastal districts between the Permian Oslo graben and the schists of the Stavanger area. Their absolute age has remained unknown, but a connexion with the Permian igneous activity in the Oslo area has been proposed<sup>1,2</sup>. Nevertheless, radioactive dating of younger Norwegian dikes has hitherto not been carried out. On this background a systematic palaeomagnetic study of the younger dikes in Norway has been undertaken by me, assuming it to be a useful tool for absolute as well as relative age determinations. So far only the extensive swarms of dolerites in the anorthosite rock complex in Rogaland have been investigated and in this communication the results are summarized.

The dolerite swarms trend west-north-west over long distances with vertical attitude reaching a maximum thickness of about 90 ft. They cut all other rocks, but where they intersect prominent fault zones slight displacements of a few feet due to later movements can be observed<sup>3</sup>. Antun, who mapped altogether 11 dikes and numbered them 1-11 from north to south, has petrographically classified the rocks into three types: trachydolerites (1-5), dolerite (6) and porphyritic dolerites (7-11). They are all olivine-bearing. Chilled margins are typical and glassy stringers are present both in margins and in apophyses. The consolidation must have been very rapid, and because of the multiple nature of the dikes Antun concludes that the intrusion act of the system must have been of short duration. Of special interest for the present investigation are the generally unaltered state of the minerals, but in the trachydolerites Nos. 1, 2, 4 and 5 some subordinate magnetite occurs as a by-product of decomposed iron-rich olivines. According to Antun the ore minerals are ilmenite and a titanomagnetite containing submicroscopic lamellae of the former.

The remanent magnetism of 44 hand samples in all has been investigated. Twenty-four samples behaved palaeomagnetically very satisfactorily. Two sites (7 samples) were obviously more or less affected by lightning. The remaining samples mostly showed internal scattered directions of natural remanent magnetism, which became either more unstable after heat treatment, or were so weakly magnetized that for practical reasons cleaning was not carried out. But even if all samples are included, the main conclusion of this investigation is not altered. So far as rocks with stable components are concerned, the results may be summarized as follows:

(1) In all dikes tested (except No. 2, where the collected site was affected by lightning) samples were found which either did not change their direction of natural remanent magnetism at all when thermally demagnetized at high temperatures, or merely contained smaller amounts of viscous magnetization which were easily removed by thermal cleaning.

(2) In the porphyritic dolerite dikes and in the dolerite dike the within-dike scatter is small. The trachydolerites, except dike No. 3, showed somewhat more scattered results probably due to the presence of the secondary magnetite mentioned above.

(3) The patterns of thermal decay of natural remanent magnetism and artificial thermo-remanent magnetism respectively are very similar for stable rock specimens, especially above 300° C.

(4) The Curie point temperature is always that for pure magnetite or slightly lower.

(5) Within an experimental error of a few degrees the directions of artificial thermo-remanent magnetism are coincident with the applied field.

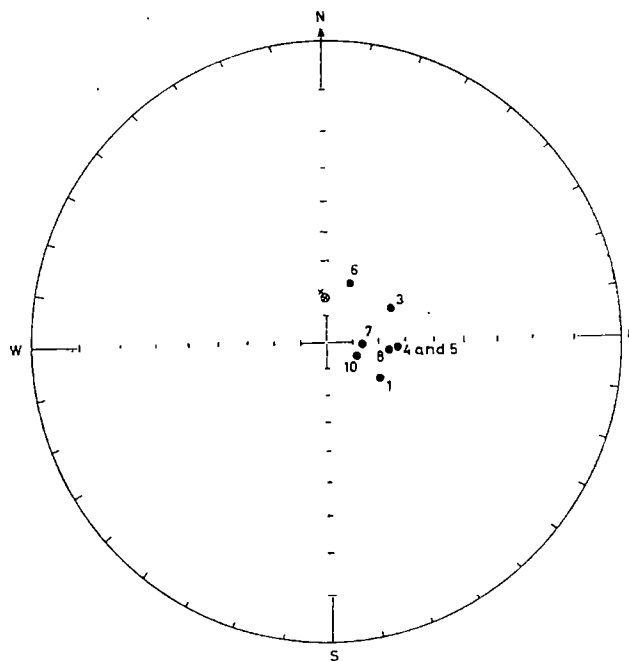


Fig. 1. Stereographic projection showing dike mean directions of younger dolerites in Rogaland, southern Norway. Only samples with stable magnetic components are included. Plots are north-seeking poles in lower hemisphere (normal). Numbers are according to Antun's numeration of the dikes. Dikes No. 4 and 5 are identical, representing a bifurcating fissure, and are therefore treated together. Dikes No. 9 and 11 are not collected. x, present field direction; ⊗, theoretical dipole field direction

(6) The stable remanent directions are mostly definitely different from that of the present Earth's field.

(7) There seems to be some connexion between magnetic directions and petrography, but more sampling is needed to clarify fully this suggestion.

(8) The intensities of artificially produced thermo-remanent magnetism are of the order of  $10^{-3}$ - $10^{-2}$  E.M.U. c.c., and therefore much less than is required, in certain cases, for a stable component anomalously magnetized in the plane of the dike<sup>3</sup>.

From the experimental evidence given here it is probably reasonable to assume that the stable component of remanent magnetism of the younger dolerites in Rogaland, southern Norway, is of thermal origin (originating when the dikes cooled), and has been preserved throughout the history of the rocks and thus contains information about the Earth's field during the time of formation of the dike system. Few time-readings of the geomagnetic field seem to be incorporated in the palaeomagnetic results, and the total span of time represented is probably considerably less than the period of secular variation. The geological indications of a short duration of the whole intrusion act are therefore probably verified.

Since the deduced palaeomagnetic field directions are representative of a geomagnetic field of fairly recent origin (Cenozoic), the considered dolerites are assumed to be of Tertiary age.

In addition, preliminary results from some other dikes from nearby districts south of Bergen seem to give the same conclusion concerning age. Prior to the present investigation, but on guess work only, a theory had been put forward in favour of Tertiary rocks in Norway.

In Tertiary times crustal unrest was characteristic of the North Atlantic region. The Scandinavian landmass must have been uplifted along fault lines in the western areas at this time<sup>4</sup>, and in Sweden and Denmark there is proof of Tertiary volcanic activity. Granulometric investigations of volcanic ash layers (Eocene) in Denmark<sup>5</sup> suggests an eruption centre in Skagerak or southern Norway. Aeromagnetic surveys over the Skagerak region

in 1962 and 1963 (carried out by the Geological Survey of Norway for the University of Bergen) have shown that a rather large magnetic anomaly exists south of Kristiansand: remnants of a possible tertiary volcanic centre are perhaps localized there.

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## GEOLOGY

### Age of the Transition in the Pre-Cambrian Atmosphere

RECENTLY published age determinations<sup>1,2</sup> together with other figures<sup>3</sup> from British Guiana can be used to give more information about the Pre-Cambrian atmosphere. Ritten<sup>4</sup> has suggested that a tentative age of 1,000 million years for the oldest geological 'red beds' indicates a provisional minimum age for the present oxygenic atmosphere. He further suggests that the transition between the latter and an earlier anoxygenic atmosphere occurred between 2,000 and 1,000 m.y. ago. Older 'red beds' occur in British Guiana [and it is postulated here that the above transition was probably between 3,000 and 2,500 m.y. ago].

The Roraima Formation<sup>5,6</sup> consists of usually flat-lying sandstones and conglomerates with notable jasper horizons enabling a three-fold classification. The formation is several thousand feet thick and covers an area of approximately 175,000 square miles in British Guiana, Brazil and Venezuela with outliers in British Guiana (Berbice) and Surinam (Tafelberg). The sandstones are usually pink and the jaspers red or green. Approximately vertical cliffs several thousand feet high define the margins, forming the pink-coloured walls to flat-topped mesas, which are such a striking feature of the Roraima Formation (Mount Roraima is the setting of Conan Doyle's *Lost World*).

The Muruwa Formation<sup>7</sup> appears to underlie the Roraima Formation, forming a low-lying belt, approximately 20 miles wide, east-west across central British Guiana. Lithologically the rock-types are remarkably similar to the Roraima Formation, differing mainly in being folded about gently plunging fold-axes.

The pink colour of the sandstones of both formations and, in particular, the deeper red of the jaspers of the Roraima Formation and the cherty mudstones of the Muruwa Formation appear mainly due to haematite and limonite. Following Ritten<sup>4</sup>, and actualistic reasoning, these sediments were probably deposited under oxygenic conditions and, as shown below, appear to be the oldest types of these deposits (unequivocally dated) so far known.

Dolerites of the Younger Basic Intrusive Group form large sills intruding the Roraima Formation. McDougal, Compston and Hawkes<sup>8</sup>, from several determinations on pyroxenes and plagioclases from these dolerites, suggest an age of intrusion of just over 2,000 m.y. Snelling and McConnell<sup>9</sup> consider the latter age is probably too high, and from determinations on pyroxenes from similar dolerites, muscovite and biotite from the associated hornfelses, and a re-interpretation of the data of McDougal *et al.*, suggests the best estimate of the age of emplacement of the intrusives to be  $1,675 \pm 100$  m.y.

However, of immediate importance to the present note is that all the present data suggest a minimum age of the Roraima Formation of about 1,700 m.y.

In addition, the Roraima Formation unconformably overlies the Barama-Mazaruni Assemblage<sup>3</sup> and some of the granites of the Younger Granite Group. It has been suggested<sup>8</sup> that a post-Roraima granite may occur in Surinam, but the evidence is not conclusive. Minor acid dykes<sup>9</sup> intrude the Roraima but no post-Roraima intrusive granites have been mapped in British Guiana. Snelling<sup>3</sup> has dated several granites from the Younger Granite Group, the oldest within the northern eugeosyncline being the Kaituma Granite ( $2,065 \pm 100$  m.y.). In addition, the Kartabu Granite of the Bartica assemblage<sup>10</sup> has been dated at  $1,960 \pm 60$  m.y. A preliminary outline of the geology of British Guiana was recently published in *Nature*<sup>11</sup>.

Two granites intruding the Muruwa Formation have been dated<sup>3</sup> at  $1,570 \pm 50$  and  $1,760 \pm 55$  m.y., but Snelling (personal communication) has reservations about these dates. However, one granite intruding the Iwokrama Formation (which appears to overly conformably the Muruwa Formation) has been reliably dated<sup>3</sup> at  $2,595 \pm 125$  m.y.

It can be clearly stated, therefore, that the Roraima Formation is older than its intrusive dolerites and must be in excess of 1,700 m.y. Geological evidence strongly suggests that the Muruwa Formation is older; this is confirmed by the oldest granite noted above, which is approximately 2,600 m.y. and gives a minimum age to the Muruwa Formation of about 2,500 m.y.

A minimum age of 2,015 m.y. has been suggested<sup>3</sup> for the main phase of the post-Barama-Mazaruni-Bartica orogeny (the last major pre-Roraima orogeny), and this age is probably also the date of emplacement of many members of the Younger Granite Group. Any Pre-Cambrian tectonism modifying the ages of the rocks underlying the Roraima Formation must, of course, be limited by the flat-lying sedimentary deposits of the Roraima Formation, which are, therefore, probably younger than 2,000 m.y.—approximately 1,800–1,700 m.y. has been suggested<sup>3</sup> for their deposition.

A tentative age of 2,000 m.y. for the youngest sediments deposited under anoxygenic conditions has been suggested by Ritten<sup>4</sup>, though he notes that these deposits may range from 2,000 to 3,000 m.y. It seems likely from the above data that an oxygenic atmosphere was extant about 1,800 m.y. ago (Roraima Formation) and at least 2,500 m.y. ago (Muruwa Formation), and that any transition from a previous anoxygenic atmosphere occurred before 2,500 m.y., probably between 3,000 and 2,500 m.y. The overlapping of these ages with the suggested range (2,000 to 3,000 m.y.<sup>4</sup>) for the sediments deposited under an anoxygenic atmosphere clearly indicates that more data are required, particularly concerning the environment of the youngest sediments deposited under anoxygenic conditions (which may not, in all cases, reflect an anoxygenic atmosphere).

The above age determinations of British Guiana rocks (except for those in McDougal *et al.*<sup>1</sup>) were made by Dr. N. J. Snelling<sup>2</sup> at the Age Determination Unit, Overseas Geological Surveys in the Department of Geology and Mineralogy, University of Oxford.

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### A Modified Staining Technique for Carbonates in Thin Section

POTASSIUM ferricyanide and alizarin red *S* are frequently used as stains for distinguishing between carbonate minerals. Potassium ferricyanide in acid solution produces Turnbull's blue with ferrous iron and it used to be thought that dolomite could be distinguished from calcite on the basis that dolomite alone contained ferrous iron. Recently this has been shown to be incorrect<sup>1</sup>, and it is now known that any carbonate mineral containing small quantities of ferrous iron will stain with potassium ferricyanide providing the carbonate can be made to react with an acid medium. Recent work<sup>2,3</sup> shows that alizarin red *S* in solution is probably the best method of distinguishing between the common carbonates.

Boiling, 30 per cent sodium hydroxide solution, as used by Freidman<sup>2</sup> for staining blocks and chips, has been found unsuitable for staining thin sections, as the mounting medium (Lakeside 70, Canada balsam) tends to dissolve. A weak hydrochloric acid solution does not attack the mounting medium. I have obtained satisfactory results with the procedure described here. A concentration of 3 per cent hydrochloric acid, or greater, at room temperature dissolves away the more soluble carbonates and hence is undesirable. At room temperature alizarin red *S* is most selective as a stain in a concentration of hydrochloric acid which falls between 1 per cent and 2 per cent. At the 1.5 per cent plus level the thickness of the thin section is greatly reduced and the staining becomes pale. At lower concentrations, the stain becomes so thick that it obscures the fine detail of the thin section. An important feature of the stain at the 1–2 per cent acid concentration is that with calcite a surface parallel to the *C*-axis is more deeply stained than one normal to that axis. This is important in fabric analysis where optic orientation patterns are required.

The potassium ferricyanide stain is successful over a range of acid concentrations, and as this solution is mixed with the alizarin red *S* solution the same acid concentration (1–2 per cent) is used. At room temperature, at these low concentrations, potassium ferricyanide does not react with siderite.

Distilled water must be used for making up all solutions and for washing off surplus stain from the thin sections after each stage of the procedure. Much more reliable results are obtained if the thin section to be stained is etched first (stage I of procedure). This pre-staining etch removes all grinding dust from the carbonate surface and is carried out for at least 10 and not more than 15 sec using 1.5 per cent hydrochloric acid. The etched section is then immersed in an acidified mixture of the two stains

(stage II). Each stain works independently, and there is no mutual interference. If the now partially stained section is immersed in an acidified solution of alizarin red *S* for a few seconds the colour differentiation of the carbonates is increased further, but it is important not to leave the section longer than 15 sec in the solution (stage III). After rapid but careful washing of the section in distilled water it is important to dry the stained surface as quickly as possible because the stain is relatively soluble in water. It is important not to touch the stained surface when drying as it is purely a surface precipitate and is easily rubbed off. To preserve and protect the now dried stain, during mounting or remounting, a layer of 'Durofix' is painted on to the surface with a soft camel-hair brush. The 'Durofix' is diluted with amyl acetate, in the ratio 'Durofix' : amyl acetate = 1 : 3, which readily evaporates, and when this layer is completely dry it has a refractive index very close to 1.54.

The details and consecutive stages of the procedure are shown in Table 1.

The distinction between dolomite and calcite, of which most ancient limestones are composed, is of considerable importance in limestone petrology. The modified staining technique described not only gives positive colour differentiation of these two important carbonate minerals but also, because of their different solubility in hydrochloric acid, gives a difference of thickness in thin section. Calcite is etched to approximately 15 $\mu$  thickness while dolomite remains at 30 $\mu$  thickness; this difference is readily observable with polarized light.

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## METALLURGY

### Identification of a Beta-Tungsten Phase in Tungsten-Rhenium Alloys

PHASE diagram equilibrium studies in the binary tungsten-rhenium system show the presence of two phases over the range 0–65 wt. per cent rhenium at 1,500° C (ref. 1). A terminal solid solution having a body-centred cubic structure exists from 0 to about 27 wt. per cent rhenium, and a phase having a tetragonal sigma-type structure is found in the composition range 43–65 wt. per cent rhenium. In this investigation, alloys containing 13–45 wt. per cent rhenium prepared by vapour deposition

Table 1

Procedure		Time	Carbonate	Result
Stage I	Etching 1.5% HCl	10–15 sec	Calcite Ferroan calcite	Considerable etch
			Dolomite Ferroan dolomite	Negligible etch
Stage II	Staining* 0.2 g A.R.S. per 100 c.c. 1.5% HCl 2.0 g P.F. per 100 c.c. 1.5% HCl Mixed in ratio A.R.S. : P.F. = 3 : 2	30–45 sec	Calcite	Very pale pink—red depending on optical orientation
			Ferroan calcite	Very pale pink—red Pale blue—dark blue Two superimposed give Mauve—purple—royal blue
			Dolomite	No colour
			Ferroan dolomite	Pale—deep turquoise depending on ferrous content
Stage III	Staining 0.2 g A.R.S. per 100 c.c. 1.5% HCl	10–15 sec	Calcite Ferroan calcite	Very pale pink—red
			Dolomite Ferroan dolomite	No colour

\* A.R.S. = Alizarin red *S*. P.F. = Potassium ferricyanide



at 500°C were found to contain a phase having a  $\beta$ -tungsten type structure not previously reported in tungsten-rhenium alloys. The stability of this phase with respect to temperature and composition is now being examined. No evidence for the presence of sigma phase or elemental rhenium was found in these alloys as deposited.

The alloys were prepared by the simultaneous hydrogen reduction of tungsten and rhenium hexafluorides at 500°C, 10 torr total system pressure, and a mole ratio of reacting gases of  $H_2:WF_6:ReF_6 = 50:6:1$ . The deposit, which formed on the inner wall of a heated copper tube, was richer in rhenium near the inlet to the deposition zone than farther downstream. Thus, alloys ranging from 4 to 46 wt. per cent rhenium were available for examination.

X-ray diffraction patterns of powder samples were obtained using a 114-mm Debye-Scherrer camera with nickel-filtered copper radiation. The phases identified in several samples of different rhenium content and the visual intensity of lines on the diffraction patterns are shown in Table 1. A body-centred cubic  $\alpha$ -tungsten (A2) structure was the principal phase in the low-rhenium deposits. The lattice parameter of this phase approaches the value for unalloyed tungsten,  $a_0 = 3.1648 \text{ \AA}$ , with decreasing rhenium content. A previously unreported cubic  $\beta$ -tungsten structure (A15) was observed in the composition range 13–45 wt. per cent rhenium. Diffraction data for the  $\beta$ -tungsten structure measured from a pattern of a 22.3 wt. per cent rhenium sample are shown in Table 2. The lattice parameter of the  $\beta$ -tungsten structure was found to be  $a_0 = 5.0182 \pm 0.005 \text{ \AA}$  at this composition.

Hardness values and impurity concentrations of the deposits are presented in Table 3. The hardness values were obtained using a 500-g load on a Kentron hardness tester. The sharp drop in hardness with decreasing

rhenium content supports the X-ray evidence of solid solution or compound formation in the deposit. The impurity content, like hardness, decreases with decreasing rhenium content. Although impurity concentrations are rather high for the higher rhenium deposits, the generally excellent purity lends credibility to the interpretation of the X-ray diffraction data. In addition to the interstitial impurities shown in Table 3, copper, magnesium, and silicon were present in trace amounts.

Superconducting transition temperatures were observed at 6.4° and 8.8° K for samples containing 6 and 12 wt. per cent rhenium, respectively. As may be seen from Table 1, the major phase in these alloys was a solid solution of rhenium in the body-centred cubic lattice of tungsten. The  $\beta$ -tungsten structure was a minor constituent in both cases. R. D. Blaugher, A. Taylor and J. K. Halm measured  $T_c$  values of 2.4° and 4.6° K for alloys containing 15 and 25 wt. per cent rhenium, respectively<sup>2</sup>. These alloys were reported to be single phase.

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## CRYSTALLOGRAPHY

### Crystal-structure of Tetragonal Strontium Oxalate

Two aspects of the structure of tetragonal calcium oxalate (weddellite), namely: (1) that the oxalate ion has the symmetry  $mmm$ ; (2) that the crystalline salt has a water content of  $2 + \text{zeolitic } X \text{ H}_2\text{O}$  ( $X \leq 0.5$ ) (ref. 1), can be examined further. Klasens, Perdok and Terpstra<sup>2</sup> described tetragonal strontium oxalate as being isomorphic with weddellite, but they gave the number of molecules of water in the former as 2.5 and in the latter as 3.

Envelope crystals of tetragonal strontium oxalate were obtained by the method of the foregoing investigators<sup>2</sup>. After storage for several months in the laboratory, a small single crystal was subjected to a crystal-structure analysis. Data were obtained with copper- $K\alpha$  radiation, with intensities monitored by a scintillation counter. Because the largest dimension of the crystal was 0.1 mm, no absorption corrections were made.

The space group is  $I4/m$ , and the cell dimensions are  $a = 12.82 \pm 0.03$ ,  $c = 7.50 \pm 0.02 \text{ \AA}$ . The measured density of  $2.36 \text{ g cm}^{-3}$  (by flotation) gives slightly more than 8 formula weights of  $SrC_2O_4 \cdot 2H_2O$  per cell; specifically, the calculated water content is 2.17 moles of water per mole of strontium. The resolution of the structure followed readily from a 3-dimensional Patterson distribution, based on 607 independent reflexions. Refinement by least squares of a structure based on  $SrC_2O_4 \cdot 2H_2O$  proceeded until the discrepancy index,  $R$ , was 0.12. A difference Fourier synthesis showed the presence of a low electron-density peak on the 4-fold axis. Placement of the appropriate fraction (0.1) of a necessarily disordered water molecule at the indicated site, as with weddellite, permitted the refinement to continue somewhat further, lowering  $R$  to 0.11. The final atomic parameters are given in Table 1.

The crystal is thus completely isomorphic with that of weddellite, even having the same zeolitic water content

Table 1. PHASES OBSERVED IN VAPOUR-DEPOSITED TUNGSTEN-RHENIUM

Rhenium (wt. %)	$\alpha$ -Tungsten $I$ (visual)*	$a_0$ (Å)	$\beta$ -Tungsten $I$ (visual)†	$a_0$ (Å)
45			S+	4.987
22			S+	5.018
13	S+	3.153	W	5.026
8	S+	3.157		
4	S+	3.160		

\*S, strong; †W, weak

Table 2. X-RAY DIFFRACTION\* DATA FOR THE  $\beta$ -TUNGSTEN STRUCTURE IN A W-22.3 WT. PER CENT RHENIUM DEPOSIT

$I$ (visual)†	$d$ (Å)	$\sin^2\theta$	$hkl$
M+	2.495	0.09549	(200)
S-	2.232	0.12494	(210)
S-	2.040	0.14276	(211)
M	1.445	0.28474	(222)
M+	1.389	0.30785	(320)
S-	1.337	0.33227	(321)
M+	1.252	0.37904	(400)
M+	1.122	0.47209	(420)
S-	$a_1$	0.49651	(421)
M+	$a_1$	0.51963	((332))
S	$a_1$	0.68325	(520, 432)
	$a_2$	0.68730	
S-	$a_1$	0.70655	(521)
	$a_2$	0.71052	
S-	$a_1$	0.75452	(440)
	$a_2$	0.75864	
M+	$a_1$	0.84796	(600, 442)
	$a_2$	0.85232	
M+	$a_1$	0.87157	(610)
	$a_2$	0.87621	
S+	$a_1$	0.89534	(611, 532)
	$a_2$	0.89984	

\*Copper- $K\alpha$ ,  $\lambda$ , 1.54051 Å. †M, medium; S, strong

Table 3. HARDNESS AND CHEMICAL ANALYSIS OF VAPOUR-DEPOSITED TUNGSTEN-RHENIUM ALLOYS

Rhenium (wt. %)	Hardness (DPH)	Carbon*	Impurities (p.p.m.) Hydrogen Oxygen	Nitrogen
46	1,775	120	11	79
29	1,960			9
38		50	8	34
22	1,730			< 5
17		< 20	2	9
13	570			< 5
10		< 20	2	9
8	370			< 5
6		< 20	2	6
5	350			< 5
4	325			< 5

\* Limit of detection 20 p.p.m.

Table 1. ATOMIC PARAMETERS OF TETRAGONAL STRONTIUM OXALATE

Atom	<i>x</i>	<i>y</i>	<i>z</i>	B (Å <sup>2</sup> )
Sr	0.301	0.196	0.0	0.8
O(1)	0.469	0.231	0.179	3.5
O(2)	0.639	0.248	0.180	1.7
O(1 <sup>1</sup> )	0.106	0.149	0.0	4.8
O(2 <sup>1</sup> )	0.390	0.013	0.0	4.7
O(1 <sup>2</sup> )	0.0	0.0	0.282	4.3
O(2 <sup>2</sup> )	0.555	0.241	0.101	1.5

( $X \leq 0.5$ ). However, the unit cell of tetragonal strontium oxalate is slightly larger, and the average distance of Sr—O, for the 8-co-ordinated strontium atom, is 2.59 Å (2.56–2.61 Å).

The C—C bond of the oxalate ion is bisected by a symmetry plane. The bond-lengths are as follows: C—C,  $1.52 \pm 0.04$  Å; C—O(1),  $1.25 \pm 0.02$  Å, C—O(2),  $1.24 \pm 0.02$  Å. The bond angles are C—C—O(1),  $117.7 \pm 1.0^\circ$ ; C—C—O(2),  $118.5 \pm 1.0^\circ$ ; O(1)—C—O(2),  $123.8 \pm 1.7^\circ$ . The oxalate ion therefore has the symmetry *mmm*, within a very small limit of error.

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## CHEMISTRY

### Octafluoroprotactinates (V)

ALTHOUGH potassium heptafluoroprotactinate (V),  $K_3PaF_7$ , has been known for several years<sup>1</sup> and a number of hexafluoroprotactinates (V),  $M_1PaF_6$ , ( $M_1 = NH_4^+$ ,  $K^+$  and  $Rb^+$ ) have been recently reported<sup>2</sup>, octafluoroprotactinates (V) have not been recorded. In an investigation of the preparation and properties of the fluoro-complexes of quinquivalent protactinium, using bromine trifluoride and aqueous hydrofluoric acid as solvents, we have isolated lithium and sodium octafluoroprotactinates (V),  $Li_3PaF_8$  and  $Na_3PaF_8$  respectively, from reactions in the latter solvent.

Solutions approximately 0.5 M in protactinium (V) were obtained by dissolving protactinium pentoxide,  $Pa_2O_5$ , previously ignited at  $750^\circ$ , in 48 per cent hydrofluoric acid. Sufficient alkali metal fluoride,  $MF$  ( $M = Li^+$ ,  $Na^+$  and  $K^+$ ), to give a 3:1 mole ratio of  $MF:Pa$  was dissolved in hydrofluoric acid, added to the protactinium (V) solution in a platinum crucible and the mixture concentrated to approximately half-volume by heating under an infra-red lamp. Addition of acetone to the mixtures containing sodium and potassium fluoride produced an immediate precipitate of octa- and heptafluoroprotactinate (V) respectively, the latter identified by its X-ray diffraction pattern<sup>3</sup>. Lithium octafluoroprotactinate (V), which was not formed under the afore-said conditions, could only be obtained by evaporating the hydrofluoric acid solution of the component fluorides to dryness and was identified from its X-ray powder pattern. The isolated products were vacuum dried at about  $100^\circ$ . Protactinium analyses were performed by dissolving the complex in 10 per cent nitric acid solution, precipitating the hydroxide and igniting it to the pentoxide at  $750^\circ$ . Fluoride in the supernatant was determined gravimetrically<sup>4</sup> as lead chloro-fluoride: found for the sodium complex: Pa, 49.38 per cent; F, 33.54 per cent; required for  $Na_3PaF_8$ : Pa, 51.1 per cent; F, 33.63 per cent.

The octafluoroprotactinates (V) are white, non-hygroscopic solids, which are soluble in dilute hydrofluoric acid, water and dilute nitric acid. It is interesting to note that no signs of hydrolysis are observed in the last two solutions, further evidence of the strong fluoride-complexing of protactinium (V). Both are immediately

hydrolysed by ammoniacal solution. The sodium salt is isostructural with<sup>5</sup> sodium octafluorouranate (V), possessing tetragonal symmetry. The lithium complex, for which there appears to be no quinquivalent niobium, tantalum and uranium analogue, is also tetragonal; owing to the presence of several weak reflexions, extra to those observed on the powder pattern of  $Na_3PaF_8$ , the X-ray powder diffraction data are best interpreted on the basis of a larger cell obtained by doubling  $a_0$ . The results of the diffraction investigations are shown in Table 1, the lithium and sodium salts having the space groups  $P4_22_12-D_2^5$  and  $I/mmm-D_{2h}^{17}$  respectively.

Table 1. UNIT CELL DATA

Compound	Symmetry	$a_0$ (Å)	$c_0$ (Å)	<i>n</i>	(g/cm <sup>3</sup> )
$Li_3PaF_8$	Tetragonal	10.386	10.89	8	4.568
$Na_3PaF_8$	Tetragonal	5.487	10.89	2	4.577

We understand from Dr. A. G. Maddock<sup>6</sup> that he and his associates, working at Cambridge, have also recently prepared  $Na_3PaF_8$  using conditions similar to those for the preparation of the tantalum (V) analogue. Our preliminary report of the preparation of the octafluoroprotactinates (V) will be supplemented by a detailed account of their chemistry, to be published elsewhere, including a full structure analysis of  $Na_3PaF_8$  and of potassium heptafluoroprotactinate (V) and the isostructural sodium and rubidium complexes.

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### Compounds containing Mercury—Iron Bonds

Hock and Stuhlman<sup>1</sup> discovered that the reaction of iron pentacarbonyl with mercuric halides yielded solid products, which they formulated as addition compounds  $Fe(CO)_4Hg(HgX_2)_2$ , containing the  $Fe(CO)_4Hg$  moiety which itself can be obtained by reaction of iron pentacarbonyl and mercuric sulphate. No structural information on these compounds is available, although Wells<sup>2</sup> has suggested that  $Fe(CO)_4Hg$  is polymeric and *trans*.

We find that although the compounds  $Fe(CO)_4Hg_2X_2$  are sufficiently soluble in polar solvents to permit recrystallization, they do not give sufficiently concentrated solutions for mol. wt. or dipole moment determination. Their elemental analyses and infra-red spectra ( $2,200$ – $175$   $cm^{-1}$ ) can be consistently explained on the basis of a monomeric structure, *cis*- $Fe(CO)_4(HgX)_2$ , in which there are two mercury-iron bonds.  $Fe(CO)_4Hg$  is insoluble in all organic solvents and is most probably polymeric; both it and the corresponding cadmium compound we also believe to have the *cis*-configuration with mercury or cadmium-iron bonds.

The pattern of carbonyl stretching frequencies is typical of that found for *cis*- $M(CO)_4L_2$  molecules. For *trans*- $M(CO)_4L_2$  only one band would be expected. The spectra of all five compounds (Table 1) are similar in the region  $620$ – $400$   $cm^{-1}$ , where  $\delta(MCO)$  and  $\nu(M-CO)$  modes absorb<sup>3</sup>, and contain more bands than would be expected for a *trans*-structure. A further, very weak, feature near  $250$   $cm^{-1}$  is common to all spectra and may be the first overtone of a  $\delta(CFeC)$  mode. The only other band in the  $Fe(CO)_4Hg$  spectrum,  $195.9$   $cm^{-1}$  (s), we assign to a mercury-iron stretching mode on the grounds that no vibrational mode of an  $M(CO)_n$  grouping is likely to absorb in that region<sup>3</sup>. Consistent with this assignment is the

Table 1. ABSORPTION FREQUENCIES (CM<sup>-1</sup>) FOR NUJOL MULLS

Assign- ment	Fe(CO) <sub>4</sub> Hg	Fe(CO) <sub>4</sub> Cd	X = Cl	cis-Fe(CO) <sub>4</sub> (HgX) <sub>2</sub> X = Br	X = I
ν(CO)	2,048 2,012 (w) 1,988 1,958 1,923	2,030 1,957 1,913 1,873	2,080 2,021†	2,082 2,020†	2,079 2,013†
δ(MCO)	u.* 804 (vs) 549 (vw) 530 (w) 516 (w)	618 (vs) 553 (w) 541 (sh) 508 (vw)	618 (sh) 604 (vs) 556 (w) 515 (m)	u. 607 (vs) 466 (vw) 509 (m)	u. 603 (vs) 472 (vw) 513 (m)
ν(M-CO)	442 (w-m) 426 (w)	456 (w-m) 432.6 (w)	436 (w-m) 423 (w)	466 (vw) 429 (w-m) 419 (w)	472 (vw) 429 (w-m) 418 (w)
Low frequency modes	257.7 (vw) 195.9 (s)	251 (vw) 226 (w) 216 (m)	202 (vs) 272.6 (vs) 252.1 (w) 219 (vw) 201 (w)	252.4 (vw) 229.3 (s) 204.9 (s) 182.8 (s)	254.3 (w) 213.4 (s) 181.6 (s)

\* u., unresolved shoulder.

† Broad band with three unresolved components: approximate frequency only.

presence of bands at 216 and 226 cm<sup>-1</sup> (ν(Cd-Fe)), in Fe(CO)<sub>4</sub>Cd, which has an equally simple spectrum. For comparison, in Hg[Co(CO)<sub>4</sub>]<sub>2</sub> and Cd[Co(CO)<sub>4</sub>]<sub>2</sub>, ν(M-CO)(sym.) is at 152 and 161 cm<sup>-1</sup> respectively<sup>4</sup>, while ν(Pt-Sn) and ν(Rh-Sn) are found<sup>5</sup> near 210 cm<sup>-1</sup>. In the compounds cis-Fe(CO)<sub>4</sub>(HgX)<sub>2</sub>, for X = Cl, Br, there are no bands in the regions in which HgX<sub>2</sub> absorbs<sup>6</sup>, disproving the original suggestion that these are addition compounds.

Vibrations of the Hg-X and Hg-Fe bonds must interact substantially, particularly for the bromide and iodide, so that it is not reasonable to make assignments to ν(Hg-Fe) or ν(Hg-X): they must be regarded as normal modes of the Fe(HgX)<sub>2</sub> framework. For X = Cl, although there must be interaction, it may be reasonable to assign the 292 and 272.6 cm<sup>-1</sup> bands to modes involving mainly Hg-Cl stretching. In that event, absorption at 219 cm<sup>-1</sup> is assigned to ν(Hg-Fe). ν(Hg-Cl) in Fe(CO)<sub>4</sub>(HgCl)<sub>2</sub> is comparable with that<sup>6</sup> in Hg<sub>2</sub>Cl<sub>2</sub> and may be taken to indicate that mercury is in oxidation state I (ref. 7).

We have also investigated the reaction of mercuric chloride with the complexes Fe(CO)<sub>5-n</sub>(PPh<sub>3</sub>)<sub>n</sub> (n = 1, 2, 3) (ref. 8). These do not add two Hg-Cl groups. A typical product is Fe(CO)<sub>2</sub>(HgCl)Cl(PPh<sub>3</sub>)<sub>2</sub>, from n = 2, analogous to that of mercuric chloride on IrCl(CO)(PPh<sub>3</sub>)<sub>2</sub> (ref. 9).

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## Optical Resolution of a DL-Amino-acid by a Stereoselective Ligand Exchange Reaction

Few investigations of the utilization of metal chelate compounds for the optical resolution of racemic compounds have so far been reported<sup>1-4</sup>. DL-Aspartic acid copper complex was optically resolved completely from its super-saturated solution by seeding it with D- or L-aspartic acid copper complexes<sup>1</sup>. Bailar *et al.* first reported the stereo-specific (stereoselective) ligand exchange reaction<sup>2</sup>, and

later they reported the partial optical resolution of DL-alanine by use of the reaction of DL-alanine with copper glutamate (optical purity 3.3 per cent<sup>3</sup>). Recently, Shibata *et al.*<sup>4</sup> reported the partial optical resolution of DL-alanine in 25.6 per cent optical purity by use of the aspartic acid cobalt(III) complex.

In this communication the optical resolution of DL-aspartic acid by the use of stereoselective ligand exchange reactions is described. Copper complexes of D- and L-alanine, D- and L-glutamic acid, and L-proline were used in this reaction. This simple procedure for the optical resolution of DL-aspartic acid resulted in 95-100 per cent optically active D- and L-aspartic acids.

Typical examples are as follows: L-alanine, 1.78 g (0.02 mole), was heated at 80° C with 6.0 g of copper carbonate in 50 ml. of water for 10 min. Unreacted copper carbonate was removed by filtration. To the L-alanine copper complex solution, DL-aspartic acid, 2.66 g (0.02 mole) in 100 ml. of water was added and the solution was filtered. The solution was allowed to stand for 30 h. The precipitated crystals were filtered and washed with cold water and ethanol. A yield of 1.27 g of D-aspartic acid copper complex was obtained. The copper complex was dissolved in 30 ml. of 2 N hydrochloric acid and was treated with hydrogen sulphide. Copper sulphide was removed by filtration and the solution was treated with charcoal. After filtration, the solution was concentrated to dryness. The dried D-aspartic acid hydrochloride was dissolved in 5 ml. of water and the pH was adjusted to 2.8-3.0 with pyridine. D-Aspartic acid crystallized. The addition of 10 ml. of alcohol resulted in 0.54 g of free D-aspartic acid. The aspartic acid was free of alanine as determined by paper chromatography and on the automatic amino-acid analyser; [α]<sub>D</sub><sup>25</sup> = -23.3, in 6 N hydrochloric acid, C = 1.27; anal. calc. for C<sub>4</sub>H<sub>7</sub>NO<sub>4</sub>: C, 36.09; H, 5.30; N, 10.52; found: C, 35.91; H, 5.41; N, 10.30.

In the same way, D-alanine copper complex (0.02 mole) was treated with DL-aspartic acid (0.02 mole). After standing for 30 h, 1.39 g of L-aspartic acid copper complex was obtained. From this, 0.54 g of free L-aspartic acid was isolated, [α]<sub>D</sub><sup>25</sup> = +23.0°, in 6 N hydrochloric acid, C = 1.36; anal. found: N, 10.48.

Similar reactions were carried out in the combination of (L-glutamic acid copper complex and DL-aspartic acid), (D-glutamic acid copper complex and DL-aspartic acid) and (L-proline copper complex and DL-aspartic acid). Results are shown in Table 1.

Table 1

Amino-acid to compose copper complex	Amino-acid to be resolved	Resolved aspartic acid* Yield (g)	Configu- ration	[α] <sub>D</sub> <sup>25</sup> ~ <sup>26</sup>	% Optical purity
L-Alanine (0.02 mole)	DL-Aspartic acid (0.02 mole)	0.54	D	-23.3	95
D-Alanine (0.02 mole)	DL-Aspartic acid (0.02 mole)	0.54	L	+23.0	93
L-Glutamic acid (0.02 mole)	DL-Aspartic acid (0.03 mole)	1.07	D	-24.7	100
L-Glutamic acid (0.025 mole)	DL-Aspartic acid (0.025 mole)	0.85	D	-24.2	98
L-Glutamic acid (0.03 mole)	DL-Aspartic acid (0.02 mole)	1.10	D	-15.6	63
D-Glutamic acid (0.02 mole)	DL-Aspartic acid (0.03 mole)	0.85	L	+24.7	100
L-Proline (0.02 mole)	DL-Aspartic acid (0.03 mole)	0.59	D	+24.1	98

\* Resolved aspartic acids were chromatographically pure. Elemental analysis of these resolved aspartic acids agreed with the theoretical value. Specific rotation of pure L-aspartic acid: [α]<sub>D</sub><sup>25</sup> = +24.72° in 6 N hydrochloric acid, C = 8.00.

The mechanism of the reaction has not yet been clarified. However, it is likely that the configuration of the entering amino-acid in the ligand exchange reaction is a mirror image structure of the amino-acid which composes the copper complex. Fig. 1 shows the possible mechanism of this reaction in which two L-amino-acids approach the copper complex composed of the two D-amino-acids.



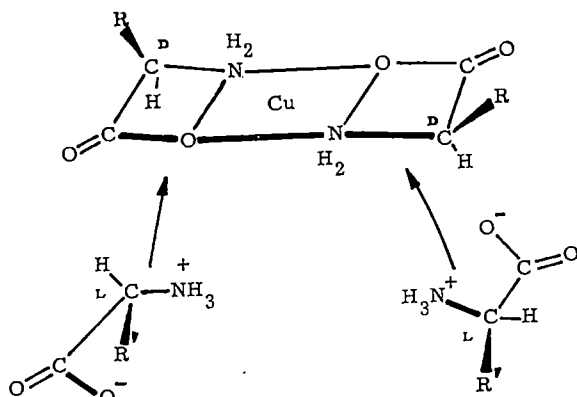


Fig. 1

The stereoselective reaction is not only interesting as a new method of optical resolution of  $\alpha$ -amino-acids, but is also interesting as an explanation of the development of optical activity of amino-acids in pre-biological systems<sup>5</sup>. Once an optically active amino-acid arose (for example by the spontaneous resolution of glutamic acid from aqueous solution<sup>6</sup>), the optical activity might be transferred to other amino-acids successively through various metal chelate compounds.

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### Line-width Alternation in the Electron Spin Resonance Spectra of *Para*-dinitrobenzene Anions in Various Mixed Solvents

As part of a general investigation of ion-solvent and ion-ion interactions by spectrometric methods we have monitored the changes in the electron spin resonance spectrum of the anion of *para*-dinitrobenzene in methanol on addition of a variety of solvents. The isotropic hyperfine coupling to <sup>14</sup>N in the two nitro-groups ( $a_{AV}^N$ ), which is particularly sensitive to changes in solvent, is plotted against the mole-fraction of added solvent in Fig. 1. It was observed, however, that of the five components associated with <sup>14</sup>N, those having  $m_N = \pm 1$  ( $m_N$  being the *Z* component of the nitrogen nuclear spin angular momentum), namely the second and fourth sets of lines, were often much broader than the remaining lines. Results are given in Fig. 2 and a typical spectrum is shown in Fig. 3a.

This phenomenon is comparable with that reported by Fraenkel *et al.* for the anions of the dinitrodurenes<sup>1</sup> and dinitromesitylenes<sup>2</sup> and for the *meta*-dinitrobenzene anion<sup>3</sup>; but these workers found no linewidth alternation for the *ortho*- or *para*-isomers in dimethyl formamide<sup>3,4</sup> even at  $-50^\circ\text{C}$ . Indeed, that for the *meta*-isomer was only barely detectable at room temperature.

Because of its marked instability the anion of *para*-dinitrobenzene was prepared by *in situ* ultra-violet

photolysis of methanolic solutions of *para*-dinitrobenzene containing 0.2–0.3 M sodium methoxide. We were unable to examine solutions containing less than about 30 per cent mole-fraction of methanol as secondary products, mainly the *para*-nitroanisole anion, obscured the spectra before they could be accurately monitored.

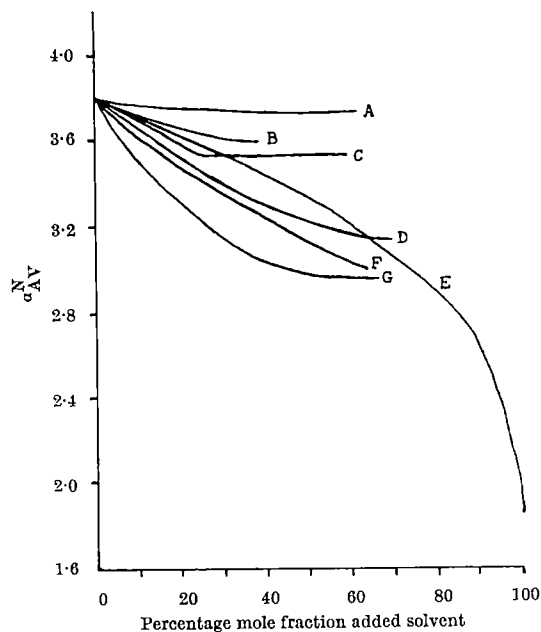


Fig. 1. Variation of  $a_{AV}^N$  for the anion of *para*-dinitrobenzene in methanol with added solvents: A, methylene chloride; B, *t*-butanol; C, diethyl ether; D, tetrahydrofuran; E, methyl cyanide; F, acetone; G, dimethoxyethane.

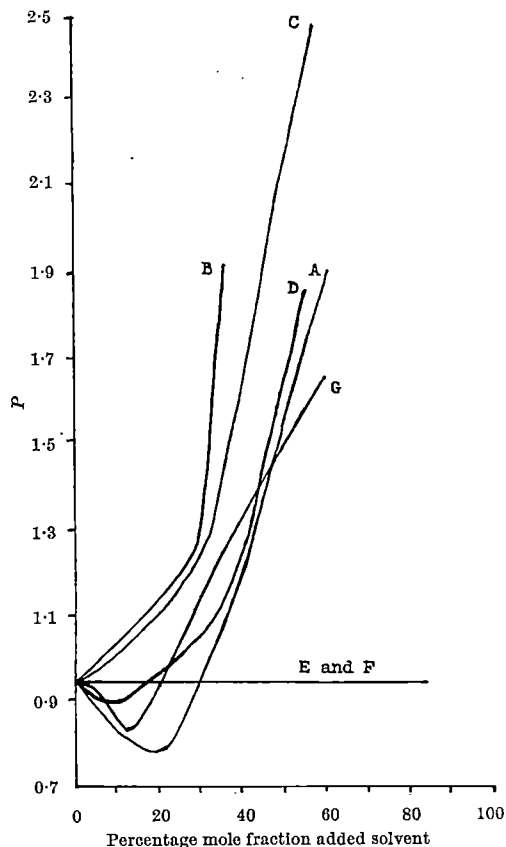


Fig. 2. Variation of line-width parameter *P* for the anion of *para*-dinitrobenzene in methanol with added solvents; same key as Fig. 1.

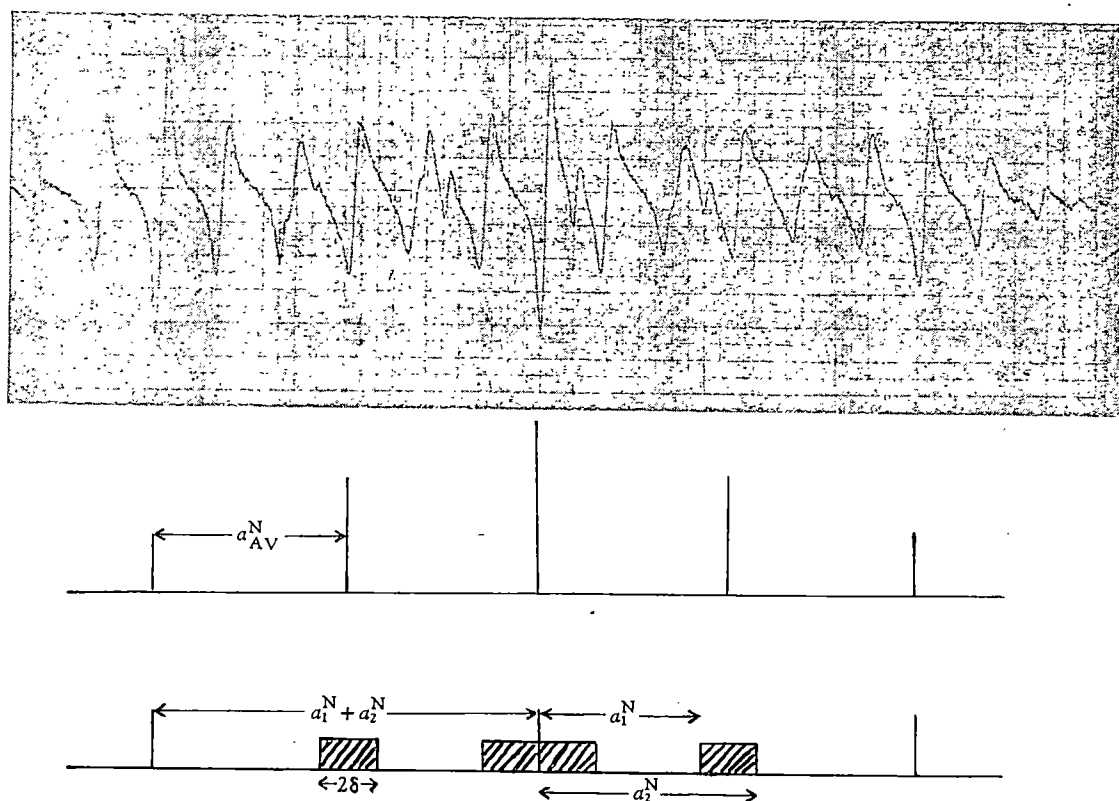


Fig. 3. *a*, A typical spectrum of the sodium salt of the *para*-dinitrobenzene anion showing line-width alternation; *b*, theoretical reconstruction showing the effect of fluctuation in the nitrogen hyperfine coupling constants  $a_1^N$  and  $a_2^N$  about the mean value  $a_{AV}^N$

$$a_1^N + a_2^N = a_{AV}^N = \text{constant}$$

$$|a_2^N - a_1^N| = 2\delta$$

In order to allow for the effect of anisotropic hyperfine interactions and  $g$ -values on the line-widths, the results in Fig. 1 have been recorded as a plot of the mean amplitude of the lines corresponding to  $m_N = 0, \pm 2$  to that of the lines corresponding to  $m_N = \pm 1$ . The value of this ratio for two equivalent  $^{14}\text{N}$  nuclei, showing a 1:2:3:2:1 intensity distribution, should be 0.83. Amplitudes of the derivative lines have been used since this is by far the most sensitive parameter to slight changes in width.

Fraenkel *et al.* have suggested that the alternation found by them for the *meta*-isomer at low temperatures stems from an asymmetric solvation which, either directly by altering spin-densities, or indirectly by encouraging an out-of-plane twist by one nitro-group, causes a fluctuation in the nitrogen hyperfine coupling constants about the mean value  $a_{AV}^N$ .

The way this affects the line-widths is depicted in Fig. 3*b*. The distance of lines 1 and 5 from the centre of the spectrum is equal to  $(a_1^N + a_2^N)$ , which is a constant, while the distance of lines 2 and 4 from the centre fluctuates between  $a_1^N$  and  $a_2^N$ . The central line will be composed of a narrow component of unit intensity superimposed on a broadened component of twice this intensity.

Our results demonstrate that unsymmetrical solvation is not the cause of this alternation for the *para*-isomer. Thus, for example, on adding methyl cyanide there is a steady decrease in  $a_{AV}^N$ , but no change in  $P$  is detectable, whereas methylene chloride causes a marked change in  $P$  but scarcely changes  $a_{AV}^N$ .

In our view the two nitro groups are likely to experience an almost equivalent solvation at all times except when in the vicinity of a cation. Thus we assign the alternation

to asymmetric ion-pair formation, the cation staying preferentially close to one or other of the nitro groups. If the ions were in direct contact then we would expect  $a_{AV}^N$  to be modified and to be a function of the size of the cation. The fact that in many instances  $a_{AV}^N$  scarcely changes at all suggests that the ions are present as solvent-separated ion-pairs<sup>5</sup> and that in solvent mixtures containing either methylene chloride, *t*-butanol or diethyl ether, the anion still comes into direct contact with methanol molecules. The marked effect of dimethoxyethane on  $a_{AV}^N$  then stems from the fact that this solvent solvates the cations, thus displacing methanol from the region between the ions.

The strong shift caused by methyl cyanide and acetone is reminiscent of the effect of these solvents on the ultra-violet spectrum of solvated halide ions<sup>6</sup> and may stem from the same cause. Thus methanol probably solvates very intimately by bonding to the oxygens of the nitro-groups, whereas methyl cyanide is expected to solvate more generally and hence more loosely, thus causing less disturbance of the spin density distribution in the anion.

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### Effect of Water Vapour on the Sensitivity of a Macro-argon Ionization Detector

As part of a comprehensive investigation into the physics of various forms of ionization detector for use in gas chromatography, it became necessary to determine the effect of water vapour on detector sensitivity. Lovelock<sup>1</sup> shows a graph which indicates that the sensitivity of a macro-argon detector was reduced 10-fold by a change in water vapour concentration from about 30 p.p.m. to about 1,000 p.p.m. v/v. At less than 30 p.p.m. of water vapour, the detector response is constant<sup>2</sup>.

A steady stream of 500 ml./min of ordinary commercial argon from the British Oxygen Co. was passed through a drying train consisting of two U-tubes filled with Linde type 13 x molecular sieve, in series with a further two U-tubes filled with phosphorus pentoxide. The water content of the issuing gas was certainly less than 1.5 p.p.m. v/v. The all-metal apparatus was continuously purged with this dry gas unless an experiment was in progress. The dry gas was then passed into a sulphuric acid saturator which produced known concentrations of water vapour<sup>3</sup>. This wet gas was fed into a power-driven syringe dilution system. The syringe was loaded with saturated diethyl ether vapour as the test substance. After injection of the ether vapour into the argon stream, the gas was passed into a metal ballast chamber, and thence into a flow splitter. 100 ml./min was fed into the macro-argon detector (Lovelock<sup>4</sup>) which was thermostatted at 50° C. On emerging from the detector, the stream passed into an electrolytic hygrometer<sup>5</sup>, in order to measure the water vapour concentration. The ionization current signal from the Pye macro-argon detector was measured with an Ekco vibrating reed electrometer type N616A, and displayed on a Honeywell potentiometric recorder. The detector was polarized with a stabilized voltage of 800 V derived from a type 532/D extra high tension power unit by Isotope Developments, Ltd.

Fig. 1 shows the response of the detector to various levels of ether vapour when dry argon was used. It confirms, in a general fashion, the results of Wiseman, quoted by Littlewood<sup>6</sup>. To obtain the results shown in Fig. 2, a constant test concentration of 5.8 p.p.m. v/v of diethyl ether vapour was used, and the concentration of water vapour varied in the argon supply. The concentration of test substance is of the same order as that of Desty, Geach and Goldup<sup>7</sup>, who used 2.6 p.p.m. of *n*-heptane in their evaluation of a flame ionization detector. It is seen that even a change in concentration of water vapour from 8 to 10 p.p.m. reduces the response of the detector to 5.8 p.p.m. of ether vapour by some 20

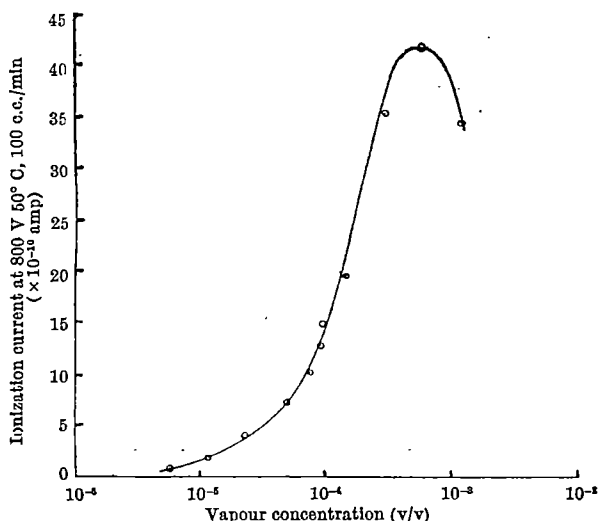


Fig. 1. Plot of ionization current against concentration of di-ethyl ether in a macro-argon detector, using dry argon

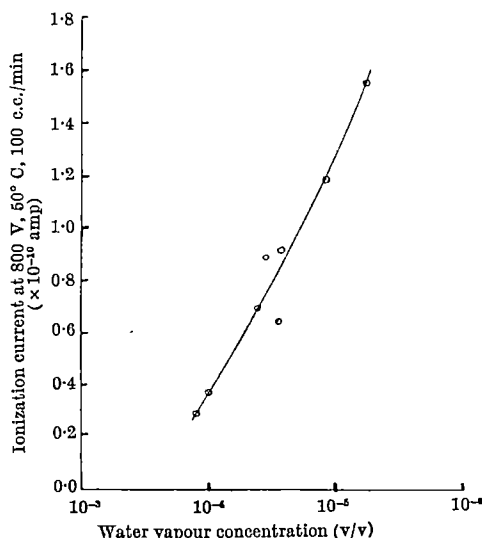


Fig. 2. The effect of various concentrations of water vapour in the argon carrier gas on the sensitivity of a macro-argon detector to a constant level of 5.8 p.p.m. of diethyl ether vapour

per cent. Hence it is clear that, for quantitative work, an effective drying train must be placed in the argon feed line to the detector, and the whole system thoroughly dried out as recommended by Evans and Scott<sup>2</sup>. The reduction in sensitivity is probably due to the attachment of electrons to water molecules to form negative ions having a lower mobility, Wilkinson<sup>8</sup>. Measurements were also made of the water vapour concentration in argon obtained straight from the cylinder, and Table 1 shows the results for several cylinders selected at random. With a cylinder having a pressure of 1,000 lb./in.<sup>2</sup> or more, the water concentration is likely to be of the order 3 p.p.m. This low level confirms the unpublished work of Hill in 1962 that the sensitivity of a micro-argon detector (Lovelock<sup>4</sup>) was not increased by placing a cold trap at -78° C in the argon line.

Cylinder No.	Cylinder pressure (lb./in. <sup>2</sup> )	Measured humidity (p.p.m. v/v)
1	1,350	2.5
2	880	1.5
3	850	6.0
4	90	10.9
4	50	15.9
4	27	35.4
5	22	4.5

Constant argon flow rate from cylinders of 500 c.c./min.

Even though dry argon is used, it may well be possible for the injection of a series of wet samples on to a column which holds back water vapour to give rise to a steady eluted concentration of water vapour which is sufficient to affect the detector sensitivity.

W. G. Pye, Ltd., kindly lent the detector to us, and the Hirst Research Centre of the General Electric Co. gave advice on the operation of the electrolytic hygrometer. This work was supported by the Sir Halley Stewart Trust.

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## BIOCHEMISTRY

## Deoxyribonucleic Acid Content of Basal Cells of Mouse Epidermis

STUDIES on mouse-ear and body-skin epidermis have led Gelfant<sup>1,2</sup> to the conclusion that there are two distinct populations of epidermal cells, one with a  $G_2$  period of a few hours, the other with a  $G_2$  period as long as five days. Bullough<sup>3</sup> has commented on the work of Gelfant and stated that his results are statistically inadequate. In view of the general uniformity in length of the  $G_2$  period in mammalian cells studied so far<sup>4</sup> of approximately 2–4 h, the existence of a population of cells with an unusually long  $G_2$  period is of some importance. It seemed worth while, therefore, to determine the distribution of DNA levels in epidermal cells, using a densitometric method, since a pronounced peak would be found at the tetraploid level if a sizeable population of cells were in the  $G_2$  state.

Pieces of skin were removed from the ears and backs of hairless mice immediately after they were killed by cervical fracture. Care had been taken not to stress the animals during the previous week<sup>5</sup>. The skins were left for 24 h at 2° C in a solution of 0.5 per cent acetic acid. At the end of this time the epidermis was peeled off the underlying connective tissue and soaked in acetic/methanol (1:3) for 30 min. The basal cells were then gently scraped off the keratin layer on to glass coverslips, squashed in a press, air-dried, and stained by the Feulgen method<sup>6</sup>, using

pararosaniline dye (Chroma). A Barr and Stroud integrating microdensitometer was used to measure the absorption of the cells at 5500 Å. Leucocytes from a mouse were smeared on the coverslips prior to staining in order to establish the diploid DNA-level. This level did not alter if leucocytes were initially exposed to acetic acid in the same way as the skin.

Histograms are shown of the DNA-levels of the basal cells of the thin epidermis of the ear in Fig. 1 (100 cells), and the multilayered epidermis of the dorsal skin in Fig. 2 (50 cells). The cells in both cases were almost entirely diploid. Scanning several hundred other cells by eye failed to reveal more than an occasional cell with a tetraploid amount of DNA. Similar distributions of DNA-levels were found in basal cells obtained from the skin of hirsute (random bred Hall Institute) mice. Since there is normally a slow turnover of basal cells<sup>7,8</sup>, it is to be expected that some cells would be in the  $G_2$  phase. However, no evidence was found of a large population in this phase. Hypodiploid nuclei were evident and could be obtained in quantity by more vigorous scraping of the keratin layer. A reduction in the DNA content of epidermal cells undergoing keratinization has been reported<sup>9</sup>.

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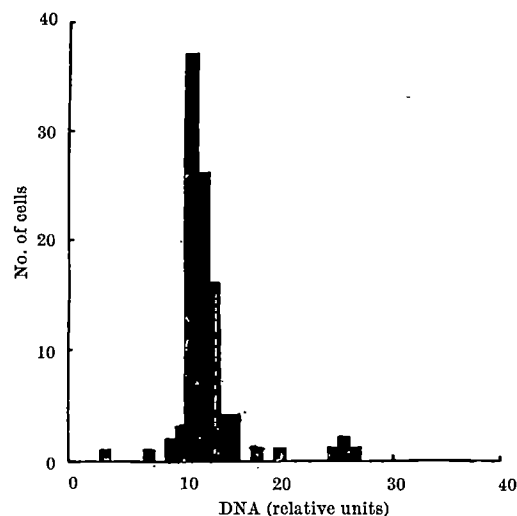


Fig. 1. DNA content of basal cells of ear epidermis

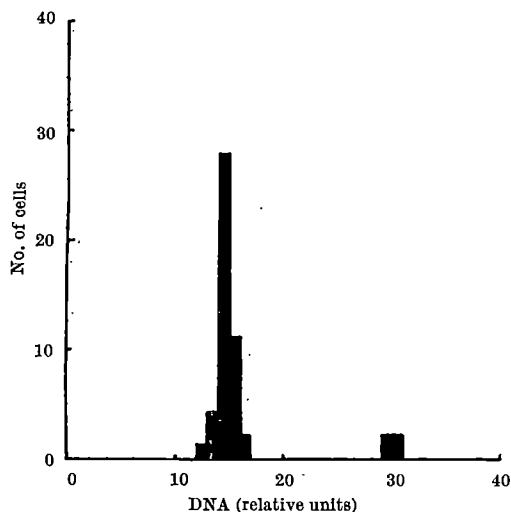


Fig. 2. DNA content of basal cells of dorsal epidermis

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## Progressive Hyperglycaemia in Experimental Obesity of Albino Rats

THE coincidence of two hereditary metabolic disorders, diabetes and obesity<sup>1,2</sup>, is quite common although their interrelationship is still a matter of speculation as there is no proof that obesity causes diabetes. On the other hand, the importance of various physiological conditions, such as obesity, puberty or pregnancy, in precipitating diabetes cannot be overlooked. Few experimental observations record their coincidence. Mayer *et al.*<sup>3</sup> showed the connexion between obesity and diabetes in an obese-hyperglycaemic strain of mice. Long<sup>4</sup> mentioned that only with great difficulty was a temporary diabetes-like state induced in hypothalamic obesity in albino rats by partial pancreatectomy. Except for the observation of Katsuki *et al.*<sup>5</sup>, who induced obesity and hyperglycaemia by gold thioglucose injection in intact mice, there is no recorded evidence that in intact animals hyperglycaemia results from obesity. While investigating the metabolic character of obese albino rats, we had already noticed that the fasting blood sugar was significantly high in these animals in comparison to control animals<sup>6</sup>.

An investigation into the carbohydrate metabolism of obese albino rats, in which obesity was induced and maintained for a longer period, was therefore undertaken to detect any correlation between obesity and hyperglycaemia.

Thirty-six male albino rats of this Institute's colony were divided between two equal groups and maintained on a standard stock diet and a 40 per cent fat diet, respectively. Both groups were treated with 'P.Z.' insulin as recorded earlier<sup>6</sup>. Glucose tolerance tests were performed periodically on the surviving rats of the control and obese groups at 7, 10 and 12 months. At 10 months, when the fasting blood glucose-level was high, blood

Table 1. CHANGES IN PYRUVIC (P) AND LACTIC (L) ACID BEFORE AND AFTER ORAL GLUCOSE (1 g/kg BODY-WT.) AT 10 MONTHS  
(Values expressed in mg per cent indicate mean  $\pm$  S.E. of the mean)

Hours of estimation	Blood pyruvic acid			Blood lactic acid			L : P	
	Control	Obese	T between means	Control	Obese	T between means	Control	Obese
0	0.31 $\pm$ 0.01	0.24 $\pm$ 0.006	4.2 *	7.5 $\pm$ 0.74	13.2 $\pm$ 1.2	4.1 *	24.2	55
1.5	0.44 $\pm$ 0.03	0.36 $\pm$ 0.004	2.3 *	14.6 $\pm$ 1.7	23.5 $\pm$ 2.5	2.9 *	33.2	65.3
3	0.5 $\pm$ 0.04	0.5 $\pm$ 0.17	0.17	13.6 $\pm$ 3.3	29.7 $\pm$ 3.3	2.8 *	27.2	59.4

\* Significant at 5 per cent level, each result represents mean of six observations.

Table 2. CHANGES IN BLOOD SUGAR AND INORGANIC PHOSPHATE (Pi) BEFORE AND AFTER ORAL GLUCOSE (1 g/kg BODY-WT.)  
(Values expressed in mg per cent indicate mean  $\pm$  S.E. of the mean)

Month at which estimation performed	Blood sugar									Blood Pi		
	7			10			12			10		
Hours of estimation	Control	Obese	T between means	Control	Obese	T between means	Control	Obese	T between means	Control	Obese	T between means
0	97 $\pm$ 3.1 (6)	121 $\pm$ 3.4 (12)	5.3 *	149 $\pm$ 1.2 (6)	162 $\pm$ 4.5 (6)	2.7 *	167 $\pm$ 3.5 (8)	177 $\pm$ 6.6 (8)	1.3	4.03 $\pm$ 0.16 (6)	4.61 $\pm$ 0.1 (6)	3.01 *
1	147 $\pm$ 2.1 (6)	166 $\pm$ 5 (12)	3.5 *	173 $\pm$ 6.3 (6)	209 $\pm$ 12.9 (6)	2.5 *	180 $\pm$ 4.4 (8)	213 $\pm$ 6.1 (8)	4.4 *	3.20 $\pm$ 0.11 (6)	2.70 $\pm$ 0.1 (6)	3.5 *
2	131 $\pm$ 4.5 (6)	129 $\pm$ 3 (12)	0.18	156 $\pm$ 13 (6)	157 $\pm$ 2.9 (6)	0.7	156 $\pm$ 3.1 (8)	171 $\pm$ 9.0 (8)	1.6	3.34 $\pm$ 0.08 (6)	2.75 $\pm$ 0.04 (6)	2.3 *
4	131 $\pm$ 4.4 (6)	150 $\pm$ 1.5 (12)	4.6 *	162 $\pm$ 12.8 (6)	161 $\pm$ 1.9 (6)	0.7	172 $\pm$ 6.1 (8)	161 $\pm$ 10 (8)	0.9	3.85 $\pm$ 0.3 (6)	3.38 $\pm$ 0.1 (6)	1.5

\* Significant at 5 per cent level, figures in parenthesis indicate the number of animals used in each observation.

inorganic phosphate was also estimated by the method of Taussky and Shorr<sup>7</sup>. Simultaneously with the glucose tolerance test, pyruvic and lactic acids in blood collected from tail vein were also estimated by the methods of Friedman and Haugen<sup>8</sup> and of Barker and Summerson<sup>9</sup>, 0, 1.5 and 3 h after glucose (Table 1). Each estimation was repeated at least twice.

Progressive increases in the fasting blood sugar-levels were noticed (Table 2) at 7, 10 and 12 months in both the groups. The values ranged from 85–106, 147–153 and 160–188 mg per cent in control, and 103–146, 145–182 and 167–222 mg per cent in the obese group at 7, 10 and 12 months, respectively. Except at 12 months, the fasting blood sugar in the obese group was significantly higher than that in control.

The peak value (1 h after glucose) in the obese group was always significantly higher than that in the control group, the ranges being 138–149, 158–199 and 169–203 mg per cent in the control, and 137–192, 173–238 and 197–238 mg per cent in the obese group at 7, 10 and 12 months, respectively. There was no significant difference in blood glucose values in the two groups afterwards. The fasting blood level of inorganic phosphate, which was significantly higher in the obese group (Table 2), came down to a significantly lower level compared to normal group 1 and 2 h after glucose. Three hours after glucose the blood level of pyruvic acid was identical in both the groups (0.5 mg per cent). Its fasting level was significantly lower in the obese group (Table 1). The fasting level of lactic acid, which was significantly higher in the obese group (13.2 mg per cent) compared to control group (7.5 mg per cent), recorded progressive significant increase to 23.5 and 29.7 mg per cent compared to control values of 14.6 and 13.6 mg per cent at 1.5 and 3 h, respectively.

The resistance of intact rats to the hyperglycaemic episode is well known. It is practically impossible to induce persistent hyperglycaemia and diabetes in intact animals of this species by means other than surgical or chemical pancreatectomy. It was reported by Ingle<sup>10</sup> that when insulin formation was suppressed in albino rats by the prolonged injection of insulin, its subsequent withdrawal led to a diabetes-like state. In the present work,

impairment of glucose tolerance or hyperglycaemia could not be explained on the foregoing basis as both groups of animals were treated similarly.

Prolonged feeding of a high fat diet causes lowering of glucose tolerance in man. Haist<sup>11</sup> reported that high fat diet caused reduction of the insulin content of  $\beta$  cells. Both the foregoing observations indicate partial functional failure of insulin apparatus on prolonged fat feeding. However, it is difficult to come to such a conclusion on the basis of our observation, particularly as changes in blood inorganic phosphate level (Table 2) strongly indicate a normal phosphorylation mechanism which is under the direct control of insulin and must always precede glycolysis.

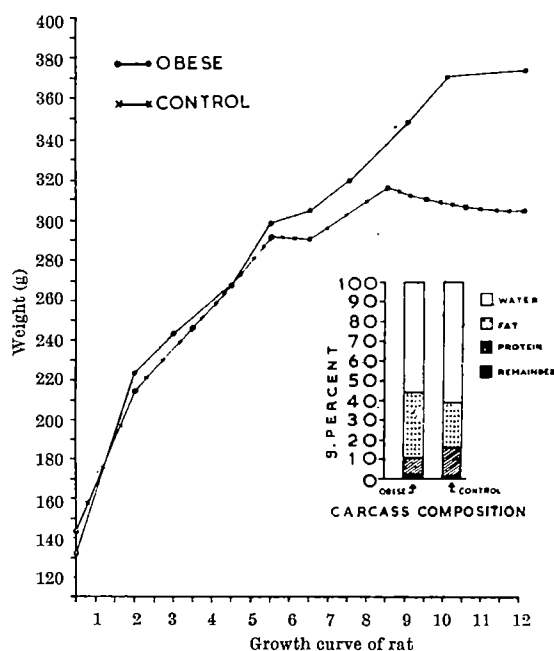


Fig. 1

The obese rat has accumulated a disproportionate mass of body fat (above 30 per cent, Fig. 1). The high metabolic activity of fat tissue in the mobilization of endogenous fat and oxidation of glucose has been unquestionably established<sup>12</sup>. That a very rapid mobilization of body fat at this site might impede glucose oxidation in muscle was conceived by Randle<sup>13</sup>. Unmistakable evidence of failure of the oxidative process to keep up with the glycolytic reaction after glucose administration is demonstrated by the rise in pyruvic acid in both groups, and by the mounting lactic acid level in the blood of the obese rats, which is significantly higher than the controls. It can be imagined that the hyperglycaemia recorded here may be due to rapid mobilization of fat resulting in the accumulation of breakdown products which might block the oxidative processes through which the products of glycolysis must pass for the complete oxidation of glucose. The importance of the present observation lies in the fact that here progressive hyperglycaemia has been a direct consequence of the induction of obesity in intact normal rats.

This material was presented, in my absence, to the Fifth Congress of the International Diabetes Federation, Toronto, July 1964.

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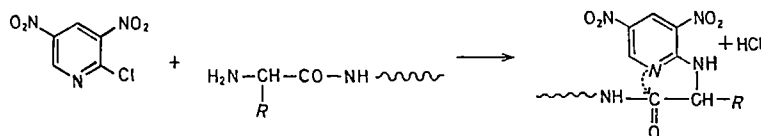
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## Determination of the N-terminal Amino-acid in Polypeptides and Proteins

THE weakest point in the DNP-method<sup>1</sup> is the destruction of the N-terminal DNP-amino-acid which occurs under the conditions of hydrolysis necessary to detach it from the protein. The optimum time of hydrolysis for an end-group analysis is the time at which DNP-peptides are no longer present; this is different with every protein, but overnight hydrolysis (16–24 h) with constant boiling HCl is routine. The extent of destruction is different with each protein, and is generally much greater than would be expected for any free DNP-amino-acid under the same conditions of hydrolysis in the absence of protein. It is not possible to correct for destruction by hydrolysis for a graded series of times and extrapolation to zero time, for much of the destruction seems to take place during the initial hours of the hydrolysis before degradation has proceeded to small fragments. Thus, although the DNP-method has been, and will doubtless continue to be, widely used for end-group analysis, it is slowly being superseded by stepwise degradation methods.

With reference to this, in a preliminary investigation we have centred our attention on halogeno-di-nitropyridines and halogeno-nitropyrimidines. Among the several reagents proposed, 2-Cl-3,5-di-nitropyridine and 2-Cl-5-nitropyrimidine are the most interesting; they react under mild conditions (pH 8.5, room temperature) to give derivatives which are very soluble in organic solvents<sup>2,3</sup>.

Evidence in the literature<sup>4</sup> suggests that internal attack by the nitrogen atom at the carbonyl carbon would facilitate the cleavage of the first peptide bond. The object of this communication is to present a new technique for the determination of the N-terminal amino-acid in peptides and proteins by means of 2-Cl-3,5-dinitropyridine:



During the course of our investigations on some dinitropyridyl-peptides (DNPyr-peptides) we have observed a noteworthy acid and basic catalysis on the cleavage of peptide bond. The following derivatives: DNPyr-Ala-Gly, DNPyr-Gly-Gly, DNPyr-Leu-Leu, DNPyr-Ala-Phe and DNPyr-Pro-Gly in HCl 6 N at 60° C or in HCOOH 90 per cent at 100° C for 2 h<sup>5</sup>, release quantitatively Gly, Gly, Leu, Phe and Gly in that order (Table 1). In these conditions the recovery of any terminal dinitropyridyl-amino-acid (DNPyr-amino-acid) is almost quantitative; on the contrary, the DNPyr-amino-acids show different stability in boiling HCl, 6 N, with a maximum of decomposition for DNPyr-Ser, DNPyr-Thr and DNPyr-Pro. Good results were obtained by performing the hydrolysis in HCl, 6 N, at 60° C with formic acid; the use of formic acid is necessary as it is a solvent for many proteins.

Table 1. RELATIVE RATE OF HYDROLYSIS OF DNPYR-PEPTIDES

Compound	Recovery of terminal DNPyr-amino-acid*	T <sub>1/2</sub>	
		(HCl 6 N 60° C)	(HCOOH 90% 100° C)
DNPyr-Ala-Gly	95	5'	5'
DNPyr-Ala-Phe	96	31'	32'
DNPyr-Gly-Gly	98	51'	48'
DNPyr-Leu-Leu	95	45'	46'
DNPyr-Pro-Gly	92	40'	42'

\* These values are corrected only for chromatographic losses.

As shown in Table 1, the rate of hydrolysis of peptide bonds of different stability (Leu-Leu or Gly-Gly) is quite similar.

For the determination of the N-terminal amino-acid in a polypeptide chain the procedure is as follows: about 1 μM of protein and an equal weight of NaHCO<sub>3</sub> are dissolved or suspended in 1 ml. of water in a 25 ml. round-bottomed flask or test-tube of convenient size. To this is added twice the volume of an ethanolic solution of 2-Cl-3,5-dinitropyridine and the mixture is shaken for 2 h at room temperature. It is then acidified with a few drops of concentrated HCl diluted with 5 ml. of water and shaken with several 5 ml. portions of ethyl acetate, decanting after each extraction; this removes ethanol, excess 2-Cl-3,5-dinitropyridine and 2-OH-3,5-dinitropyridine, and leaves an aqueous suspension of the insoluble DNPyr-protein.

The hydrolysing acid (HCl 6 N with 30 per cent formic acid) is then added and hydrolysis is effected for 8–10 h at 60° C. The reaction mixture is transferred to a test-tube, diluted with water and extracted with four 5-ml. portions of ethyl acetate; the combined ethyl acetate extracts are then washed with small portions of water to remove ε-DNPyr-lysine peptides, the washings being returned to the aqueous phase. After evaporation of the ethyl acetate extracts the residue is quantitatively transferred to the paper chromatogram with small portions of acetone: the best two-dimensional system is that of Levy<sup>6</sup>. The end-group spot is then cut out and dropped into a 2 × 15 cm test-tube: 1 per cent sodium bicarbonate is added and the tube placed in a water bath at 60° C for 30 min. The optical density at 340 mμ (360 mμ in the case of DNPyr-Pro) is read in a Beckman model DU spectrophotometer against an extract of blank paper<sup>7</sup>.



The application of our method to known proteins has confirmed its validity so far as the quantitative determinations of glycine and phenylalanine in insulin and of lysine in lysozyme are concerned.

This method has three particular advantages: (a) the condensation with the terminal amino group is quantitative and relatively free from side reactions; (b) the DNPy-amino-acids produced are bright yellow, which is very helpful for their micromanipulation, chromatography and photometric estimation; (c) the terminal DNPy-amino-acids are quantitatively recovered after hydrolysis of the polypeptide chain.

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### Breakdown of Caffeine in the Leaves of *Coffea arabica* L.

THE caffeine content of older *Coffea* leaves decreases after an initial increase in young leaves<sup>1</sup>. There are two possible explanations of this fact; first, a chemical transformation of caffeine; secondly, a translocation from the leaf to other organs. The object of these investigations was to find out if there is a breakdown of caffeine in the *Coffea* leaves and what compounds are formed. Four differently labelled caffeine molecules were synthesized, two with <sup>14</sup>C in the purine ring (pos. 2 and 8) and two with <sup>14</sup>C in the methyl groups (N1-methyl <sup>14</sup>C and N7-methyl <sup>14</sup>C).

To determine whether there is a caffeine breakdown in the leaves, a radioactive caffeine solution was fed through the petiole to isolated *Coffea* leaves (feeding time 8 h). After 10 days the leaves were dried and pulverized, and the total radioactivity of the dry powder was determined. Part of the dry powder was extracted and the extract was chromatographed (Whatman No. 1, *n*-butanol / NH<sub>3</sub> (*d*: 0.91) / H<sub>2</sub>O, 86:5:14). The total activity of caffeine was determined. In older leaves there was a large difference between the total activity of dry powder and the total activity of caffeine still present. This difference is accounted for by the breakdown products of the preceding 10 days of incubation. My experiments showed that the breakdown rate of endogenous and of fed caffeine was almost the same.

Single leaves were fed in a plastic chamber through which air was sucked. After passing through the chamber the CO<sub>2</sub> was absorbed by a KOH solution. The older leaves released measurable amounts of radioactive CO<sub>2</sub> when they were fed with ring-labelled as well as with methyl-labelled caffeine. These experiments showed that a complete breakdown of the caffeine molecule in the metabolism of the leaf is possible.

To determine the different breakdown products, *Coffea* leaves, still connected with the tree, were fed with radioactive caffeine through the mid-rib (feeding time 1-2 days). Ten days later the leaves were gathered, frozen with liquid air, pulverized and extracted with 80 per cent ethanol. The concentrated extract was co-chromatographed with expected breakdown products (2-dim., Whatman No. 1; butanol / acetic acid / water (4:1:1); ethanol / acetic acid / water (81:5:14). X-ray papers were pressed on the chromatograms and exposed for 2 months. The developed co-chromatographed compounds

on the paper chromatograms and the radioactive spots on the X-ray papers were compared. The radiograms of C2- and C8-labelled caffeine did not show essential difference in the arrangement of spots. The feeding experiments with N1-methyl <sup>14</sup>C-caffeine showed the same arrangement of radioactive compounds as the experiments with N7-methyl <sup>14</sup>C-caffeine. The position of radioactive compounds on the radiograms is shown in Fig. 1. Three unknown substances, X, Y and Z, appeared not only in experiments with ring-labelled but also with methyl-labelled caffeine.

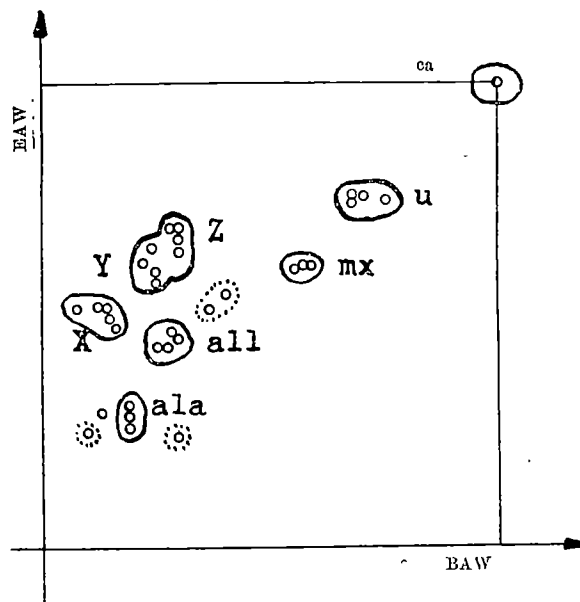


Fig. 1. Summary of the radiograms of the caffeine breakdown products. BAW, ethanol / acetic acid / water (81:5:14); BAW, butanol / acetic acid / water (4:1:1); all, allantoin; ala, allantoic acid; u, urea; mx, methyl-xanthine; X, Y and Z, unknown substances; ca, caffeine

In order to identify the breakdown products of caffeine I performed several groups of experiments. In the first group I investigated whether *Coffea* leaves degrade caffeine to methylated xanthines and uric acids, as do some mammals. No radioactivity was detectable in the different mono- and dimethyl uric acids. A radioactive spot coincided with 3- or/and 7-methyl-xanthine. In the second group of investigations allantoin, allantoic acid and urea were co-chromatographed with extracts of C2- and C8-feeding experiments. In both experiments the production of large amounts of radioactive allantoin and smaller amounts of active allantoic acid and urea could be demonstrated. The extracts in feeding experiments with methyl-labelled caffeine showed no activity in different amino-acids connected with Cl-metabolism (serine, citrulline, arginine, methionine).

After feeding experiments with C2- and C8-labelled caffeine the radioactivity was localized in the allantoin molecule. Alkaline and acidic hydrolysis degraded allantoin first to allantoic acid and to urea plus glyoxylic acid. After these hydrolyses the extract was chromatographed and the chromatogram autoradiographed. The whole activity of allantoin was recovered in urea. The identity of this urea was proved in a parallel trial by degradation with the enzyme urease. This experiment demonstrated that only the C-atoms of the ureido-groups of allantoin were radioactive. This fact indicated that allantoin is a real breakdown product of caffeine.

The experiments showed the path of carbon 2 and 8 of caffeine. There are a number of unknown intermediates between caffeine and allantoin. Allantoin is the precursor of allantoic acid and urea. The release of <sup>14</sup>CO<sub>2</sub> by the

leaves makes it probable that radioactive urea is hydrolysed by urease in the leaves of the *Coffea* tree. A more detailed report of these experiments will be published later<sup>2</sup>.

This work was supported by the Atomic Energy Commission of the Swiss National Research Foundation.

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### Solubilization of Keratins by Reaction with Ethylene Oxide

THE reactions of keratins and other proteins with epoxides have been described by several investigators<sup>1</sup>. The chemical modification of wool by reaction with mono- or di-functional epoxides in aqueous or alcoholic solution at or near room temperature has also been studied. In general, the acid and alkali solubility is decreased by reaction with di-functional epoxides, but increased if a monofunctional epoxide is employed<sup>2</sup>. We have recently found, however, that quite different results are obtained if wool is heated with ethylene oxide and a small amount of *N,N*-dimethylaniline with no solvent, as described by Haas *et al.*<sup>3</sup> for making hydroxy-ethyl nylon. Under these conditions, the wool fibres are disintegrated and the product is soluble in water. Part of the ethylene oxide does not react and part is converted to polyethylene glycol which can be extracted from the aqueous solution with methylene chloride or toluene, although the latter solvent forms troublesome emulsions which are much more persistent than those formed by methylene chloride. A very small part of the ethylene oxide apparently becomes attached to the solubilized wool.

As an example, 0.960 g wool cloth was sealed in a glass tube with 10 ml. ethylene oxide and 0.1 g *N,N*-dimethylaniline in a nitrogen atmosphere (no special precautions were taken to include or exclude moisture) and heated at 80° C for 25 h. The viscous, syrupy reaction product was washed out of the tube with water, and the resulting aqueous solution was exhaustively extracted in a continuous extractor with methylene chloride. The syrupy extract weighed 6.2 g. The aqueous phase was clarified by centrifuging; the residue in the centrifuge tube, on drying, gave a solid that weighed 2.9 mg. The weight of the water-soluble, methylene chloride-insoluble fraction, estimated by drying an aliquot to constant weight, was 1.724 g. John J. Bartulovich, of this laboratory, found by ultracentrifugation (in a preliminary determination by the Archibald method) that the material not extracted from the aqueous solution by methylene chloride had a weight average molecular weight of about 10,000. Wilfred H. Ward, also of this laboratory, found that the material passed through a dialysis membrane, which indicated that the molecular weight cannot greatly exceed 10,000. The precise extent of degradation of the protein thus remain to be determined.

Other fibrous proteins solubilized by this procedure are: mohair, ovokeratin, cattle horn, and silk fibroin. In one trial with fibroin, complete solubilization was obtained, but in others only one-fifth or one-fourth of the silk was solubilized. The insoluble silk remaining after the reaction with ethylene oxide and *N,N*-dimethylaniline retained the fibrous appearance and structure.

When wool that has received a polyamide treatment applied by the interfacial polymerization procedure<sup>4</sup> is used, the wool is solubilized and the polyamide sheath is undissolved.

Work is in progress on the nature of the water-soluble reaction product resulting from the reaction of ethylene

oxide with the protein, and the procedure may be useful in fundamental keratin studies.

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### Estimation of Urinary Oxalate by the Method of Isotope Dilution

DURING the course of a series of metabolic experiments in which 1-<sup>13</sup>C-ascorbic acid and 1-<sup>13</sup>C-glycine were fed to a normal adult<sup>1</sup> and hyperoxaluric patients<sup>2</sup> daily samples of urinary oxalic acid were isolated as calcium oxalate for estimation of the <sup>13</sup>C enrichment. It was found expedient to determine the daily levels of excretion by the method of isotope dilution, using the specific <sup>14</sup>C-activity of the oxalate samples prior to combustion to CO<sub>2</sub> for mass spectrometric analysis. The amounts of <sup>14</sup>C and <sup>13</sup>C added were low and hence did not interfere with the measurement of <sup>13</sup>CO<sub>2</sub>. Previously reported methods for calcium oxalate estimation have the disadvantage that they depend on the complete recovery of oxalate from a sample of the urine<sup>3-7</sup>. Using the method of isotope dilution, however, the only exacting requirement is the purity of the final product, which is checked by repeated reprecipitation to constant specific activity.

Twenty-four-hour urine collections were made in 40 ml. of 6 N HCl, and after the addition of 1 : 2-<sup>14</sup>C-oxalic acid (1 µc., 0.2 µg) sufficient concentrated HCl was added to bring the concentration to 2 N and the whole was continuously extracted for 16 h with peroxide-free ether. The ether phase was evaporated in the presence of water (5 ml.) and the aqueous residue was treated with 0.880 NH<sub>4</sub>OH (1–2 ml.). Saturated CaCl<sub>2</sub> (0.5 ml.) was added and the precipitated (COO)<sub>2</sub>Ca.H<sub>2</sub>O was purified by solution in 2 N HCl (5–7 ml.), decolorization with 'Norit' and precipitation with 0.880 NH<sub>4</sub>OH and acetic acid at pH 5.2 (brom-cresol purple). Samples were counted by the liquid scintillation technique (Nuclear Chicago Instrument, model 725) using 100-µl. aliquots (taken from the HCl solution prior to re-precipitation) and a dioxan-based phosphor (10 ml. 'Panax', type T.E.D.). At the concentration used (0.1–0.5 mg of (COO)<sub>2</sub>Ca.H<sub>2</sub>O) no quenching due to calcium oxalate was observed, and the counting efficiency was 51 per cent. The disintegrations per min were calculated from an efficiency curve established by adding different volumes (25–300 µl.) of 2 N HCl to a known radioactive standard (Packard, Standard Source Toluene <sup>14</sup>C) in 10 ml. of phosphor and applying the 'Channels Ratio' method<sup>8</sup>. The purification process was repeated until the specific activity remained constant (usually three re-precipitations were adequate). In some cases the purity of the final product was also checked by dissolving 1–2 mg in 2 N H<sub>2</sub>SO<sub>4</sub> (5 ml.) and determining the oxalic acid by the method of Hodgkinson and Zarembski<sup>4</sup>. In any isotope dilution analysis the total amount of substance present is inversely proportional to its specific activity and the amount of urinary calcium oxalate was calculated from its specific activity, compared with that of a standard prepared from the same 1 : 2-<sup>14</sup>C-oxalic acid.

During metabolic experiments lasting 19 and 15 consecutive days<sup>2</sup>, a normal adult gave daily (COOH)<sub>2</sub>.2H<sub>2</sub>O excretions of 41.8 mg. (S.D. ± 4.6 mg) and 26.7 mg (S.D. ± 4.6 mg) respectively, whereas a hyperoxaluric



child studied for 28 consecutive days gave  $82.1 \text{ mg}$  ( $S.D. \pm 23 \text{ mg}$ ) per day. Values obtained for 3 consecutive days on the same hyperoxaluric child by the method of Archer *et al.*<sup>3</sup> were  $63.9 \pm 4.8$ ;  $40.0 \pm 2.5$  and  $37.7 \pm 2.4$ , while the isotope dilution method applied to the same urine samples gave values of  $80.8 \pm 2.8$ ;  $72.4 \pm 0.8$  and  $58.1 \pm 0.6$  respectively, expressed as  $\text{mg}$  of  $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$  per 24 h. Although it is possible that oxalogenic compounds in urine may produce as much as 10 per cent of the total oxalate during the acid-ether extraction period<sup>4</sup>, we suggest that the higher values obtained by isotope dilution are largely a reflexion of the sensitivity of the method.

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### Disruption of Mast Cells by a Component of Eosinophil Granules

EOSINOPHILS are phagocytic cells, and granules adjacent to the phagocytic vacuole rupture<sup>1</sup>. Eosinophil granules contain a number of acid hydrolases together with basic protein and peroxidase<sup>2</sup> and during phagocytosis these substances are discharged from the disrupted granules into, or alongside, the phagocytic vacuole. The acid hydrolases released from eosinophil granules probably have a digestive function, but the significance of the other granule components is not known. This work reports the presence in eosinophil granules of a substance capable of causing the disruption of mast cells. The active principle appears to be associated with the enzyme peroxidase.

Eosinophils were obtained from rats of the Long Evans strain, using the methods previously described of peritoneal lavage and centrifugation of peritoneal cells in albumin<sup>3</sup>. Contaminating red cells present in the eosinophil cell button were lysed by the addition of 2 ml. of a 0.2 per cent sodium chloride solution. The cells were suspended in the hypotonic saline solution for 15 sec. after which time the tube was filled with physiological saline and centrifuged. Eosinophils constituted approximately 90 per cent of the cells present, the remainder being mononuclear cells. Basic protein and peroxidase were extracted into solution from the eosinophil granules by the addition to intact cells of 2 ml. 0.05 M acetate buffer at pH 3.7. The supernatant solution obtained after centrifugation was added to 10 ml. 0.2 per cent ammonium hydroxide solution and a precipitate was formed.

The precipitate was redissolved in 0.05 acetate buffer at pH 4.5 to a concentration of approximately 10 mg protein/ml. and applied to a 'Pevikon' block. 'Pevikon powder' C870 (Stockholms Superfosfat Fabriks Aktiebolag, Stockholm) was mixed with a small volume of 0.05 acetate buffer at pH 4.5 and moulded into a block, 12 in.  $\times$  4 in.  $\times$  0.5 in., for electrophoresis. Electrophoresis was performed for 8 h at 250 V and 10 m.amp. when the block was cut into sections 0.5 in. in width, and

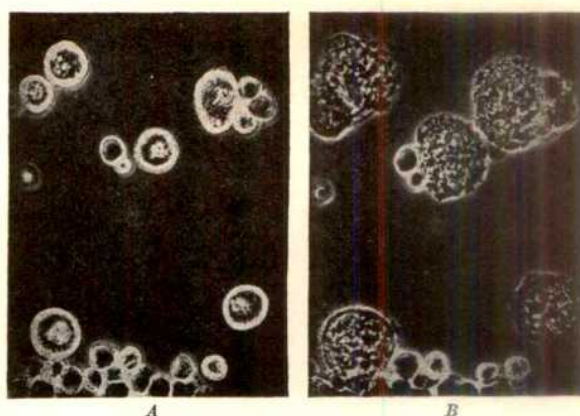


Fig. 1. (A) The appearance of 6 normal rat mast cells by phase contrast microscopy. (B) Same cells following introduction of an isotonic solution containing the peroxidase rich eluate at pH 7. Granules have escaped from the swollen cells.

eluates were obtained. Protein estimations on the eluates were performed by the method of Lowry *et al.*<sup>4</sup> and peroxidase was estimated by the guaiacol method of Chance and Maehly<sup>5</sup>. The activity of the peroxidase present was recorded in protein equivalents of crystalline horse-radish peroxidase. Crystalline horse-radish peroxidase, batch 62602A, was obtained from Nutritional Biochemical Corporation, New Jersey. Fresh mast cells were obtained from the peritoneal cavity of rats and were not separated from other peritoneal cells<sup>6</sup>. One drop of mast cells suspended in physiological saline was added to a mixture consisting of one drop of eluate, one drop of 0.2 sodium phosphate buffer at pH 7, and three drops of saline. Those eluates found to cause mast cell disruption were diluted in saline to the limit at which disruption of added mast cells occurred. The eluate containing the maximum amount of peroxidase was concentrated by ultrafiltration and re-applied to a 'Pevikon' block. Electrophoresis was repeated on a 'Pevikon' block at pH 5.8 in 0.05 M phosphate buffer. The peroxidase-rich fraction obtained after the second electrophoresis was submitted again to electrophoresis at pH 5.8. Eosinophil peroxidase has an absorption band in the Soret region maximal at 415 m $\mu$  (to be published) and the various preparations of peroxidase were tested for absorption at 415 m $\mu$  and 280 m $\mu$  using a Zeiss model P.M.Q. absorptiometer.

Results are shown in Table 1 of the estimations of protein and peroxidase concentrations in the eluates obtained from the 'Pevikon' block.

Table 1

Distance from origin towards cathode (in.)	Protein concentration in eluate ( $\mu\text{g}/\text{ml.}$ )	Peroxidase concentration in eluate ( $\mu\text{g}/\text{ml.}$ )	Disruption of added mast cells
0.0	0	0	0
0.5	0	0	0
1.0	0	0	0
1.5	0	0	0
2.0	5	0	0
2.5	155	28	0
3.0	94	36	0
3.5	16	50	+
4.0	90	108	+++

Maximum protein concentration was found in the eluate obtained 2.5 in. from the origin towards the cathode and maximum peroxidase concentration was found 4 in. from the origin. Mast cell disrupting activity was found in the eluate 4 in. from the origin (see Fig. 1) and weak activity against mast cells was found in the eluate at 3.5 in., the ratio of the absorption at 415 m $\mu$  to that at 280 m $\mu$ , for the various peroxidase preparations, was as follows:

Acid extract of eosinophils before electrophoresis	0.06
Peroxidase-rich fraction after first electrophoresis	0.45
Peroxidase-rich fraction after second electrophoresis	0.72
Peroxidase-rich fraction after third electrophoresis	0.80



Disruption of mast cells occurred when eluates obtained from each electrophoresis procedure were added to mast cells. The minimum protein concentration which caused mast cell disruption in these eluates was approximately  $1 \times 10^{-5}$  mg/ml. It is not known whether the substance causing disruption of mast cells is peroxidase but the absorption ratio of the fraction obtained after the third electrophoresis compares closely with the 430/280 ratio of 0.82 obtained by Agner for crystalline myeloperoxidase<sup>7</sup>.

It is known that eosinophils are attracted to sites of antigen-antibody reaction<sup>8</sup>, and granule lysis occurs during phagocytosis by eosinophils of antigen-antibody complexes. Liberation of granule components from the cells to the surrounding tissues has not been shown to occur but it seems probable. The component of the eosinophil granule active against mast cells may then be released to the surrounding tissues, causing disruption of local mast cells.

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## PHYSIOLOGY

### Interpretation of Skin Impedance Measurements

HUMAN skin presents a high impedance to alternating current of low frequency<sup>1</sup>. The impedance is greatly reduced if the surface layers of the skin are abraded<sup>2</sup>. When the frequency of the applied a.c. is raised above 10 c/s, the impedance falls; the equivalent circuit is a polarization impedance in parallel with a resistance<sup>3</sup>. The impedance of rabbit or pig skin is less than that of human skin, and is also less dependent on frequency (Fig. 1).

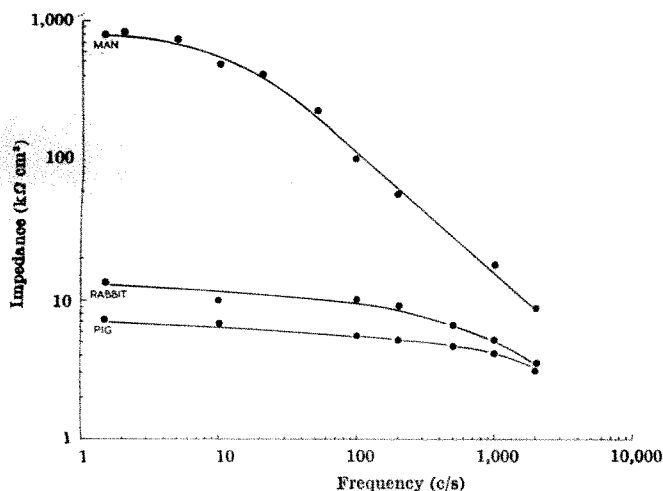


Fig. 1. Impedance of skin in contact with 0.9 per cent sodium chloride as a function of frequency of applied alternating current

I have investigated how the impedance to low-frequency a.c. recorded from skin is influenced by the way in which contact is made with the skin surface. A circular metal plate (0.2–14 cm<sup>2</sup>) was pressed down at 100 g/cm<sup>2</sup> on the dry-shaved dorsal surface of a man's forearm in a cool environment, and the impedance between this plate and a 15-cm<sup>2</sup> silver plate moistened with Cambridge electrode jelly on the volar surface was measured by a bridge technique, using 1.5-c/s a.c. at 1 V root mean square. Higher voltage produced non-linearity in response. Immediately on contact between the metal and the skin the impedance was very high ( $\gg 10$  M $\Omega$ ); it fell rapidly to reach a nearly steady value ( $Z$ ) within 10 min of contact. This steady value was inversely related to the area ( $A$ ) of the metal plate; the best fit was given by  $Z = kA^{-1.28}$ . As the divergence from inverse proportionality was small, results were expressed in impedance  $\times$  area, specific impedance (Table 1). Silver, aluminium and iron plates all gave the same specific impedance on the same skin region, and so did mercury, confined in a 1.5 cm<sup>2</sup> 'Perspex' ring. Powdered gold applied between a metal plate and the skin did not affect the impedance, nor did variation of the contact pressure in the range 30–300 g/cm<sup>2</sup>. Obstruction of the hair follicles with silicone grease before applying the metal plate raised the impedance slightly ( $Z_1/Z_0 = 1.32$ , 5 per cent fiducial limit 0.94–1.70 in 9 experiments), but placing similar grease spots between the hairs also raised the impedance slightly ( $Z_1/Z_0 = 1.18$ , 5 per cent fiducial limit 0.99–1.29).

Table 1. SPECIFIC IMPEDANCE OF SKIN TO 1.5-C/S A.C.: MEAN AND 5 PER CENT FIDUCIAL LIMITS

Species	Skin contact	Impedance (k $\Omega$ cm <sup>2</sup> )
Man, forearm	Silver	6,800 (5,200–8,400; 22 expts.)
	Agar gel	480 (150–810; 6 expts.)
	Saline	880 (590–1,170; 9 expts.)
Pig, flank	Saline	12 (8–16; 11 expts.)
Rabbit, flank	Saline	18 (11–26; 9 expts.)

With damp electrodes quite different results were obtained. Similar experiments to the aforementioned were performed, except that the dry-metal contact was replaced by 0.9 per cent sodium chloride in agar gel (area 0.16–3.5 cm<sup>2</sup>) to which electrical connexion was made both by voltage-recording and current-carrying silver wires. The impedance recorded was much less than with the dry electrode (Table 1), and fell only slightly with time of contact; after 5 min it was constant. The specific impedance (impedance  $\times$  area) was least for the smallest area; over the whole area range it varied by a factor of 1.8.

0.9 per cent sodium chloride solution, confined in a 4-cm<sup>2</sup> silastomer ring on the skin, gave a rather higher impedance than the agar gel; the difference was not significant (Table 1). A similar experimental arrangement on the clipped flank skin of an anaesthetized rabbit or pig showed a much lower impedance (Table 1).

The reciprocal of specific impedance, the specific admittance, is the current flow per unit E.M.F. and area. If the skin is adequately hydrated and the a.c. applied is of sufficiently low frequency, this should theoretically be proportional to the skin permeability to the current-carrying ions within the stratum corneum. The admittances of human, pig and rabbit skin under 0.9 per cent sodium chloride are in the ratio 1:73:49, while their permeabilities to Na<sup>+</sup> ions are in the ratio 1:27:42 (ref. 4). Thus both the animal skins are more permeable to ions and more conductive than human skin.

When the skin surface is not deliberately hydrated, the admittance should be a measure of the hydration of its surface layers provided that the contact between the electrode and the outermost cells of the stratum corneum is adequate. Since addition of contacting materials (gold dust, mercury) or increase of pressure does not increase the current flow, this is probably so. The small effect of greasing the hair follicles indicates that most of the current flows through the exposed epidermis in human skin.

The high specific impedance observed with small dry electrodes, and the low specific impedance observed with small wet electrodes, could both be due to lateral movement of water within the stratum corneum, in the first case drying the area under the electrode and in the second dampening the surrounding area.

These results indicate the conditions under which electrical impedance through skin can be interpreted in terms of the physical parameters of the stratum corneum.

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### Retinal Ganglion Cell Activity in Cats during Dark Adaptation

THE existence in complete darkness of a tonic retinal discharge is well known<sup>1-3</sup>. There still exist, however, some questions as to its origin and functional significance.

In an attempt to investigate the possible origin of the tonic dark discharge we have measured as a function of time the firing rate of single retinal ganglion cells [ $r(t)$ ] from the moment of light cut-off until the retina returns to the condition of dark adaptation. By correlation of this activity with the classical light threshold changes during dark adaptation some conclusion may be drawn as to the effect of receptor activity on tonic dark discharge.

Results reported here are based on measurements of approximately 100 retinal cells from approximately 50 cats. Each cat was pre-trigeminally sectioned<sup>4</sup>, injected with curare to prevent movement, and placed under artificial respiration. Rectal temperature and blood pressure were monitored during the experiments. The eye was opened (cornea and lens removed) and 5–10  $\mu$  steel microelectrodes placed in the retina. Most probably, all recordings were from large ganglion cells<sup>4</sup>. The entire retina was illuminated from a light source approximately 20 cm from the eye. Only those recordings were used in which a single cell's activity could be easily distinguished on the basis of amplitude. Amplified pulses were fed to a Beckman 7360 counter and observations made of the firing rate as a function of time. To measure light threshold changes (no attempt was made to measure absolute threshold) we applied bursts of light every 30–60 sec. The bursts were synchronized with the pulse counter to facilitate detection of small rate changes and lasted 300 msec. Threshold was taken to be the smallest value of light-burst intensity which produced a noticeable change in firing rate.

The general procedure was as follows: (1) light threshold and average firing rate were measured for the dark-adapted eye; (2) strong light (4,000 lux) was applied for some minutes; (3) firing rate and threshold were recorded, beginning from the moment of light cut-off.

Our results may be divided into two parts, concerning respectively the transient  $r(t)$  immediately following the onset of darkness, and the long-time (tonic) rate variations. Transient responses of on-cells differed markedly from those of off-cells, while long-time dark discharge behaviour did not significantly differ between the two types of cell.

The figure shows a typical result for transient behaviour. In the off-cell the transient after darkness has two prominent features: (a) a short (5–10 sec) large initial burst of activity; (b) a second, longer-lasting hump beginning after approximately 20 sec. In the on-cell the transient following darkness is an abrupt drop in rate followed by an exponential increase to a steady state rate  $R_0$ . The form is approximately  $r(t) = R_0 (1 - \exp - t/\tau)$ . In

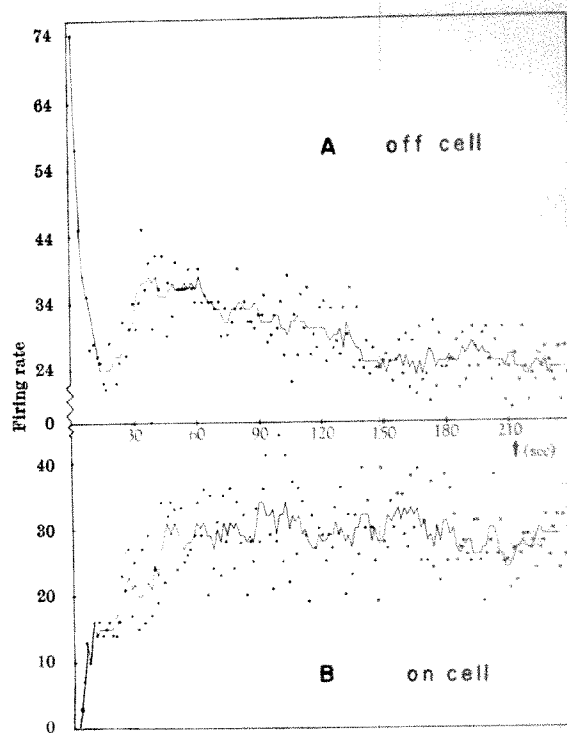


Fig. 1. Typical ganglion cell firing rate transients  $r(t)$  in discharge per sec plotted against time. For both examples strong retinal illumination of more than 5 min was turned off at  $t=0$ . The points represent actual readings every two seconds. The solid curve (except for the first several points) represents a short-time average (over 10 sec) calculated as a visual aid. The firing rate of the off cell (A) during the preceding illumination was 0 discharges per sec, while that of the on cell (B) was 48 discharges per sec. Note that the off cell plot ordinate does not begin at zero.

the example shown in Fig. 1,  $R_0$  is approximately 30 discharges per sec and the time constant  $\tau$  is approximately 30 sec.

Long-time rate changes after the onset of darkness were measured for as long as unit firing was countable. This was usually 30–45 min, sometimes 60–80 min, and in two or three cases for up to 2 h. In summary, we observed no systematic trends. After the initial transient the cell average firing rate remained relatively constant for a very long time.

The measurements of light threshold changes during dark adaptation revealed two salient features: (1) The threshold to light burst was almost always a monotonically decreasing function of time. The time it took for the threshold to become sensibly constant was in agreement with classical results (approx. 30 min) and always was at least 10 times as long as the duration of the firing rate transient. For this purpose the duration of the firing rate transient was defined as the time necessary for the firing rate to return to a condition indistinguishable from that prior to light exposure. (2) There was never any observable correlation between variations in light threshold and variations in firing rate after the transient period. We made no measurements of threshold during the transient period.

Summing up, the time-course of threshold changes tested by means of short pulses of light and the time-course of the spontaneous firing rate of the same ganglion cell are completely different. From this observation, some conclusions can be drawn. First, any process of dark adaptation, either limited to the receptors<sup>6</sup> or involving the neural elements of the retina<sup>7,8</sup>, does not influence the ganglion cell dark discharge, at least after the transient period. Secondly, the dark discharge is likely to represent the spontaneous activity of de-afferented retinal neurones. Thirdly, since there is good evidence that the visual threshold during the process of

dark adaptation is determined neurally rather than at the receptor level<sup>7,8</sup>, it is likely that the dark deafferentation occurs between the bipolar and the ganglion cell.

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### Intracellular Action Potentials associated with the Beating of the Cilia in Ctenophore Comb Plate Cells

IN ctenophores the tall columnar cells which bear the large cilia are about  $100\mu \times 8-12\mu$ , arranged in groups along the combs<sup>1</sup>. The large size of the cells makes it possible to record intracellular potentials which occur at each beat of the cilia. Although there is a good deal of previous work on the relations between membrane potential and ciliary activity in Protozoa<sup>2</sup>, no accounts of intracellular potentials from metazoan ciliated cells have come to hand.

Pieces of comb plate are cut from specimens of the Venus's girdle, *Cestus veneris*, about 50 cm long, and firmly held down in a wax dish by means of fine cactus spines. Usually the cilia then beat slowly at regular intervals and can also be aroused to give a single beat by a very gentle tap to the bench. Under direct observation a KCl-filled glass microelectrode (which must be 30-100 M ohms to be successful) is lowered into the cushion of ciliated cells just at the region of the ciliary basal bodies. Negative-going extracellular potentials, in the form of small peaks of a few millivolts and about 0.1 sec duration, are recorded before the electrode enters a cell. They vary greatly in height as the electrode presses to different extents against the cell. Resting potentials up to 40 mV are recorded as the electrode enters; at the same time the action potentials reverse in sign and now have a long falling phase (Fig. 1). The maximum height of action potentials was 27 mV at 15° C. The action potential is similar whether the beat is spontaneous or set off by a minute vibration. At this first attempt technical difficulties prevented successful recording of the mechanical movement and it is not possible to say whether the electri-

cal change starts before or after the beat of the cilia. The beat is, however, terminated in 0.2-0.4 sec and the potential change lasts much longer. Action potentials are graded, the smaller ones corresponding with weaker beats and larger ones with stronger beats. When two beats occur in quick succession the action potential of the second stands on the first as in Fig. 1b, although the two mechanical beats are quite separate. All cells have similar responses. No pacemaker or generator potentials have been seen. Depolarization of the cell by damage with the electrode causes the cilia of that cell, and of neighbouring cells, to beat rapidly. This can lead to a continual quivering of a small group of cilia. Although it is impossible to identify individual cells from which recordings are made in these circumstances, the quivering of cilia is found when the resting potential declines away to nothing. These small movements are not propagated.

This is the tissue for which the term "neuroid transmission" was first coined<sup>3</sup>, meaning propagation from cell to cell, but the results reported here fail to elucidate the mechanism by which the groups of ciliated cells are co-ordinated among themselves or between groups. It seems reasonable to conclude that the depolarization of the cell sets off the beat of the cilia of that cell. The ciliated cells lie close against each other, and their closely apposed membranes in some places closely resemble electrical synapses without vesicles<sup>4</sup>, as do some muscle fibre contacts in ctenophores. The beat of the ciliated cells is inhibited by a nerve net which spreads over the whole ectodermal surface and the ciliated cells have synapses on them only at their basal ends, far from the cilia<sup>4</sup>, showing that the whole cell must be electrically active for them to be effective. Post-synaptic potentials are presumably so attenuated that they are not observable in records from the ciliated ends of the cells.

This work was carried out at the Stazione Zoologica, Naples, and I thank the director, Dr. P. Dohrn, for the facilities which made it possible. Among his staff, I particularly thank Dr. R. A. Chapman, whose apparatus was used for the intracellular recordings.

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### Metabolic Effect of Epinephrine on the $Q_{O_2}$ of the Arrested Isolated Perfused Rat Heart

SYMPATHETIC amines increase the oxygen consumption of the myocardium and it has usually been assumed that this is related primarily, if not exclusively, to the increase effected in rate and contractility. The present investigation demonstrates an increase in myocardial oxygen consumption due to epinephrine during potassium-induced cardiac arrest, that is, in the absence of any chronotropic or inotropic effect. This metabolic increase in oxygen consumption is accompanied by an increase in glycerol release into the perfusion medium, suggesting that the rate of utilization of endogenous lipids rises.

Fed male albino rats were decapitated, and their hearts removed and perfused following the method of Morgan *et al.*<sup>1</sup>. 15-30 c.c. of modified Krebs' bicarbonate buffer<sup>2</sup> was used, equilibrated with 95 per cent  $O_2$  and 5 per cent  $CO_2$ .  $Q_{O_2}$  was calculated from arterio-venous  $pO_2$  differences, measured with a Clark  $O_2$  macroelectrode, and from the  $O_2$  solubility constant, flow rates being measured directly. Perfusions were carried out at 37.5° C.

In the first series of experiments, using beating hearts (Series A, Table 1),  $Q_{O_2}$  was measured at 20 min in 11

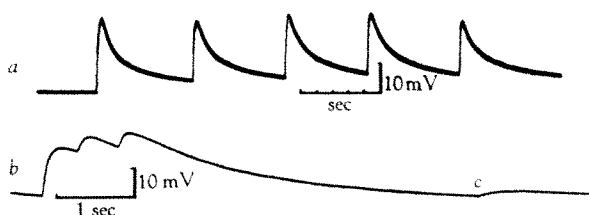


Fig. 1. Intracellular records from ciliated comb plate cells. a, At each beat there is a positive-going action potential with a rapid rising phase and a slow falling phase; b, at 5 times the recording speed, three beats, artificially initiated by small vibrations, follow each other rapidly, forming a staircase; c, the graded nature of the action potentials is shown by a small spontaneous potential, which was accompanied by a weak beat of the cilia.



Table 1. EFFECT OF EPINEPHRINE IN BEATING AND ARRESTED PERFUSED RAT HEARTS

Conditions	$Q_0$ (ml. $O_2$ /min/g dry wt)	No.	Glycerol release ( $\mu$ g/g dry wt/h)	No.
Beating hearts (Series A)				
Control	$0.58 \pm 0.04^*$	10	$122 \pm 35^*$	6
Epinephrine	$0.92 \pm 0.03^*$ ( $P < 0.005$ )	11	$290 \pm 35^*$ ( $P < 0.01$ )	6
Arrested hearts (Series B)				
Control period	$0.28 \pm 0.02^*$	8		
After addition of epinephrine	$0.41 \pm 0.02^*$ ( $P < 0.005$ )†			

\* Standard error of the mean (S.E.M.).

† Calculated from S.E.M. of differences between paired values from single hearts (mean difference  $0.14 \pm 0.02$ ).

control hearts and in 12 hearts perfused from zero time with medium containing epinephrine,  $0.1 \mu\text{g/ml}$ . Some of these perfusates were then analysed to determine free fatty acids (FFA) and glycerol release. In a second set of experiments (Series B, Table 1), the hearts were arrested by using a perfusate containing  $31.3 \text{ mM K}^+$  (reducing the concentration of  $\text{Na}^+$  to maintain isotonicity).  $Q_0$  was measured at 15 min, by which time a stable baseline control  $Q_0$  had been reached. Epinephrine was then added to the perfusate ( $0.1 \mu\text{g/ml}$ ) and  $Q_0$  was measured again in the same preparation after 15 min. FFA were measured by Dole's titration method<sup>3</sup>. Glycerol was determined enzymatically<sup>4</sup>.

As shown in Table 1, there was a significant epinephrine stimulation of  $Q_0$ , of 58 per cent over control values in the beating heart (Series A). This was accompanied by a significant increase in glycerol release. There was no demonstrable release of FFA.

The hearts arrested with potassium (Series B) showed a lower control  $Q_0$ , presumably due to the lack of contractile activity. It should be noted that whereas  $10\text{--}12 \text{ mM K}^+$  caused arrest in the absence of epinephrine, a potassium concentration of  $31.3 \text{ mM}$  was necessary to maintain the hearts in arrest in the presence of epinephrine. The same concentration of potassium was used in the control period. Epinephrine produced a highly significant (46 per cent) stimulation of  $Q_0$ .

Though epinephrine caused a comparable percentage elevation of  $Q_0$ , of approximately 50 per cent in both the beating and the arrested hearts, the absolute value of the increment in arrested hearts ( $0.13 \text{ ml/min}$ ) was much smaller than that in beating hearts ( $0.34 \text{ ml/min}$ ). If it is assumed that the metabolic effect is independent of chronotropic and inotropic effects, the data suggest that about one-third of the increment in beating hearts could be accounted for by such a metabolic effect, at least partially dissociable from effects on work function. Recent investigations using the isolated dog heart have also indicated such a dissociation between effects of epinephrine on contractility and on oxygen consumption under somewhat different experimental conditions<sup>5,6,11</sup>.

The stimulation of glycerol release strongly suggests an increase in the rate of breakdown of glycerides or phospholipids under the influence of epinephrine. Epinephrine activation of adipose tissue lipase has been well documented<sup>7</sup>, and Randle has reported increases in FFA production in muscle on incubation with epinephrine<sup>8</sup>. Alousi and Mallov have reported increases in lipoprotein lipase activity in the hearts of rats treated chronically with epinephrine, but they did not observe any changes on acute administration of epinephrine<sup>9</sup>.

Previous investigations in this laboratory<sup>10</sup> have shown that perfusion of the rat heart with a high concentration of FFA induces an elevation of  $Q_0$ . The mechanism by which this is brought about is not as yet clear. However, these results and the present results, taken together, suggest a possible explanation for the metabolic increment in  $Q_0$  caused by epinephrine. It may be that increased breakdown of endogenous myocardial lipid, releasing FFA intracellularly, may be the initiating cause for the increase in  $Q_0$  caused by epinephrine, independently of chronotropic and inotropic effects. The latter are, of course, the

major factors leading to elevated myocardial oxygen consumption in response to epinephrine. It may be that epinephrine stimulation of lipolysis may be an important concurrent effect, providing available substrate to support the increased energy requirements of a heart beating more rapidly and more vigorously.

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### Ergothioneine and Central Neurones

Crossland, Woodruff and Mitchell<sup>1</sup> have recently identified the 'cerebellar excitatory factor'<sup>2,3</sup> as ergothioneine. This factor had been found in extracts of the cerebellum from several mammalian species<sup>3</sup> and it was said to have a potent excitatory action on the electrical activity of the cerebellum and the cerebral cortex when injected into the carotid blood stream<sup>2,3</sup>. The authors therefore concluded that this factor may play a significant part in non-cholinergic synaptic transmission, in the cerebellum and other parts of the central nervous system.

We have tested the action of ergothioneine on cerebellar and cerebral cortical neurones by direct application from micropipettes. The method was similar to that used in a previous study of cerebral neurones<sup>4</sup>. The multi-barrelled micropipettes contained in one barrel a  $0.3 \text{ M}$  solution of ergothioneine hydrochloride (Koch-Light Laboratories), with the pH adjusted to a value between 4.8 and 6.5. The micropipettes were filled in a few minutes with fresh solutions, or with solutions kept frozen for several days, by centrifugation. Like the cerebellar factor<sup>3</sup>, ergothioneine is stable in acid solutions at room temperature, particularly in the absence of oxidizing agents<sup>5</sup>. The micropipettes were generally used on the day they were filled.

Four cats anaesthetized with 'Dial' compound (Ciba, Ltd.) were investigated. In each case, the micropipettes were inserted into the cerebellar cortex in several areas in and near the vermis. The action of ergothioneine was tested on a total of 70 cells firing spontaneously or as a result of excitation by small amounts of L-glutamate applied from another barrel of the pipette<sup>4</sup>. Ergothioneine was released by relatively large outward current ( $100\text{--}200 \text{ nA}$ ) for periods of at least 1 min. In most cases it did not affect significantly the excitability of the cells (Fig. 1). Approximately 1 cell in 10 showed some signs of excitation, but the effects were mostly weak and slow, requiring large doses of ergothioneine and long applications. There was no evidence that acetylcholine-sensitive cells might be more readily affected by ergothioneine, nor did the latter tend to inhibit strongly the neuronal discharge.

A number of cerebral cortical cells were also tested in two of the cats, 10 in the sensorimotor cortex, and 4 in the pyriform cortex, with even more negative results (Fig. 2).

We conclude from these observations that ergothioneine is unlikely to play a very important part as a transmitter in a widespread system of non-cholinergic synapses in the cerebellum and the cerebral cortex. This does not exclude

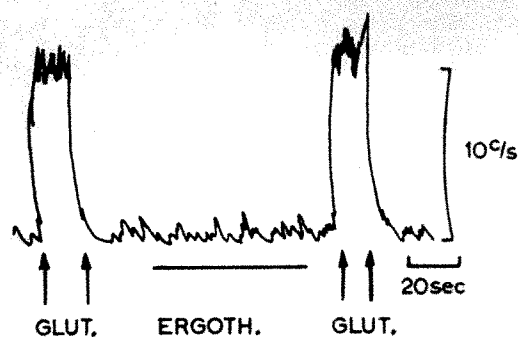


Fig. 1. Frequency of discharge of neurone in vermis of a cat's cerebellum. The spontaneous firing was enormously accelerated by L-glutamate, released between each pair of arrows (22 n.amp), but a large application of ergothioneine (100 n.amp), indicated by the horizontal line, had no clear action. The substances were released from different barrels of the micropipette by iontophoresis

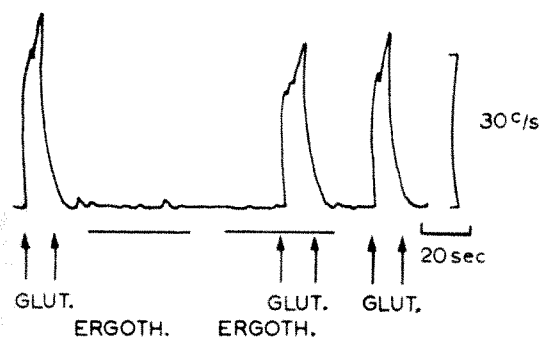


Fig. 2. Frequency of discharge of neurone in posterior sigmoid gyrus of a cat's cerebral cortex. Between each pair of arrows, L-glutamate was released from one barrel of micropipette (40 n.amp) and during periods shown by horizontal line ergothioneine was released from another barrel (265 n.amp at first signal, 65 n.amp at second); this neither excited nor depressed the cell

the possibility that it affects neuronal function by a slower and more diffuse kind of mechanism.

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### Comparison of Methods for Determination of the Sodium Content of Sweat

MEASUREMENTS of the intake and urinary output of salt under conditions of heavy sweating as obtained by Conn *et al.*<sup>1</sup>, Malhotra *et al.*<sup>2</sup> and our unit<sup>3</sup> permitted the conclusion that sodium loss in the sweat could not be as high as reported by several other authors<sup>4-6</sup>. This implies that methods employed for salt determination in the sweat give results which are considerably higher than the true salt content. The aim of the experiments reported here was to compare the reliability of these various methods.

Forty-six young men, aged 18-24, partly or fully acclimatized, and 2 farmers, aged 33 and 34, respectively, living on a 'naturalist' diet (almost devoid of sodium) were examined. Sweat was collected by three methods simultaneously: (1) Whole-body sweat (B.S.), according to the method of

von Heyningen *et al.*<sup>7</sup> with the exception that the subject was not dried throughout the experiment; (2) Arm sweat (A.S.), by enclosing the arm from the elbow down in a polyethylene bag, admixture of palm sweat being avoided by placing the hand in a rubber glove; (3) Filter paper pad sweat (P.S.) by the method reported by Dole *et al.*<sup>8</sup>.

Total sweat volume was determined by weighing the subject before and after the experiment, adding water intake and subtracting urine output. The experiments were performed in a dry fibreglass tub (160 × 160 × 60 cm). Each experiment, except four which were interrupted by the subject's inability to continue, lasted 90 min. The subjects were exercised (step test) part of the time. Climatic conditions were constant during each experiment but varied from experiment to experiment. (Dry bulb, 37°-48° C; wet bulb, 25.5°-34.5° C, relative humidity 27-48 per cent.) Double aliquots of sweat from each sample were examined for sodium by flame photometry. Results are given in m.equiv./l.

Table 1. COMPARISON OF SODIUM CONCENTRATION OF B.S. AND A.S. BY INCREASING SODIUM CONCENTRATION (IN m.equiv./l.).

m.equiv./l.	No. of subjects*	B.S.	A.S.	Difference %
5-15	10	11	19.9	79†
15.1-25	13	21.2	28.8	36†
25.1-45	16	35.1	41.9	19†

\* 7 subjects were dispersed in higher concentration groups, which were too small to be included. †  $P < 0.01$

Table 2. COMPARISON OF SODIUM CONCENTRATION OF B.S. AND A.S. ARRANGED BY RELATIVE HUMIDITY (IN m.equiv./l.).

Relative humidity	No. of subjects	B.S.	A.S.	Difference %
below 33%	8	29*	52	79†
above 33%	38	29†	36.3	25†

\* S.D. ± 13.8. † S.D. ± 15.8. ‡  $P < 0.01$

Table 3. COMPARISON OF SODIUM CONCENTRATION OF B.S., A.S. AND P.S. IN 2 'NATURALIST' FARMERS (IN m.equiv./l.).

Subject	B.S.	A.S.	P.S.	Difference B.S.-A.S. %	Difference B.S.-P.S. %
A	3.3	8.0	15.9	142	382
B	5.2	17.0	21.0	227	304

The mean sodium concentration ( $\pm$  standard deviation) in the sweat of the first 46 subjects was: B.S. 29 ( $\pm 15.6$ ), A.S. 38.5 ( $\pm 20.4$ ) and P.S. 38.4 ( $\pm 23.5$ ). Only 40 subjects were tested by the last method. Mean sodium concentration of B.S. was 33 per cent lower than by the two other methods ( $P < 0.01$ ). Differences between B.S. and A.S. were found to be highest when sodium concentration was low (Table 1) and when relative humidity was low (Table 2). Greatest differences were found in the two 'naturalists' (Table 3), who had an extremely low salt concentration (3.3 and 5.2 m.equiv./l. B.S., respectively).

The B.S. method must be considered, on theoretical and practical grounds, to be the correct one. Results by other methods are on the high side and in certain circumstances the error increased considerably. This seems to exclude the possibility of using the A.S. or P.S. methods with a corrective factor as Weiner suggested<sup>9</sup>.

The differences between the methods probably depend on multiple factors, including physiological and physical ones. Buettner has shown that water is reabsorbed by the skin when its vapour pressure in the air exceeds 21 mm Hg and when salt concentration is below 5.5 per cent<sup>10</sup>. This may account for the fact that the closer the external conditions approach the micro-climate within the bag or the pad, the smaller the differences between the methods. This would also explain Ladell's observation that no difference exists between A.S. and B.S. collected within a water-bath<sup>11</sup>.

In the experiments reported here, sodium concentration in whole-body sweat was considerably smaller than concomitantly-obtained samples of arm bag sweat and filter paper pad sweat. The difference was not constant but increased considerably with decrease of sodium in the sweat and with decrease of the relative humidity of

the air. The bag and pad methods are not suitable for determination of the sodium content of sweat.

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### Oestrogen and Gonadotrophin in the Blood of Dogs during Bone Fracture Healing

PREVIOUS reports have dealt with variations in the activity of thyroid glands<sup>1</sup> and changes in the levels of androgens, follicle-stimulating and luteinizing hormones<sup>2</sup> as a result of bone fracture. The present investigation is concerned with changes in oestrogens and gonadotrophic hormones (FSH and LH) in the blood of dogs during the healing of bone fractures. Twenty-five mature anoestrous non-pregnant female dogs were used. Mid-diaphyseal fractures of the left radius were made by an open technique. Animals were anaesthetized using chlorpromazine hydrochloride and thiopental sodium. The radius in the dog was firmly fixed at its proximal and distal extremities to the ulna, which was left intact and acted as a natural splint.

At weekly intervals after fracture, 50 ml. of blood was collected by jugular venipuncture from each of 5 animals. The animals were afterwards killed and the uterus and ovaries were examined to exclude pregnancy and cyclic ovarian activity. The blood was allowed to clot, and sera belonging to animals of the same group were pooled. Groups of animals were examined at one, two, three, four and six weeks after fracture.

Oestrogenic substances were separated<sup>3</sup> and assayed biologically<sup>4</sup>. The methods used for the isolation and assay of gonadotrophic hormones were as described earlier<sup>5</sup>. The results obtained, which are shown in Table 1, were analysed statistically. The standard error of the mean was computed and 't' tests were performed to detect differences between groups.

Significantly high levels of oestrogenic substances were observed by the end of the second week after fracture and were continued until the end of the sixth week. This early rise in serum oestrogen, which was not preceded or accompanied by appreciable changes in either FSH or LH, favours the hypothesis of the existence of extra-ovarian

sources for oestrogen, such as the adrenal cortex. Adrenal corticotrophic hormone is shown to induce the production of relatively small amounts of oestrogen by the adrenals<sup>6</sup>. However, the changes in serum FSH and LH detected in the subsequent stages of the healing process could be explained on the basis of the findings which showed that oestrogen increased the synthesis and release of LH. At the same time, it induced a drop in FSH content in the blood and in the pituitary gland<sup>6</sup>. This was the case during fracture healing, where increased oestrogen formation elevated serum LH without significantly affecting serum FSH.

It is generally accepted that bone resorption takes place during the early stages of fracture healing<sup>7</sup>, this coincides with the period when the rise in serum oestrogen was insignificant. The increased level of oestrogens during the second week favours an increased bone formation<sup>8</sup> and mineral deposition<sup>9</sup>. Oestrogenic substances are known to possess a general metabolic action on bone<sup>10</sup>. It is believed that oestrogen possesses a specific stimulating effect on osteoblasts which in turn form protein matrix on which calcium and phosphorus are deposited as hydroxyapatite to form normal bone. Animal experiments have shown that ovariectomy decreased callus formation and the breaking strength of bone<sup>11</sup>. On the other hand, the extraneous administration of oestrogens hastened fracture healing<sup>12</sup>.

It could be concluded that a rise in oestrogen level takes place in the blood of dogs after the lapse of one week following bone fractures, and that this hormone is physiologically essential for the normal process of fracture repair.

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### PHARMACOLOGY

#### N<sup>4</sup>-substituted N<sup>1</sup>-(3-Dimethylaminopropyl)-piperazines: a New Series of Compounds active against *Trypanosoma cruzi* Infections in Mice

RECENT reviews<sup>1-3</sup> on the treatment of *Trypanosoma cruzi* infections (Chagas's disease, South American trypanosomiasis) in man emphasize the lack of a satisfactory mode of therapy.

We wish to report the synthesis and biological evaluation of a series of compounds of the general structure:



Table 1. OESTROGENS, LUTEINIZING (LH) AND FOLLICLE STIMULATING (FSH) HORMONES IN THE SERUM OF BITCHES DURING BONE FRACTURE HEALING

Weeks after fracture	Oestrogen (mouse uterus wt., mg/100 g body-wt.)	LH (No. of corpora haemorrhagica per mouse)	FSH (wt. of mouse ovary, mg/100 g body-wt.)
Normal	63.50 ± 2.25	0.31 ± 0.170	37.14 ± 2.36
One	73.37 ± 5.66	0.20 ± 0.140	32.95 ± 3.28
Two	94.29† ± 4.16	0.10 ± 0.002	31.84 ± 2.57
Three	79.90† ± 2.36	1.27* ± 0.250	38.52 ± 3.32
Four	81.98† ± 2.15	1.36* ± 0.430	42.35 ± 3.13
Six	79.34* ± 3.50	0.50 ± 0.260	39.06 ± 2.80

± Standard error.

\* Significant at 95 per cent confidence level.

† Significant at 99 per cent confidence level.



which has shown a high degree of chemotherapeutic activity against the *B* strain of *T. cruzi* infection in mice<sup>4</sup>. Table 1 lists some of the more active members of this series and exemplifies the broad variation in *R* that is possible with minimal effect on the chemotherapeutic activity.

The compounds were usually obtained as the water-soluble hydrochloric, maleic, or fumaric acid salts. Water-insoluble 4,4'-methylenetris(3-hydroxy-2-naphthoic acid) salts of selected compounds were prepared for special investigation.

In contrast with the relative non-specificity of *R* in structure I, modifications of the *N*<sup>1</sup>-(3-dimethylamino-propyl)piperazine moiety have resulted in a considerable loss of activity against experimental *T. cruzi* infections in mice. Thus, replacement of the dimethylamino group by a diethylamino, di-isopropylamino, piperidino, or benzylmethylamino moiety, or of the 1,3-trimethylene chain by an ethylene, 1,2-propylene, or 1,4-tetramethylene chain reduced the activity to less than one-fifth of that of compound *A* (Table 1).

Screening of the compounds of structure I against *T. cruzi* was carried out as follows<sup>4</sup>: 6–8-week-old Manor strain female albino mice were inoculated subcutaneously with approximately 50,000 parasites (from infected donor mice), the median survival time of untreated mice being 13–16 days. Compounds to be tested were administered by drug-diet on days 6–12 post-inoculation. The principal criterion of chemotherapeutic activity was the survival time of treated mice relative to untreated mice, but examination of the peripheral blood and of various internal organs for parasites was also done in some experiments. Activity was unaffected when the compounds were administered by gavage, or by subcutaneous or intraperi-

toneal injection. Table 2 shows the survival data for infected mice treated with some of the compounds listed in Table 1.

The parent compound (*I*, *R* = H) was completely inactive on dosage-levels up to 512 mg/kg/day.

Compound *E* was directly compared with two compounds reported<sup>5–7</sup> to possess some activity against *T. cruzi* in mice, namely, 6-methoxy-8-(4-amino-1-methyl-butylamino) quinoline diphosphate (primaquine) and racemic 5-morpholinomethyl-3-(5-nitrofurfurylidene-amino)-2-oxazolidinone (furaltadone). The results are summarized in Table 3.

Preliminary investigations with compound *E* in the treatment of the *B* strain of *T. cruzi* infection in dogs have given favourable results. Long-term toxicity and metabolism investigations in several species of experimental animals are now being carried out.

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Table 1	
Compound	<i>R</i>
<i>A</i>	7-chloro-4-quinolyl
<i>B</i>	4-pyridyl
<i>C</i>	5-nitro-2-thiazolyl
<i>D</i>	2-benzothiazolyl
<i>E</i> *	4-acetamidophenyl
<i>F</i>	4-n-butoxyphenyl
<i>G</i>	3-chlorophenyl
<i>H</i>	4-benzenesulphonamidophenyl
<i>I</i>	<i>o</i> -tolyl
<i>J</i>	4-nitrophenyl
<i>K</i>	2,4-dichlorobenzoyl
<i>L</i>	2-phenyl-2H-1,2,3-triazole-4-carbonyl

\* The generic name is piperamide.

Table 2			
Compound	Approx. dosage drug diet mg/kg/day free base	Median survival time (days)	% Survivors of 10 mice 40 days* post- inoculation
<i>A</i>	4	> 40	60
	19	> 40	70
	79	> 40	80
<i>B</i>	4	26.5	30
	18	> 40	90
	84	> 40	90
<i>D</i>	3	18.5	0
	18	23	20
	63	33	40
<i>E</i>	4	26.5	20
	18	> 40	60
	78	> 40	80
<i>L</i>	4	34	40
	17	> 40	60
	74	> 40	70
Controls	—	14.5	0

\* Maximum observation period post-inoculation. In some experiments, survival times in excess of one year were observed.

Table 3				
Compound	No. mice	Unit dose* mg/kg free base	Median survival time (days)	% Survivors 90 days† post-inoculation
<i>E</i>	15	2.5	> 90	80
	10	10	> 90	90
Primaquine	15	2.5	15.5	13
	10	10	19	40
Furaltadone	15	2.5	16	0
	10	10	20.5	20
Controls	—	—	14.5–16.5	0

\* Treatment by gavage, three times daily on days 6 and 7 and twice daily on day 8 to day 12 inclusive, post-inoculation.

† Maximum observation period.

## Mechanism of Norepinephrine Binding

A NUMBER of sympathomimetic amines chemically related to norepinephrine (Fig. 1), which have either a catechol or  $\beta$ -hydroxyl group, can be stored in the vesicles of sympathetic nerves<sup>1</sup>. Octopamine, which lacks only the *m*-hydroxyl group of norepinephrine, is released by nerve stimulation<sup>2</sup>, and is depleted by drugs which deplete norepinephrine<sup>3</sup>. Although bound to particles with similar sedimentation characteristics as those which store norepinephrine<sup>4,5</sup>, octopamine appears to be stored mainly in a compartment which has a rapid turnover.

The ability of drugs to release several sympathomimetic amines was examined in an attempt to gain insight into the role of hydroxyl groups of norepinephrine in tissue binding of this catecholamine.

Osborne-Mendel rats weighing 180–200 g received 20  $\mu$ c. of *dl*-norepinephrine-7-<sup>3</sup>H (5,600 mc./mM), 10  $\mu$ c. tyramine-<sup>3</sup>H (1,500 mc./mM), 10  $\mu$ c. of *m*-tyramine-<sup>3</sup>H (1,500 mc./mM) or 20  $\mu$ c. of dopamine-<sup>3</sup>H (3,000 mc./mM) intravenously. Animals which received dopamine were given disulphiram (400 mg/kg), 1 h prior to the labelled amine, to prevent conversion of the administered compound to norepinephrine<sup>6</sup>. Tyramine (10 mg/kg), guanethidine (20 mg/kg) or reserpine (2.5 mg/kg) was administered intramuscularly after the labelled amine, at the times indicated in Table 1. When the drugs were administered, only the  $\beta$ -hydroxylated derivatives of tyramine-<sup>3</sup>H (octopamine and *m*-octopamine) were in the hearts<sup>1</sup>. The rats were killed 2 h after administration of the labelled amines, and the hearts removed and homogenized in 10 ml. of cold 0.4 N perchloric acid. Amines present in the protein-free supernatant were analysed as previously described<sup>7,8</sup>.

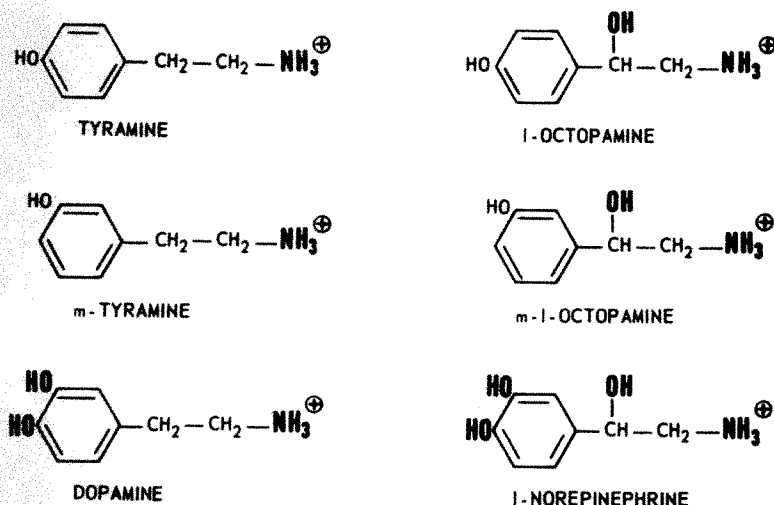
As expected, tyramine, guanethidine and reserpine were not equally effective in depleting norepinephrine from the rat heart. In the interval of drug action in these experiments, tyramine depleted by 50 per cent, guan-

Table 1. EFFECTS OF DRUGS ON TISSUE CONTENT OF NOREPINEPHRINE-<sup>3</sup>H, DOPAMINE-<sup>3</sup>H, OCTOPAMINE-<sup>3</sup>H AND *m*-OCTOPAMINE-<sup>3</sup>H IN THE RAT HEART\*

Drug	Norepinephrine		Dopamine		Octopamine		<i>m</i> -Octopamine	
	mμc./heart	% Control	mμc./heart	% Control	mμc.	% Control	mμc.	% Control
None	660 ± 51	100	5.52 ± 0.25	100	14.55 ± 0.90	100	5.16 ± 0.60	100
Tyramine (10 mg/kg, 1 h)†	332 ± 20	50	0.74 ± 0.10	13.4	0.60 ± 0.15	4.1	0.45 ± 0.07	8.7
Guanethidine (20 mg/kg, 1 h)†	424 ± 16	64	0.80 ± 0.09	14.5	0.21 ± 0.015	1.45	0.16 ± 0.015	3.1
Reserpine (2.5 mg/kg, 30 min)†	82 ± 7.7	12.4	0.16 ± 0.014	2.9	0.12 ± 0.015	0.86	0.10 ± 0.015	1.93

\* Results are the means ± S.E.M. for groups of six animals.

† Dose and time of administration after the labelled amine.

Fig. 1. Phenylethylamine derivatives related to norepinephrine. The structures believed to play a part in binding of the amines in sympathetic nerves are shown in bold type. Tyramine and *m*-tyramine are not retained in the tissues<sup>4,5</sup>

ethidine by 36 per cent, and reserpine by 87 per cent catecholamine (Table 1). After treatment with these drugs more dopamine, octopamine or *m*-octopamine was released. Octopamine-<sup>3</sup>H and *m*-octopamine-<sup>3</sup>H were depleted most effectively; dopamine disappearance, however, was not so great as that of the phenolic amines (Table 1). Reserpine was the most effective depleting agent for all the amines. In these experiments guanethidine appears to deplete phenolic amines more effectively than tyramine, but the two drugs appear to be equally effective in lowering tissue levels of dopamine-<sup>3</sup>H (Table 1).

These results suggest that the catechol structure and the β-hydroxyl group of norepinephrine may be important in its binding. Dopamine is less easily released than octopamine or *m*-octopamine, suggesting that the catechol group is more important than the β-hydroxyl group for resistance to depletion. Tyramine and guanethidine appear to be equally effective (at this interval of drug action) in depleting dopamine, but guanethidine is more effective in lowering tissue levels of the phenolic β-hydroxylated amines.

While it is generally agreed that there are several mathematical compartments for norepinephrine in the nerve endings<sup>9</sup> the nature of these compartments has not been defined. Crout<sup>10</sup> has suggested that the apparent compartments may be a consequence of differences in anatomical location of similar vesicles within the sympathetic nerves. The differences in turn-over rate and availability to release by tyramine are explained on the basis of differences in proximity of the vesicles to the neuronal membrane. There are alternative hypotheses, however, not based on anatomical localization, which may explain the existence of several storage sites for norepinephrine. The differential action of drugs on the release of amines related to norepinephrine suggests that chemical structure rather than anatomical location may be the determining factor in the ease of release of these amines. Different binding sites may be located in different

types of vesicles or there may be differences in the affinity of norepinephrine to several binding sites within a single vesicle. To our knowledge, no evidence has been presented which indicates that there is more than one type of vesicle for norepinephrine storage in the sympathetic nerves.

Stjärne<sup>11</sup>, however, has presented evidence that there is more than one type of norepinephrine storage in vesicles isolated from bovine splenic nerve. His results, and those presented here, suggest that the pools of norepinephrine may be related to different modes of binding of the catecholamine. Phenylethylamine derivatives may be bound through a catechol or β-hydroxyl group (Fig. 1) (ref. 1). Norepinephrine, which has both, may therefore be bound in a number of ways. This may account for the apparent pools of this catecholamine in the sympathetic nerve endings.

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## PATHOLOGY

### Induction of Rat Mammary Cancers by Cancer Chemotherapeutic Agents

HUGGINS *et al.*<sup>1</sup> demonstrated that multiple mammary adenocarcinomas could be induced rapidly and invariably in female rats of the Sprague-Dawley strain by gastric instillation of 3-methylcholanthrene. Later, 7,12-dimethylbenz(a)anthracene was found to induce the same cancer<sup>2</sup>. Shellabarger *et al.*<sup>3</sup> induced similar mammary cancers in rats by sub-lethal whole-body γ-irradiation. Recently, Huggins and Fukunishi<sup>4</sup> noted that mammary carcinomas elicited either by X-irradiation or by 7,12-di-

methylbenz(a)anthracene had similar enzyme constitutions and hormone-dependency. Huggins and Yang<sup>5</sup> have postulated that both radiation and polynuclear aromatic hydrocarbons exert their oncogenic effect by selective changes in the nucleic acids of mammary cells. In an experiment described here, it was noted that mammary cancers can arise also in rats receiving anti-cancer drugs, including biological alkylating agents. Although the biological alkylating agents have been shown to induce cancers in rats, these have usually been sarcomas arising at the site of a subcutaneous injection<sup>6</sup>.

As part of an experiment designed to assess the toxicity of simultaneous carcinogen (3-methylcholanthrene) and drug administration, a group of 32 female Sprague-Dawley rats, aged 56 days, and another group of 32 rats, aged 97 days, received each of the following compounds: *N,N',N''*-triethylenethiophosphoramidate ('ThiotepaRx') 0.82 mg/kg, three times a week; methotrexate 0.29 mg/kg, three times a week; vinblastine sulphate 0.08 mg/kg, once a week; they also received cyclophosphamide ('CytoxanRx') at 0, 3.8, 7.6 or 11.5 mg/kg, and 5-fluorouracil at 0, 3.8, 7.6 or 11.5 mg/kg, three times a week. These animals did not receive polycyclic hydrocarbon carcinogen. The vinblastine sulphate was given once on each of five consecutive weeks; the other drugs were given the first, second, fourth and fifth weeks. No drug was given after the fifth week. The animals were kept in groups of 8 in stainless steel cages (separate from the group of animals which received 3-methylcholanthrene together with the anti-cancer drugs) in a well-ventilated room at 25°C. They were fed a standard chow diet with fresh lettuce three times a week and raw liver once a week. Drugs were given intraperitoneally and the amount given was based on the mean weight of the animals in each group at the start of the experiment. The animals were weighed and palpated for tumours every 10–14 days for 84 days. No tumours were detected during this interval, but appeared later in some of the animals. The animals were killed at 147 days. In the younger age group (56 days), 7 (24 per cent) of the 29 surviving rats had a total of 9 tumours; and 2 (7 per cent) of the 28 survivors in the older group (97 days) had a total of 2 tumours at the end of 147 days (Table 1). Three tumours were about 1 cm in diameter; the others were smaller. Microscopically all the tumours were mammary adenocarcinomas indistinguishable from those induced by 3-methylcholanthrene.

Table 1. INCIDENCE OF CANCERS IN TREATMENT GROUPS

Number of cancers Age-group		Thlo.	Metho.	Treatment Vinbl.	Cyclo.	5-FU
I 56 days	II 97 days					
2	0	+	+	+	0	+
0	2	+	+	+	+	0
7	0	+	+	+	+	+

The incidence of spontaneous mammary carcinomas in rats is quite low; only 1–2 per cent of rats of this strain develop such cancers over the course of a year or longer<sup>4,7,8</sup>. Thus the observed incidence of mammary cancer in this experimental group is greater than the expected spontaneous rate; moreover, the younger animals had a higher incidence than the older rats. The latter finding is especially interesting since the 50–70 day period is the time of maximum susceptibility of rats of this strain to mammary cancer induced by chemical carcinogens<sup>2</sup>. For these reasons it seems most likely that the anti-cancer drugs caused these neoplasms. It cannot be decided from this experiment which of the drugs were responsible, since all the animals received methotrexate, vinblastine sulphate and triethylenethiophosphoramidate. Two of the animals with cancers also received cyclophosphamide but not 5-fluorouracil, whereas one received 5-fluorouracil but not cyclophosphamide. The other animals received combinations of various levels of both cyclophosphamide and

5-fluorouracil in addition to the three other drugs noted here. The polyfunctional alkylating agents, cyclophosphamide and triethylenethiophosphoramidate, may be involved, since the radiomimetic effect of the nitrogen mustard alkylating agents is well known. Recently, 5-fluorouracil has been shown to have an anti-androgenic effect<sup>9</sup>, but whether this played a part in the observed carcinogenesis also remains to be established. An experiment designed to distinguish the carcinogenic effects of the drugs given singly and in combination in animals of different sex and age has been initiated.

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### Intracellular Location of Tissue Thromboplastin and Possible Relation to Fibrin Deposits in Human Neoplasms

IN 1958 O'Meara and Jackson<sup>1</sup>, using histochemical techniques, described deposits of fibrin in human malignant neoplasms. Fibrin has been demonstrated also in a number of experimental animal neoplasms by immunological and radioisotopic methods<sup>2,3</sup> and in a variety of human malignant neoplasms by the fluorescent antibody technique<sup>4</sup>. While fibrin may occur in haemorrhagic areas, in blood vessels and in necrotic areas in the vicinity of tumours, it also occurs in the extracellular spaces of tumours.

It is thought that the proliferating tumour cells release coagulative or thromboplastic substances which react with the proteins of the extra-cellular fluid to alter fibrinogen to fibrin. Thermolabile thromboplastins have been demonstrated in crude extracts of human malignant tumours, normal colon and normal chorion<sup>5,6</sup>. Boggust *et al.*<sup>7</sup> have isolated and investigated the properties of coagulative material from chorion and cancer tissue extracts by chromatography on DEAE cellulose and by starch-gel electrophoresis.

In order to learn more about the mechanism of fibrin deposition in tumours an attempt was made to determine the intracellular location, if any, of thromboplastic material in chorion cells with a view to a subsequent investigation of tumour cells. Chorionic membrane was chosen for the initial investigations since it does not contain blood vessels<sup>8,9</sup> and cell constituents from it are therefore unlikely to be contaminated with plasma coagulative factors.

Human placentae were collected on to saline ice immediately after delivery and the chorion was removed from the amnion. The washed chorionic membrane (22.4 g, 20 ml.) was cut into small pieces and homogenized with a glass and 'Teflon' homogenizer in 80 ml. of 0.25 M sucrose buffered to pH 7.2 with McIlvaine's phosphate citrate buffer. The homogenate (100 ml.) was passed through 16-mesh nylon bolting cloth (obtainable from Wm. Dell and Son, Ltd., 48 Weston St., London, S.E.1).



Table 1. DETAILS OF DILUTIONS OF THE PELLETS WITH 0.25 M SUCROSE TOGETHER WITH NITROGEN AND PHOSPHORUS VALUES ON THE DILUTED PELLETS. Summation of the fractions to the homogenate is also shown

Fraction	Volume of pellet (ml.)	Volume of sucrose added (ml.)	Final volume (ml.)	Nitrogen (mg/ml.)	Total nitrogen in suspended pellet (mg)	Phosphorus ( $\gamma$ /ml.)	Total phosphorus in suspended pellet (mg)
Debris and nuclear	22.5	58.3	80.8	1.25	100.4	75.5	6.10
Mitochondrial	0.5	1.3	1.8	1.25	2.25	106.5	0.19
Microsomal	0.5	1.3	1.8	3.20	5.76	484.0	0.87
Soluble			230.0	0.55	126.5	46.0	10.58
			83.9		234.9		17.75
Homogenate			84.0	2.75	251.0	212.0	17.8

After removing an aliquot (7.0 ml.) the filtered homogenate, now measuring 83 ml., was submitted to differential centrifugation<sup>10</sup> to obtain debris and nuclear fraction (700g), mitochondrial fraction (5,000g) and microsomal (57,000g) fraction. Fraction purity was checked by phase-contrast microscopy. The nuclear, mitochondrial and microsomal pellets after washing were diluted with sucrose proportional to their respective spun volumes; the details of these dilutions are shown in Table 1.

Nitrogen and phosphorus determinations were carried out on the filtered homogenate, the diluted nuclear, mitochondrial, and microsomal fractions, and also on the soluble (final supernatant) fractions. All operations were carried out at 0°–4° C and metal contact and chelating agents were avoided so far as possible.

The homogenate, suspended pellets and soluble fraction were then further diluted with 0.85 per cent saline and tested for their coagulative activity in a number of coagulation systems. Sucrose can be shown to be inert in coagulation systems and its effect is one of dilution only.

In the first system pooled human plasma was diluted 1 in 6 with 0.85 per cent saline and phosphate citrate buffer (pH 6.5) as previously described<sup>5,6</sup>; this system was designed to simulate extracellular fluid protein concentrations such as might exist in neoplasms. The calcium requirement is critical and must be titrated out beforehand for each batch of plasma<sup>5,6</sup>. 0.5 ml. of the cell fraction diluted with 0.85 per cent saline was added to 0.5 ml. diluted plasma and about 0.15 ml. of 2 per cent calcium chloride, and the highest dilution of the cell fraction giving a firm clot after 30 min at 37° C was recorded as the end point. A typical result is shown in Fig. 1.

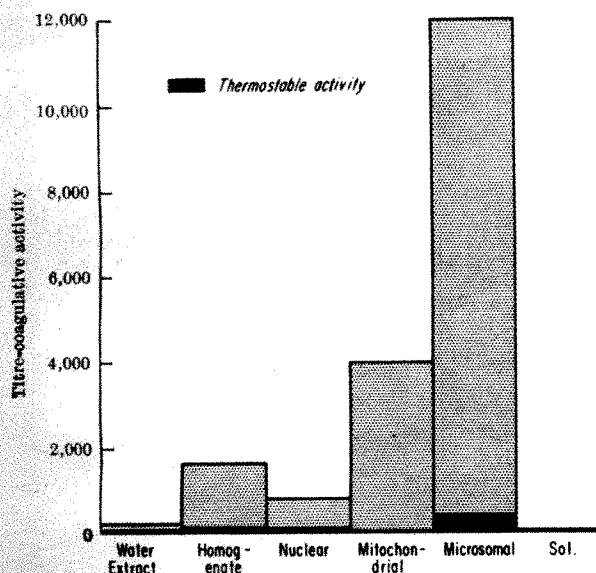


Fig. 1. The highest saline dilutions of heated and unheated chorion cell fractions which will produce clotting in 30 min at 37° C in a recalcified dilute plasma system at pH 6.5. 'Water extract', a simple water extract of intact chorion

The diluted cell fractions were also heated at 60° C for 30 min and then tested as above, and the levels of thermostable thromboplastins are shown with the thermolabile thromboplastins in Fig. 1.

Efficient non-destructive homogenization procedures are capable of releasing very potent coagulant material from cells. The microsomal fraction (Fig. 1) shows enormous activity compared with the other fractions. The activity in the mitochondrial fraction is probably largely due to contamination by microsomes. The absence of thromboplastic activity in the soluble fraction indicates that activity is associated with a particle which is completely sedimented at 57,000g. It might be argued that the absence of activity in the soluble fraction is due to dilution by the relatively large volume of the suspending medium, but by spinning down the homogenate directly at 57,000g and sampling the supernatant for coagulative activity it can be shown that this is not the case. The relatively low activity in the homogenate may be due to an inhibitor or to physical binding of small particles.

Table 2 shows the nitrogen and phosphorus concentrations at the dilutions of greatest coagulative activity for each of the cell fractions. It will be seen that the high nitrogen and phosphorus levels in the soluble fraction are not associated with coagulative activity whereas low concentrations in the particulate microsomal fraction are associated with activity.

Table 2. NITROGEN AND PHOSPHORUS CONCENTRATIONS IN 0.5 ML. OF DILUTED CELL FRACTION AT THE HIGHEST CELL FRACTION DILUTIONS SHOWING THROMBOPLASTIC ACTIVITY, AS SHOWN IN FIG. 1

	Thermolabile titre	Nitrogen $\gamma$	Phosphorus $\gamma$
Homogenate	1,600	0.86	0.06
Nuclear	800	0.78	0.04
Mitochondrial	4,000	0.16	0.01
Microsomal	12,000	0.13	0.02
Soluble	1	275.0	23.9

The homogenate and other fractions (diluted with sucrose as described in Table 1) were then further diluted with saline, and 0.1 ml. of the dilutions were added to previously frozen platelet-free plasma and M/40 calcium in the conventional 1-stage prothrombin time of Quick. The clotting times of the various dilutions are shown in Fig. 2.

Taking the 30-sec level as a moderate degree of activity in this test it will be seen from Fig. 2 that the microsomal fraction is active at a dilution of 1/800 compared with the mitochondrial fraction (1/95), nuclear fraction (1/10), homogenate (1/30), and soluble fraction (1/4). The slope of the microsomal curve is quite different from that of the other fractions, and it is evident that these small particles retain the ability at very high dilutions to shorten the clotting time of recalcified plasma. These and other results will be reported in detail elsewhere.

Table 3. CONCENTRATIONS OF NITROGEN AND PHOSPHORUS IN 0.1 ML. OF THE DILUTED CELL FRACTIONS AT THE 30-SEC LEVEL OF THROMBOPLASTIC ACTIVITY, AS SHOWN IN FIG. 2

	Dilution	Nitrogen $\gamma$	Phosphorus $\gamma$
Homogenate	35	7.86	0.61
Nuclear	10	12.50	0.75
Mitochondrial	95	1.33	0.11
Microsomal	800	0.40	0.06
Soluble	4	13.75	1.15

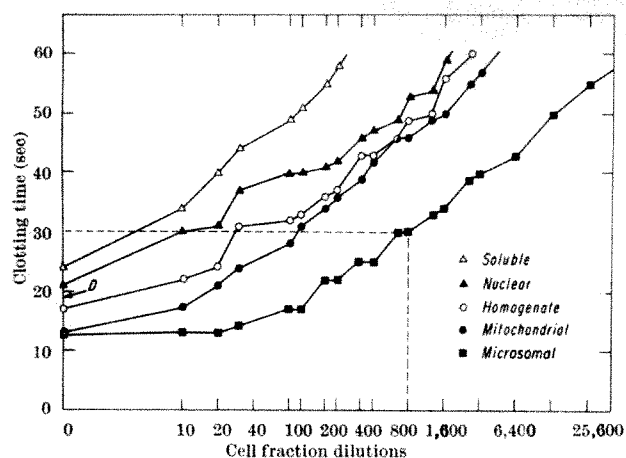


Fig. 2. Clotting time of saline-diluted chorion cell fractions (0.1 ml.) when added to 0.1 ml. of previously frozen platelet-free plasma and 0.1 ml. calcium chloride (M/40). D, Difco brain thromboplastin. Saline clotting time, 85 sec. Sucrose clotting time, 110 sec. Abscissa, logarithmic scale

In Table 3 are shown the nitrogen and phosphorus levels at the 30-sec level of activity. It will be seen that the relatively high nitrogen and phosphorus levels in the particle-free soluble fraction are not associated with coagulative activity.

There has been persistent difficulty with the chemical identification of tissue thromboplastin in cells, and theoretically this could be related to a particulate nature; differential centrifugation may be useful as a preliminary step in purification. The findings here described indicate that thermolabile thromboplastic material is located principally in a minute particle in chorion cells. Preliminary observations suggest that the coagulative activity is associated with a particle rather than with soluble material.

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## IMMUNOLOGY

### Role of Non-specific Protein in the Sensitization of Red Blood Cells by Antigen

BOTH the bis-diazotized benzidine (BDB)<sup>1</sup> and tanned cell haemagglutination<sup>2</sup> techniques are considered to be among the most sensitive methods for the detection of antibody<sup>3,4</sup>. However, whether or not both these haemagglutination techniques (BDB and tanned cells) are equally sensitive with respect to any particular anti-

Table 1. EFFECT OF PROTEIN CONCENTRATION ON THE SENSITIZATION OF RED BLOOD CELLS IN THE BDB HAEMAGGLUTINATION TECHNIQUE

BSA-anti-BSA system

Mg BSA used in sensitization	Titre*
0.063	128,000
0.125	128,000
0.25	128,000
0.50	128,000
1.00	128,000
1.00 + 0.25	128,000
1.00 + 0.5	128,000
1.00 + 1.0	128,000
1.00 + 2.0	32,000
1.00 + 4.0	16,000
1.00 + 8.0	8,000
1.00 + 16.0	†

\* The term 'titre' used here and in the following tables represents the maximum dilution of the antiserum capable of causing agglutination of sensitized red cells.

† Could not be determined due to non-specific agglutination.

gen-antibody system and therefore can be used interchangeably has not yet been elucidated. In fact, evidence obtained in this laboratory<sup>5</sup> would tend to suggest that, in certain cases, it is advantageous to use the BDB technique and in other cases the tanned cell technique, in order to achieve maximum sensitivity. In view of this finding, it became obvious that a better understanding of the mechanisms underlying these two haemagglutination reactions would be most appropriate. The results of initial experiments reported in this communication indicate that the two techniques differ in their ability to detect antibody when sensitization of the red cells with specific antigen is carried out in the presence of extraneous protein.

The two antigens used were bovine serum albumin (BSA) (Pentex, Inc., Kankakee, Illinois) and human  $\gamma$ -globulin (HGG) (Pentex, Inc.).

Antisera were obtained from adult New Zealand white rabbits immunized with these two antigens. The immunization schedule consisted of three 1-ml. injections, at intervals of ten days, of the antigen emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan) to a concentration of 50 mg/ml. The rabbits were bled from the marginal ear vein ten to twelve days after the last injection of the antigen. The clotted bloods were centrifuged, the sera decanted off and stored at  $-10^{\circ}\text{C}$  until used.

The BDB and tanned cell techniques were used essentially as described by Stavitsky and Arquilla<sup>1</sup>, Boyden<sup>2</sup> and Gordon *et al.*<sup>6</sup>. The sole modification in the BDB haemagglutination technique was the addition of non-specific protein (for the particular system involved) to the red-blood-cell-antigen mixture before the addition of BDB. When the tanned cell technique was used, the non-specific protein was added to the antigen prior to the addition of the tanned cells.

As can be seen in Table 2, the addition of HGG to the BSA-red-cell mixture prior to the addition of the BDB had the same effect as the addition of similar amounts of BSA (Table 1), that of markedly diminishing the antibody titre (anti-BSA) obtained with the cells sensitized with this BSA-HGG solution. This effect was especially noticeable in the presence of very large quantities of the non-cross-reactive protein (HGG) which resulted in the red cells being markedly sub-optimally sensitized with

Table 2. EFFECT OF PROTEIN IMPURITIES ON THE SENSITIZATION OF RED BLOOD CELLS IN THE BDB HAEMAGGLUTINATION TECHNIQUE

Total protein variable, specific antigen constant					
BSA-anti-BSA system			HGG-anti-HGG system		
Mg BSA used in sensitization	Mg HGG added to BSA prior to sensitization	Titre	Mg HGG used in sensitization	Mg BSA added to HGG prior to sensitization	Titre
1	0	128,000	1	0	12,800
1	0.25	128,000	1	0.25	6,400
1	0.50	128,000	1	0.50	3,200
1	1.0	128,000	1	1.0	1,600
1	2.0	32,000	1	2.0	0
1	4.0	16,000	1	4.0	0
1	8.0	8,000	1	8.0	0
1	16.0	*	1	16.0	0

\* Could not be determined due to non-specific agglutination.

BSA. At very high relative concentrations of HGG (16 mg), all the sensitized red cells were non-specifically agglutinated in the diluent and therefore no titre could be determined. On the other hand, even relatively small quantities of BSA were capable of completely inhibiting the sensitization of the red cells with HGG (Table 2).

With the tanned cell technique, neither BSA nor HGG was capable of inhibiting the sensitization of the cells with HGG and BSA, respectively, in the concentration used (Table 4). This result was anticipated since the optimal sensitization of the tanned cells does not appear to be dependent on the concentration of the specific antigen within the wide range of concentrations used in these experiments (Table 3).

Table 3. EFFECT OF PROTEIN CONCENTRATION ON THE SENSITIZATION OF RED BLOOD CELLS IN THE TANNED CELL HAEMAGGLUTINATION TECHNIQUE  
BSA-anti-BSA system

Mg BSA used for sensitization	Titre
0.33	1,280,000
0.33 + 0.33	1,280,000
0.33 + 1.33	1,280,000
0.33 + 5.33	1,280,000
0.33 + 10.66	1,280,000
0.33 + 21.33	*

\* Could not be determined due to non-specific agglutination.

Table 4. EFFECT OF EXTRANEEOUS PROTEIN ON THE SENSITIZATION OF RED BLOOD CELLS IN THE TANNED CELL HAEMAGGLUTINATION TECHNIQUE  
BSA-anti-BSA system

BSA-anti-BSA system			HGG-anti-HGG system		
Mg BSA used in sensitization	Mg HGG added to BSA prior to sensitization	Titre	Mg HGG used in sensitization	Mg BSA added to HGG prior to sensitization	Titre
0.33	0	1,280,000	0.33	0	6,400
0.33	0.083	1,280,000	0.33	0.083	6,400
0.33	0.166	1,280,000	0.33	0.166	3,200
0.33	0.33	1,280,000	0.33	0.33	3,200
0.33	0.66	1,280,000	0.33	0.66	3,200
0.33	1.33	1,280,000	0.33	2.66	3,200
0.33	5.33	1,280,000	0.33	5.33	3,200
0.33	10.66	*	0.33	10.66	3,200
0.33	21.33	*	0.33	21.33	3,200

\* Could not be determined due to positive controls.

The experiments reported in this communication were primarily concerned with the effect of non-specific protein on the sensitization of the red cell with the specific antigen. It is probable that the difficulties encountered when interpreting the results obtained with the haemagglutination techniques reside in our sparse knowledge of the mechanism of both haemagglutination techniques (BDB and tanned cell). Such information is considered to be especially important in view of the finding that, with many antisera, the BDB haemagglutination technique may give a much higher titre than that obtained with the tanned cell method (with the same antiserum) and vice versa. At times, one of the techniques may give no titre at all<sup>5</sup>.

It was demonstrated that HGG, in high concentration (HGG:BSA = 8:1), was capable of affecting the sensitization of the red cells with BSA, using the BDB technique, to the extent that the anti-BSA gave low titres when tested with the cells sensitized with this HGG-BSA solution. The effect of BSA on the HGG system was far more marked. In this case, the addition of BSA to the HGG was capable of affecting the sensitization of the cells with HGG to the extent that they would not be agglutinated in the presence of anti-HGG (Table 2). BSA was capable of exerting this effect at a relatively low concentration with respect to HGG (BSA:HGG = 2:1). On the other hand, neither the specific nor non-specific protein, in the range of concentrations used, had any adverse effect on the specific sensitization using the tanned cell technique (Tables 3 and 4). That is, HGG could not inhibit the optimal sensitization of red blood cells with BSA, even in high concentration, and vice versa. One might therefore conclude that different antigens will be affected differently, in so far as their capacity to sensitize red cells is concerned, by the presence of non-specific

protein. Furthermore, it would appear that the sensitization of red cells, using tannic acid, is less affected by extraneous protein than is the sensitization using BDB. This finding should be considered by those investigators working with organ extracts as antigens. Since these extracts are mixtures of many protein antigens, the optimal sensitization of red cells with respect to any one of these antigens may be inhibited by a heterologous protein or antigen in the solution. The results of this investigation suggest that the tanned cell haemagglutination technique would be less susceptible to non-specific inhibition of sensitization than would cells using the BDB technique.

It would therefore be advisable to use both haemagglutination techniques as screening procedures for the detection of antibodies before committing oneself as to the presence or absence of antibodies.

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### Localization of Erythropoietin in Glomeruli of Sheep Kidney by Fluorescent Antibody Technique

THE kidney has been shown by several workers<sup>1-3</sup> to produce an erythropoietic substance. Investigations of the renal site of erythropoietin production, however, have been controversial and inconclusive<sup>4-6</sup>. The known antigenic properties of erythropoietin<sup>7</sup> prompted us to try a line of investigation using the fluorescent antibody technique. Anti-erythropoietin serum was prepared by injecting a young Dutch rabbit with concentrated sheep erythropoietin in complete Freund's adjuvant. (Step III Sheep Erythropoietin Lot No. K 147192 with specific activity of 11.7 units per mg protein was supplied by the U.S.P.H.S. Hematology Study Section.) 0.4 ml. of the antigen-adjuvant emulsion containing 4.8 mg (56 units) erythropoietin was injected intradermally into the footpads (0.1 ml. into each pad) of the rabbit on the first day of the investigation. One week later a second dose of 0.4 ml. (4.8 mg protein) was injected intradermally into 4 separate sites on the back of the neck. A control rabbit was also injected in the same way with Freund's adjuvant alone. Two weeks later the rabbits were bled and the sera separated.

*In vitro* activities of the antisera were determined by Ouchterlony gel-diffusion<sup>8</sup> and immuno-electrophoresis. Absorption investigations were carried out by mixing the antisera of the immunized rabbit with an equal volume of a 1:4 dilution of normal sheep serum in saline. The unabsorbed rabbit antisera contained at least 8 distinct precipitin lines when tested by immuno-electrophoresis against concentrated (24 mg protein/ml.) erythropoietin. After absorption with appropriate concentration of sheep serum only a single line was discernible.

*In vivo* neutralization of the biological activity of sheep erythropoietin was demonstrated in the polycythaemic mouse assay<sup>9</sup>. Erythropoietin-serum mixtures were mixed and left at 4° C overnight before injection into the mice. The mean per cent <sup>55</sup>Fe incorporation in red cells



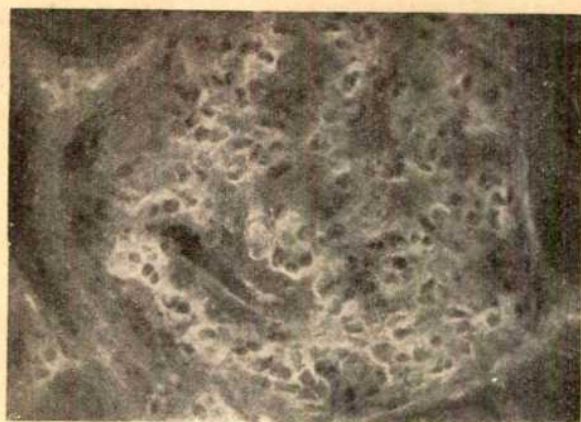


Fig. 1. Fluorescence photomicrograph of rabbit kidney section treated with anti-erythropoietin serum and stained via the double sandwich method. Cytoplasmic fluorescence can be seen in the cells of the capillary walls in the glomerular tuft. ( $\times c. 200$ )

in polycythaemic mice (6 in each group) were: (a) saline,  $0.44 \pm 0.07$ ; (b) control rabbit serum mixed with 3.0 units sheep erythropoietin,  $11.5 \pm 2.2$ ; (c) unabsorbed antisera with 3.0 units erythropoietin,  $11.1 \pm 0.78$ ; (d) antisera absorbed with normal sheep serum plus 3.0 units erythropoietin,  $3.33 \pm 0.98$ . The absorbed antiserum produced a marked neutralization of the effects of 3.0 units of sheep erythropoietin on  $^{59}\text{Fe}$  incorporation in RBC of polycythaemic mice when compared with that of the control serum; while the unabsorbed antiserum did not significantly affect the biological activity of erythropoietin.

For the immuno-fluorescent investigations the 'double sandwich' method, previously used for the biological localization of renin<sup>10</sup>, in which specific antibody globulin applied to the corresponding tissue antigen is identified by means of a second layer of fluorescent anti-globulin serum, was used. Unfixed frozen sections of normal sheep kidney ( $5\mu$ ) were air-dried, treated with the antisera, washed with buffered saline, then incubated for 45 min at  $37^\circ\text{C}$  with a fluorescein isothiocyanate-labelled sheep anti-rabbit globulin serum, washed with saline, mounted in glycerol and examined with a fluorescence microscope. Fig. 1 shows the intense fluorescent staining of the capillary walls of the glomerular tuft with the absorbed antiserum. Control sections in which serum from the Freund adjuvant-injected rabbit was substituted failed to show this specific fluorescence; only the expected background fluorescence was found. Neutralization of anti-erythropoietin serum with sheep erythropoietin before the application of the antibody to the tissue reduced the glomerular staining considerably. Other capillaries not associated with glomerular tufts did not fluoresce. Treatment of sheep lung, liver and spleen by the same techniques using both absorbed anti-erythropoietin and control rabbit serum did not result in a significant amount of fluorescence. Absence of significant staining of these organs indicates that the antibody in the absorbed antiserum reacted specifically with glomeruli.

We were unable to distinguish epithelial from endothelial cells in the centre of the glomerular tuft in our preparations stained with fluorescent antibody. Therefore, it was not possible to identify the cells in the capillary wall which were reacting with the erythropoietin antibody. The juxtaglomerular area in our kidney sections did not appear to be fluorescing. However, we did not stain the juxtaglomerular cells with a tinctorial stain for granules. This does not rule out the possibility that increased fluorescence may be seen in association with the increased juxtaglomerular cell granularity reported following a potent erythropoietic stimulus<sup>5</sup>.

The results provide evidence that the site of erythropoietin elaboration is the glomerular tuft. The intense

fluorescence of the capillary wall suggests that erythropoietin is either produced or stored in these cells. The fact that the erythropoietin antibody was predominantly anti-glomerular, with complete absence of renal tubular staining, is good evidence that the renal tubules are not a site of production of erythropoietin.

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### Reduction of Anaphylactic Shock in Bursectomized Chickens

IN chickens, the production of circulating antibody to soluble or bacterial antigens depends on the presence of the bursa of Fabricius (BF) during embryonic and early post-natal life<sup>1</sup>. The BF appears to have little influence on tissue immunity<sup>2</sup>.

Injections of bovine serum albumin (BSA) will induce anaphylactic shock in chickens<sup>3,4</sup>. Our data demonstrate that chemical or surgical bursectomy will suppress anaphylactic shock in chickens.

The experimental chickens were from a strain of New Hampshires developed at our Experiment Station by Prof. L. J. Dreesen. Surgical bursectomy was performed 1 day or 2 weeks after hatching. Chemical bursectomy was performed by dipping fertile eggs in 1,280 mg of testosterone propionate per 100 ml. of ethyl alcohol<sup>5</sup>. The BF obtained by bursectomy was washed with a solution of bacitracin and then immediately transferred to the subcutaneous portion of the breast of either the same chick (autotransplantation) or a different one (homotransplantation). The transplanted BF disappeared within 4 weeks and was replaced by connective tissue.

Table 1. THE RESPONSE OF 5-WEEK-OLD SHAM-OPERATED AND BURSECTOMIZED BIRDS TO A SECOND INTRAVENOUS INJECTION OF BOVINE SERUM ALBUMIN

Treatment	Anaphylactic symptoms					'Anaphylactic index'
	-	+	++	+++	f	
<b>Trial 1</b>						
Sham operated	0	0	1	3	0	2.75
Bursectomized						
Chemically	8	0	0	0	1	0.44
Surgically at 1 day	11	1	1	0	0	0.23
Autotransplanted, 1 day	1	5	0	0	0	0.83
Homotransplanted, 1 day	6	1	0	0	0	0.14
<b>Trial 2</b>						
Sham operated	0	2	6	6	1	2.40*
Bursectomized						
1 day	14	2	0	0	0	0.13
2 weeks	2	2	7	2	0	1.69
Homotransplanted at 2 weeks						
Bursectomized at 1 day	5	1	0	0	0	0.17
Bursectomized at 2 weeks	2	1	2	1	0	1.33
Autotransplanted at 2 weeks						
Bursectomized at 2 weeks	2	4	3	1	0	1.30

\*  $P < 0.01$ .



All birds were sensitized at 5 weeks of age with a single intravenous injection of 40 mg of BSA per kg of body-weight. A second injection of BSA, one-third the amount of the initial injection, was administered intravenously 7 days later. All birds were classified on the basis of how they responded to the second injection of BSA. A negative reaction would indicate no response. A 'slight' positive (+) response was characterized by an open beak, strenuous panting, feather fluffing, and repeated trials of defaecation. A 'mild' response (++) was recorded when the birds exhibited, in addition to these symptoms, muscular weakness, sitting on the floor and refusing to move on stimulation. A severe response (+++) included the foregoing symptoms together with convulsions. Arbitrary values of 1, 2, 3 and 4 were assigned to the slight, mild, severe and fatal (f) symptoms, respectively. The 'anaphylactic index' was calculated by multiplying the number of birds in each category by the assigned value for that category, summing, and dividing by the total number of birds<sup>6</sup>.

Removal of the BF significantly suppressed the anaphylactic symptoms (Table 1). Bursectomy at one day was more effective than at 2 weeks of age. Chemical bursectomy prevented the occurrence of anaphylactic symptoms with the exception of one chick which revealed a BF at autopsy. Neither autotransplantation nor homotransplantation was able to restore the bursectomized bird's ability to respond to a second injection of BSA.

Hypersensitivity of the delayed type is not influenced by bursectomy<sup>7</sup> while immediate hypersensitivity, anaphylaxis, is markedly reduced by bursectomy (Table 1). Once again, this emphasizes the specific part the bursa plays in avian immunity. The avian thymus significantly influences tissue immunity and has little influence on the production of circulating antibody<sup>2,8</sup>. We believe that thymectomy would significantly alter delayed hypersensitivity, but would not influence immediate hypersensitivity, anaphylaxis, in the chick.

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### Effects of Antigenic Dissimilarity between Mother and Foetus on Placental Size in Mice

THE mammalian foetus must differ antigenically from its mother, in normal circumstances, the exception being in highly inbred strains. Consequently, except in matings between inbred mammals, the foetus and the foetal part of the placenta may be regarded as a homograft, and a homograft reaction against the foreign antigens might be expected. However, the antigenically different foetus and placenta are maintained in the uterus throughout pregnancy without eliciting any apparent rejection reaction, although the immunological competence of the mother must be significant during gestation.

Billington<sup>1</sup> showed that placental size tends to be greater when mother and foetus are antigenically dissimilar. He found a significantly larger size for the placentae of F1 hybrid foetuses between two inbred strains and showed, by transplantation of pure-bred eggs from one of these strains into mothers of the other strain, that this increase in size was not due to heterosis.

It was concluded that if the size of the placenta is affected by antigenic dissimilarity between mother and conceptus, this might be due to either a reaction of the maternal mucosa to the trophoblast, or a reaction of the trophoblast to a mucosa which lacks some of its antigens. An experiment was designed to investigate this problem further, and the preliminary results indicate that antigenic dissimilarity does evoke a reaction between mother and foetus, the extent of this reaction being directly proportional to the degree of immunological dissimilarity existing between them.

The mice used were young adult virgin females of the C57BL strain, mated at about 90 days of age. Four groups of mice were used. Mice of group 1 were injected, within 24 h of birth, with about 8,000,000 A2G male spleen cells in about 50  $\mu$ l. of Tyrode's solution, into the anterior facial vein, to render them tolerant. They were mated with A2G males. Mice of group 2 were injected intraperitoneally, at 6 weeks and 9 weeks of age, with half a spleen equivalent suspended in about 0.5 ml. of Tyrode's solution, to render them immune. These also were mated with A2G males. Mice of group 3 were untreated, and were mated with A2G males as controls with hybrid litters. Mice in group 4 were untreated, and mated with C57BL males as control with inbred litters.

Skin grafts were used to test tolerance and immunity, using the method of Gottfried and Padnos<sup>2</sup>. The mean survival time for the control grafts of A2G male skin on to C57BL females was found to be 10 days  $\pm$  2 days. Survival for longer than 14 days was considered as confirming tolerance, and rejection before the seventh day was considered to indicate immunity. Two animals were successfully tested in this way in group 1, and four in group 2. In these cases, tolerance and immunity, respectively, were confirmed.

The mothers were killed 17½ days after finding the vaginal plug. The placentae and foetuses were removed from the uterus, weighed and counted. Litters containing any reabsorbing foetuses were not included in the statistical analysis; and, since the size of the litter has been shown to affect individual placental size<sup>3</sup>, abnormally small litters of four or less were also excluded. Six litters were excluded on these grounds. All the remaining litters contained seven or more healthy foetuses.

The placentae were blotted on filter paper dampened with normal saline solution, and were weighed to the nearest mg on a torsion balance. The results are set out in Table 1.

Table 1

Mother	Foetus	No. of litters	No. of conceptuses	Mean size of litter	Mean wt. of placentae, and S.E., in mg
Tolerant	C57BL/A2G	8	65	8.13	88.42 $\pm$ 0.83
Immune	C57BL/A2G	9	69	7.67	111.51 $\pm$ 1.24
Control	C57BL/A2G	11	85	7.73	98.36 $\pm$ 0.76
Control	C57BL/C57BL	7	64	9.14	83.53 $\pm$ 0.78

It will be apparent that the placentae of the hybrid foetuses in group 3 are significantly larger than those of the inbred foetuses in group 4 although, since the litters of group 4 were larger, correction for litter size would tend to diminish this difference. This finding confirms Billington's claim that hybrid foetuses have larger placentae than inbred foetuses in C57BL mothers. The mean placental weights in both groups, when allowance is made for litter size, are smaller than those recorded by Billington for the same strains; a difference possibly due to variation in weighing technique.

The placentae of the tolerant group are smaller than those of the control hybrids, the difference being significant ( $P < 0.001$ ), allowance for litter size being unnecessary as it is nearly the same in both groups. Also, the placental weights of the tolerant group do not differ significantly from those of the control inbred group, and if allowance is made for difference in litter size, they would tend to be even smaller.

The placentae of the immune group are larger than those of the control hybrids ( $P < 0.001$ ), allowance for litter size again being unnecessary.

These results confirm the finding that hybrid fetuses tend to have larger placentae than pure-bred fetuses in C57BL mothers. They further show that prior immunization of the mother to the paternal antigens results in a significant increase in placental size whereas, in mothers rendered tolerant of the paternal antigens, the placentae are significantly smaller than in untreated mothers, and are indeed as small as, or possibly even smaller than, those of inbred fetuses in similar mothers.

Since the fetuses in the tolerant, immune and hybrid control groups were genetically similar with respect to the relation between mother and foetus, the observed differences in placental size must be due to the experimental condition of the mothers, depending on whether she was tolerant, immune or untreated. The tolerant mothers differ from the control mothers only in that they lack the potentiality, which the untreated mothers possess, to react to the specific foetal antigens. The difference in placental size must therefore be due primarily to this difference in the mothers. Some reaction to the foetal antigens by the control mothers, that does not take place in the tolerant mothers, must account for the differences in placental size, presumably by providing in the controls a more favourable mucosal environment for the invasion of the trophoblast than in the tolerant animals.

Pre-immunization of the mother to A2G male antigens must result in some response of the uterine tissues to the implanting hybrid embryos which enables them to establish larger placentae. Whether this response is cellular or serological in nature, or both, is unknown.

It follows that, if placental size depends on the extent of trophoblastic invasion, the latter must be affected by the maternal response in such a way that it is more extensive in immune, and less extensive in tolerant, than in untreated mothers. Histological investigation of the placentae, when this can be completed, may throw light on the mechanism involved.

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### Failure of some Hill Lambs to absorb Maternal Gamma-globulin

THE ability of rats to absorb proteins across the gut normally persists until 20 days of age, but can be ended earlier by the administration of certain corticosteroids<sup>1,2</sup>. As a result of this observation and an investigation of mortality data for suckling rats, it was suggested that a premature loss of absorption might be induced by mothers during stress<sup>3</sup>. Because of the severe winter of 1962-63 many hill ewes experienced long periods of undernourishment and climatic stress during pregnancy. The opportunity was taken, therefore, to examine lambs which died before weaning in hill flocks on the A.R.C. Animal Breeding

Research Organization's farms at Stanhope and Blythbank in Peeblesshire. Post-mortem examination was by the procedures described by McFarlane<sup>4</sup>. Blood samples were taken from the hearts and the serum proteins separated by electrophoresis on paper for 16 h (2 m.amp/5 cm width) in a sodium barbiturate-sodium acetate buffer (pH = 8.6;  $\mu = 0.1$ ) using 0.05 ml. serum samples. The strips were dyed with bromo-phenol blue and the concentration of each component measured with a photo-electric scanner.

Table 1. OCCURRENCE OF MILK IN THE GUT AND SERUM  $\gamma$ -GLOBULIN

Age when sampled (days)	Dead lambs without $\gamma$ -globulin		Dead lambs with $\gamma$ -globulin		Live lambs born indoors	
	Milk present	Milk absent	No. $\gamma$ -globulin*	Ave. $\gamma$ -globulin*	No. $\gamma$ -globulin*	Ave. $\gamma$ -globulin*
<1	3	12	2	17.0	3	6.7
1	9	7	5	19.7	4	9.5
2	9	1	10	18.3	4	9.1
3 or 4	7	1	15	18.7	2	7.0
5-7	4	2	7	24.7	5	22.1
8-12	1	3	6	20.8	4	24.1
13-19	—	—	7	15.2	7	16.2
20-24	—	—	9	20.2	4	13.9
25-43	—	—	9	18.6	5	18.5

\* Expressed as percentage of total serum proteins.

Observations were made on 259 lambs; 92 had died before, during or very shortly after birth and will not be considered further. Another 20 had died on the day of birth but had survived long enough to walk. Results from these and older lambs are summarized in Table 1, which includes, for comparison, average serum  $\gamma$ -globulin levels in healthy lambs which were born to ewes kept indoors and adequately fed during pregnancy. Normal lambs can absorb proteins only during the first 2 days of life, and any premature loss of this ability would have to be initiated *in utero*. As lambs are generally born without appreciable quantities of  $\gamma$ -globulin, the amounts of this component which have been absorbed from the colostrum can be easily determined in very young lambs, but analysis is complicated in older lambs by the presence of increasing amounts of autogenous protein.

Many of the lambs dying at ages up to 10 days had no  $\gamma$ -globulin in their sera. It would be expected that some new-born lambs would have no opportunity to suck because of desertion by the mothers or for other reasons. This partly accounts for the high proportion dying on the first day without absorbing  $\gamma$ -globulin. Over the rest of the period there were no significant differences either in the proportion with milk in the gut, or in the amount and distribution of the milk, between the groups of lambs with or lacking  $\gamma$ -globulin respectively. Some lambs dying during the first few days after birth had no milk, but low levels of  $\gamma$ -globulin; this may have been autogenous protein in some cases but in others was probably due to the ingestion of milk which was not detected at post-mortem examination. As the ages of the lambs increase, the presence of milk becomes decreasingly reliable as evidence that some was in the gut at the time when absorption normally occurs. It seems clear, however, that a considerable number of lambs obtained milk during this period yet failed to absorb any  $\gamma$ -globulin. All lambs in this category were born to Scottish Blackface ewes at Stanhope, a farm providing a particularly severe environment with mainly heather-covered hills rising to 2,700 ft. No additional features were observed which distinguished these lambs or their mothers from those of other groups.

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## RADIOBIOLOGY

### Effect of Whole-body X-irradiation on $^{14}\text{C}$ -Leucine Incorporation into Proteins of Cell Nuclei of Regenerating Rat Liver

X-IRRADIATION has been shown to interfere with some biosynthetic processes in the cell nucleus of the regenerating liver. A disturbance of DNA synthesis<sup>1-3</sup> and of nuclear RNA turnover<sup>4</sup> was noted when animals were irradiated with moderate doses of X-rays in the early phase of liver regeneration (2-6 h after partial hepatectomy in the case of RNA), but no effect was observed after X-irradiation in the later stages within 24 h after operation. Similarly, an inhibitory effect of X-irradiation on the total protein turnover in the 23-h regenerating liver was reported to occur in animals exposed to lethal doses of X-irradiation 2-6 h after partial hepatectomy, but not in those irradiated 18 h after operation<sup>5</sup>. The present communication reports the effect of whole-body X-irradiation, at different time intervals within 24 h after partial hepatectomy, on amino-acid incorporation into proteins of cell nuclei isolated from a 24-h regenerating liver.

Animals used in this study were male Wistar rats of approximately 2 months and 160-170 g. Partial hepatectomies were performed according to Higgins and Anderson<sup>6</sup>. The rats were whole-body irradiated with a dose of 900 r. (measured in air), the X-ray machine operating at 220 kV and 15 m.amp., with 0.5 mm Cu and 1 mm Al. The target distance was 40 cm and the dose rate 82 r./min.

The liver was freed from erythrocytes by perfusion with cold physiological saline followed by 0.25 M sucrose-2 mM  $\text{CaCl}_2$ . The 10 per cent tissue suspension in 0.25 M sucrose-5 mM  $\text{CaCl}_2$  was treated in a hand-operated ball type homogenizer<sup>7</sup> until almost all cells were broken. Nuclei were separated from other cell fractions by repeated centrifugation in a discontinuous density gradient of 0.25 M and 0.34 M sucrose, each solution containing 2 mM  $\text{CaCl}_2$ . Nuclear suspensions in isotonic sucrose were checked for purity by phase-contrast microscopy. Those containing more than 5 per cent whole cell contaminants were rejected. Nuclei were incubated for 90 min in the presence of  $^{14}\text{C}$ -leucine (Fig. 1). After that time or at shorter intervals the reaction was stopped by adding an equal volume of 20 per cent trichloroacetic acid. Precipitated protein was purified according to Allfrey *et al.*<sup>8</sup>. Weighed aliquots were suspended in acetone and plated on Whatman No. 50 paper. The activity was counted in a gas

flow counter (Nuclear, Chicago). The results were adjusted at infinite thickness.

The time course of  $^{14}\text{C}$ -leucine incorporation into nuclear proteins during a 90-min incubation of nuclei isolated from a 24-h regenerating liver is shown in Fig. 1.

Irradiated rats received 900 r. of X-rays at 2, 6 and 16 h after partial hepatectomy. They were killed 24 h after operation. Each irradiated animal was paired with a sham-irradiated control for simultaneous incubation of nuclei. All animals were fasted from operation to killing. In these experiments the time course of  $^{14}\text{C}$ -leucine incorporation was not followed, but only 90-min incubation values were recorded. The experimental results are summarized in Table 1.

Table 1. EFFECT OF 900-R. X-IRRADIATION ON  $^{14}\text{C}$ -LEUCINE INCORPORATION INTO PROTEINS OF ISOLATED CELL NUCLEI OF 24-H REGENERATING LIVER

Time of irradiation after hepatectomy (h)	C.p.m. at infinite thickness Controls	C.p.m. at infinite thickness Irradiated	t-test
2	454 $\pm$ 33	344 $\pm$ 23	$P < 0.05$
6	432 $\pm$ 30	326 $\pm$ 26	$P < 0.05$
16	501 $\pm$ 34	499 $\pm$ 33	No difference

For each time interval eight paired rats were used.

As shown in Table 1,  $^{14}\text{C}$ -leucine incorporation into proteins of regenerating liver nuclei was inhibited by 20-25 per cent when irradiation was given 2 or 6 h after partial hepatectomy. No inhibition was observed when animals were irradiated 16 h after operation.

There is a striking similarity between the effect of X-irradiation on amino-acid incorporation at tissue level<sup>5</sup> and at nuclear level, suggesting that the mechanisms of nuclear and cytoplasmic protein synthesis are closely interdependent in growing liver cells.

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### Hereditary Cataract induced by X-irradiation of Young Rats

IN a previous investigation<sup>1</sup> we showed that about 5 per cent of the offspring of 8- and 17-day-old rats which had been subject to 50, 150, 300 and 500 r. of total-body X-irradiation were dwarfs. At weaning the body-weight of the dwarf animals was half that of the controls of corresponding age, but this difference between normal and dwarfed offsprings decreased with age. In the  $F_2$  (from the  $F_1$  dwarfed generations) almost the same percentage of dwarfed animals was observed, but, in addition, in  $F_3$  some offsprings were found with bilateral cataract.

The cataracts were observed in the  $F_3$  generation of a 17-day-old female irradiated with 150 r. of total-body X-irradiation. At the age of 70 days, this female was mated with a control male and produced  $F_1$  and  $F_2$  progeny with normal eyes. In the  $F_3$  generation about 25 per cent had cataracts although all animals were not affected at the same time: at weaning about 75 per cent of the mutant offsprings showed bilateral cataracts while one or both lenses of 25 per cent appeared to be normal. At 70 days of age, bilateral cataracts were present in all mutants. The breeding tests have established that this mutation is inherited as a simple recessive factor: the mating between cataractous male and female gave 100 per cent of cataractous offspring while the mating between

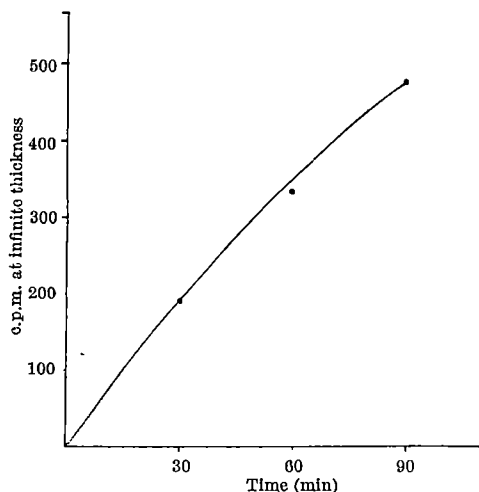


Fig. 1. Incorporation of  $^{14}\text{C}$ -leucine into proteins of isolated cell nuclei of 24 h regenerating rat liver. Incubation at 37°C with constant shaking. Incubation medium: 1 ml. nuclear suspension, 1  $\mu\text{C}$ . DL-1- $^{14}\text{C}$ -leucine (6.4 mc./mmole), 0.20 M sucrose, 0.025 M sodium phosphate buffer (pH 7.3), 0.02 M glucose, 0.02 M NaCl, 5 mM  $\text{MgCl}_2$ , in a total volume of 2 ml.

## BIOLOGY

## Yield Depression in Wheat due to High Nitrate Applications, and its Alleviation by Molybdenum

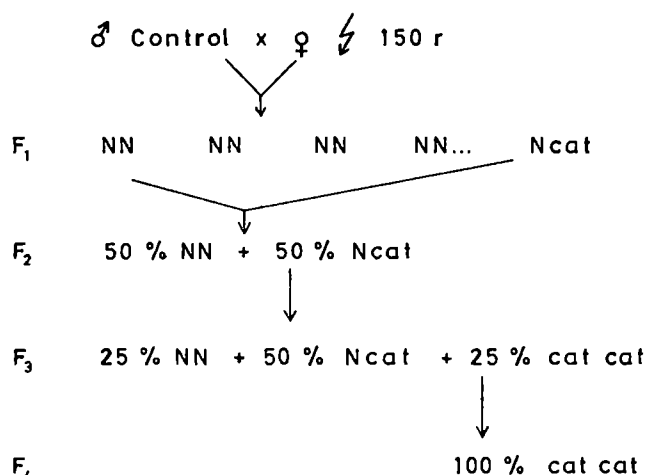


Fig. 1. Mechanism of mutation transmission

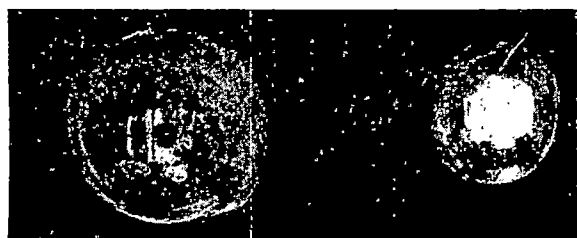


Fig. 2. Normal and cataractous eye

an animal with cataract and a normal one resulted in a normal progeny the offsprings of which showed an incidence of cataracts in a ratio of 1/4. In the tests for detecting sex linkage, non-consistent deviation was found in the distribution of mutant animals between the sexes, and there is therefore no reason to suppose a sex-linked gene. Although the growth of the cataractous animal is normal, their fertility seems to be reduced. The transmission of this recessively inherited mutation is illustrated by Fig. 1.

For the gross dissections, the eye was cut with a razor blade and examined under a dissecting microscope. The cataractous eyes are always smaller than the normal ones (Fig. 2) and the weight of the lens is about five to ten times less than the weight of the controls at 70 days of life. Histological examination will be carried out to determine the relation between our X-ray-induced cataract mutation and the spontaneous mutations of this type described by other authors. In 1939, Bourne and Gruneberg<sup>2</sup> reported a spontaneous hereditary cataract in the rat which, however, was a recessive retinal abnormality and only one-half of the animals developed cataract after the third month. A spontaneous cataractous condition inherited as a simple dominant has also been described in the rat by Smith and Barrentine<sup>3</sup>. On the other hand, the mechanism of the development of this induced cataract will be analysed by biochemical methods to explain the role of polyol, glucose and fructose, as well as the changes in the lens cell membrane permeability, in this phenotype.

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Most soils in southern Australia were initially of low nitrogen status, and bare fallows were generally used to provide mineral nitrogen for cereal crops. In more recent years a ley system based on subterranean clover (*Trifolium subterraneum* L.) pastures has been introduced to counter decline in soil fertility, weed infestation and other factors. Much higher levels of available nitrogen now occur than under previous conditions, particularly in the season immediately following the ley.

In 1962, wheat (*Triticum aestivum* L., var. 'Bencubbin') was grown on several soils in pots in the glasshouse. Four weeks after sowing, the plants developed symptoms ranging from leaf-tip scorch to death of tillers, and growth was reduced. The most severe damage occurred in plants growing on red-brown earths which had been under fallow in the field prior to collection. Eventually the plants recovered, and matured, but no measurement of the extent of damage was possible since all plants were affected.

In 1964, wheat (var. 'Bordan') was grown in pots on a yellow-brown podzolic soil on which subterranean clover was known to respond to applications of molybdenum. Sodium nitrate at four rates (0, 40, 80, 160 p.p.m. nitrogen in soil) and sodium molybdate at two rates (0, 1.5 p.p.m. molybdenum) were applied to the soil in factorial combination with nine replications, together with a basal dressing of the other essential nutrients. The plants were observed at intervals for symptoms, and after seven weeks the tops were harvested, and weighed after drying at 100° C.

Leaf damage similar to that observed in the 1962 experiment occurred in the absence of molybdenum, but not in its presence. Yields of dry matter are presented in Table 1.

Table 1. DRY MATTER YIELDS OF WHEAT PLANTS AS AFFECTED BY MOLYBDENUM- AND NITRATE-LEVELS

Nitrate treatment (p.p.m. N)	Dry matter yields (g)	
	- Molybdenum	+ Molybdenum
0	0.81	0.86
40	2.79	3.00
80	3.49	3.89
160	2.89†	4.25* ‡

\* Difference between - Mo and + Mo significant at  $P < 0.001$ .

† Difference between N 80 and N 160 in the absence of Mo significant at  $P < 0.001$ .

‡ Difference between N 80 and N 160 in the presence of Mo not significant.

In the presence or absence of molybdenum, the maximum yield was obtained with a nitrate application equivalent to 80 p.p.m. nitrogen in the soil (N 80). The higher concentration of applied nitrate depressed the yield in the absence, but not in the presence, of applied molybdenum. Where yield was depressed, the plants were chlorotic and carried many damaged leaves. At the highest rate of nitrate addition (N 160) the added molybdenum overcame not only a possible deficiency in molybdenum supply from the soil but also prevented symptoms of damage and malfunction.

A similar depression in yield and correction with molybdenum application were obtained on a red-brown earth, which would not be expected to be deficient in molybdenum<sup>1</sup>.

It is known that molybdenum is a component of nitrate reductase<sup>4</sup>. Plants supplied with nitrate require more molybdenum than plants supplied with ammonium, and nitrate accumulates in molybdenum-deficient plants<sup>5-7</sup>. The results presented here, however, show that increasing the supply of nitrate to wheat plants increases the response to applied molybdenum. The mechanism of the yield depression with high nitrate dressings and of its alleviation by application of molybdenum to the soil is not known. Among the possibilities which we are now examining

Table 2. EFFECT OF NITRATE AND MOLYBDENUM SUPPLY ON PROTEIN AND NITRATE NITROGEN IN WHEAT PLANTS

Nitrate treatment (p.p.m. N)	- Molybdenum			+ Molybdenum		
	Nitrate N (% dry matter)	Protein N (% dry matter)	Total protein nitrogen (mg/pot)	Nitrate N (% dry matter)	Protein N (% dry matter)	Total protein nitrogen (mg/pot)
0	0.02	1.63	13	0.02	1.58	14
40	0.07	1.88	52	0.03	1.69	51
80	0.13	2.50	87	0.07	1.97	77
160	0.89	3.56	103	0.50	3.42	145

are: (1) a reduction in molybdenum uptake by the plants due to the high nitrate application, as suggested by Stout *et al.*<sup>8</sup>; (2) an increased requirement for nitrate reductase, and hence for molybdenum, within the plant. The results for nitrate and total protein analyses (Table 2) suggest that there was sufficient molybdenum for nitrate reduction at least up to N 80.

At the highest level of nitrate supply (N 160), nitrate accumulated in the plants, and in the absence of applied molybdenum there was a depression in growth. It is of course possible that the nitrate concentrations in the visibly damaged tissues were much higher than the levels reported in Table 2, which refers to the whole tops.

Few responses by wheat to molybdenum applications have been reported for soil culture, so the occurrence of a response on red-brown earths is of interest because they are widely used in Australia for wheat growing.

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### Glycollic Acid Metabolism and the Movements of Stomata

THE effect of carbon dioxide-free air in producing stomatal opening is well known, but practically nothing is known concerning the biochemistry of its action. Some recent work has revealed that the metabolism of glycollic acid may be implicated in stomatal movements and an attempt has been made to explain the response to low carbon dioxide tension in terms of a promotion of glycollic acid synthesis<sup>1-3</sup>. Stomatal opening in response to low carbon dioxide levels appears to have been considered purely as a light reaction; it is stated<sup>3</sup> that "low concentrations of carbon dioxide in light are necessary in order to obtain large stomatal widths". The fact is, however, that opening due to the removal of carbon dioxide can occur in the absence of light. This was observed in 1948 by Heath for *Pelargonium*<sup>4</sup>, and by several authors since, including Stålfelt<sup>5</sup>.

Recent experiments here have provided abundant evidence that the same is true in *Xanthium pennsylvanicum*. In this species some night opening occurs towards the end of a 16-h night<sup>6</sup> and this enables a stream of either carbon dioxide-free or ordinary air to be forced through the leaf. The former treatment produces quite wide stomatal opening. In Table 1 is shown, for six different leaves, the opening brought about by air free of carbon dioxide compared with that for air containing 0.03 per cent carbon dioxide. There were no humidity differences between the treatments, dry air being used in each. Another experiment (Fig. 1) demonstrated that

opening induced in this way can continue for several hours with only a small amount of re-closure. In Table 2 is shown, for six leaves, the degree of opening in white light of 15,000 lux in ordinary air compared with the maximum brought about by air free of carbon dioxide in darkness. Also shown is the degree of opening after 5 h of the latter treatment. It is not unexpected that a stream of air free of carbon dioxide through the leaf in darkness is somewhat less effective than high-intensity light for producing opening; flushing the sub-stomatal cavity cannot be expected to achieve a lowering of carbon dioxide tension as efficiently as does photosynthetic consumption within the guard cells in light of high intensity. A falling off in aperture similar to that in Fig. 1 is sometimes found in this species in light, and the behaviour in air free of carbon dioxide in darkness therefore appears to resemble quite closely the behaviour in light.

Zelitch<sup>8</sup> proposes that a glycollic acid-glyoxylic acid shuttle would oxidize pyridine nucleotides and enable photophosphorylation to proceed and provide adenosine triphosphate needed for the 'pumping' of water into the guard cells. However, since opening can occur so readily in darkness, participation of photosynthetic phosphorylation for adenosine triphosphate synthesis must be ruled out, at least in opening due to removal of carbon dioxide.

In the experiments<sup>3</sup> where high partial pressures of carbon dioxide in light were found to inhibit the synthesis of glycollic acid and also stomatal opening, the effect of the carbon dioxide was presumably to modify the products of photosynthesis in the manner which is now well established<sup>8</sup>. Stomatal opening in response to low carbon dioxide tensions is not, however, easily interpreted in terms of glycollic acid being a product of photosynthesis under such conditions. This is because opening is favoured down to extremely low carbon dioxide concentrations, and zero concentration does not cause closure<sup>9</sup>, while for glycolate production there is an optimum carbon dioxide concentration and below this, as the concentration approaches zero, production falls off<sup>10</sup>. Experiments<sup>3</sup> performed with concentrations of 1.8 per cent and 0.03 per cent representing 'high' and 'low' carbon dioxide

Table 1. STOMATAL OPENING WITH A STREAM OF AIR (0.03 PER CENT CARBON DIOXIDE) OR AIR FREE OF CARBON DIOXIDE PASSING THROUGH THE LEAF

Leaf	Dry air (0.03% CO <sub>2</sub> )	Dry CO <sub>2</sub> -free air
1	0.35	5.70
2	0.20	7.05
3	0.30	7.20
4	0.10	5.60
5	0.00	4.85
6	0.15	5.75

Leaves 1-6 represent six replications. The order of application of the two treatments was randomized. The figures indicate 'degree of opening' on the scale of the recording resistance porometer (ref. 11).

Table 2. STOMATAL OPENING IN LIGHT COMPARED WITH THAT IN AIR FREE OF CARBON DIOXIDE IN DARKNESS

Leaf	Maximum opening in 15,000 lux	Opening in CO <sub>2</sub> -free air in darkness	
		Maximum	After 5 hours
1	8.25	7.05	5.50
2	8.20	5.95	3.75
3	8.30	5.20	4.80
4	8.20	5.40	4.50
5	7.95	6.65	5.85
6	8.30	7.30	6.40

Leaves 1-6 represent six replications. The figures indicate 'degree of opening' on the scale of the recording resistance porometer (ref. 11).



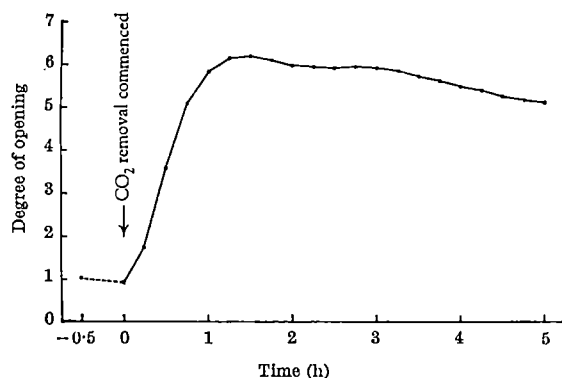


Fig. 1. Stomatal opening in darkness in response to a stream of  $\text{CO}_2$ -free air passing through the leaf. Mean of observations on six leaves. Broken line indicates degree of opening in ambient air before treatment commenced. Ordinate scale: arbitrary units of total pressure minus pressure across the leaf, in a recording resistance porometer (ref. 11)

tensions are rather unsatisfactory since the stomatal response is at physiological levels<sup>9</sup>, and in light 0.03 per cent represents a 'high' concentration under natural conditions.

That there is some evidence<sup>3</sup> of glycolic acid metabolism being implicated in stomatal movements is not disputed. It is clear, however, that opening due to low carbon dioxide tensions does not depend on illumination, and this appears to rule out the mechanism so far suggested<sup>3</sup>.

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### External Stimulus for the Natural Spawning of *Xenopus laevis*

In 1934<sup>1</sup> and 1935<sup>2</sup>, as a result of field observations and a statistical investigation of variation in the spawning date of the common frog, *Rana temporaria*, I showed that the observed relations were very unlike what would be expected if the weather were acting directly on the frogs, and suggested that the weather acted on the algae of the ponds. In 1961<sup>3</sup> a more extensive investigation lent support to this conclusion.

This work has been continued as an experimental investigation, using *Xenopus laevis*. Three aquaria, each containing about 30 l. of water, and divided into two sections by a partition, were so connected that a continuous circulation of water was maintained through them. Weed from natural ponds was placed in the upstream section from time to time. In a second series of experiments, a fourth aquarium was placed in the open air. It contained no frogs, and was used to receive the added weed. In this series, therefore, there was no physical contact between the frogs and the added weed, but the stream of water flowed through this aquarium. Temperature was kept at 23° C,

and one-half of the indoor arrangement was brightly illuminated by a fluorescent lamp, close to the water level, the other half being covered by a dark cloth. Each section contained one pair of frogs. The position of each pair was decided by a table of random numbers, and was changed once a week. In the second series, the position of the light and the presence or absence of added weed was also decided in this way. There was no substantial difference between the two series, and Table 1 is based on both. Spawning was attributed to weed when it occurred within four days from the addition, a decision based on statistical evidence that there was a peak on the second day, but that results after the fourth were rare.

Table 1

	Light		Dark		Total
	Weed	No weed	Weed	No weed	
Contribution to $\chi^2$	20 (4.25) 58.5	5 (11.75) 3.9	5 (4.25) 0.13	2 (11.75) 8.15	32 70.68

The numbers in brackets are the results expected on the null hypothesis.  $P < 0.001$ .

It can be seen that the results depart very much from chance, on the assumption that spawnings would, in that case, be distributed in proportion to the illumination and to the frequency of additions of weed. Most of the contributions to  $\chi^2$  come from the class 'weed and light', the converse 'no weed and no light' accounting for almost all the rest.

Another series of tests was then carried out, in which uni-algal cultures (usually about 3 l.) were used instead of natural collections. The cultures were of various kinds: two species of *Chlamydomonas*, a species of *Oedogonium* normally found in the aquaria, an unidentified unicellular species from a frog pond, *Chlamydomonas* cultures killed by heating to 60°, and the soil-water medium used for the *Oedogonium* and some of the *Chlamydomonas* cultures, and suspected of having activity from the algae it contained. This medium was active three weeks after sterilization. A culture of another unicellular form was not effective.

The results are shown in Table 2. In this series, there were more spawnings not associated with 'weed' than in the earlier experiments. Part way through, however, another light had been placed so that all the aquaria were illuminated, and of the 10 non-associated spawnings, 8 were from this period. Otherwise, there was little difference from the results with the mixed weed. The table is constructed on the assumption that each day constituted a trial with six replicates, whether there were any positive results or not. Since the critical period was four days, the total period of 157 days was multiplied by 6 for the grand total, and the weed occasions by 24 for the weed total. No result, positive or negative, was omitted. By the method of Fisher and Yates<sup>4</sup>,  $P < 0.005$ , so that the result is highly significant.

Table 2

	Weed	No weed	Total
	Spawn	Spawn	Spawn
No spawn	18 (9.3) 294 (302.7)	10 (18.7) 620 (614.3)	28 914
	312	630	942

$P < 0.005$

The joint action of light and weed, not necessarily in the presence of the frogs, but merely upstream from them, points to the action of a water-soluble substance, probably an algal metabolite.

The literature on the natural spawning of *X. laevis* is not extensive and is usually considered to be conflicting. Some observations by Bles<sup>5</sup> seem to have been overlooked. His paper seems to show that he sometimes added a pure culture of *Chlamydomonas* to his aquaria to induce spawning, and that he suspected that some event in the microflora influenced his animals. Vanderplank<sup>6</sup> showed the effect of light. Grimm<sup>7</sup> rightly concluded that in his experiments spawning was associated with water changes.

There was no such association in my experiments, but Grimm added filamentous algae when he changed the water in order to destroy the residual chlorine in the city water. Taken together, the present results and those in the literature appear to confirm the suggestion arrived at on the basis of the field observation on *R. temporaria*.

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## ENTOMOLOGY

### Effect of 5-Fluorouracil on the Development of the Scales of *Ephestia*

THE bodies and wings of the Lepidoptera are covered with scales, which are homologous to flattened hairs. They are derived by a rather complicated mode of division and growth processes from epidermal cells. Their development in *Ephestia* has been examined in detail by Stossberg<sup>1,2</sup>. Since each scale is the product of a single cell, it might be a suitable system for the investigation of developmental mechanisms.

In a first attempt to gain some information regarding the succession of developmental events involved in the establishment of the final structure of the scale and their control mechanisms, it was decided to test the influence of 5-fluorouracil (5-FU) on the developing scale. As an analogue of uracil, 5-FU has been reported to become incorporated into RNA, thus leading to the synthesis of partially or totally modified RNA<sup>3,4</sup>.

Pupae of known ages were treated with 5-FU by implanting crystals into the body cavity. The method of implantation has been described elsewhere<sup>5</sup>. The pupae were allowed to continue development at 25° C until the 14th pupal day, when the animals were dissected. All animals were alive at this time. The front wings were dissected out, fixed shortly afterwards in 10 per cent formalin and squashed on a slide by applying gentle pressure to the overlying coverslip. The preparations were then examined under a phase contrast microscope. In each age group, 10 animals were treated and 5 more were killed immediately at the time of operation to check the developmental stage of the scales at the time of treatment.

Table 1 and Fig. 1 show briefly and schematically the normal development of the scales related to the age of the pupa as given in hours in the left column of Table 1. The Arabic numbers indicate the number of animals the scales of which have reached the stage shown in Fig. 1. The outgrowth of scales starts at 54–60 h after pupation. At 72 h, the round process flattens (stage II) and fine longitudinal elements become visible within the granular cytoplasm. At 108 h, the formation of the distal teeth is indicated (stage III). At 126–132 h, the shape is completed (stage IV) and from this time on the scale enlarges

proportionately. In stage V the cytoplasm becomes vacuolized and degenerates, leaving a rigid, fully outgrown but still unpigmented scale, which becomes strengthened by thick trabeculae (stage VI). On the 13th day, in stage VII, the pigmentation of the scale is completed<sup>1,2,6</sup>.

Table 1. NORMAL DEVELOPMENT OF SCALES ON THE WING OF *Ephestia* (Five animals for each time, stages as shown in Fig. 1)

Time after pupation (h)	No. of animals at different stages
42–48	5–0
54–60	3–0 2–I
66–72	3–I 2–II
78–84	2–I 3–II
102–108	3–II 2–III
126–132	5–IV
150–156	4–IV 1–V
174–180	5–V
198–204	5–VI
222–228	5–VI
270–276	4–VI 1–VII
294–300	5–VII

Table 2 summarizes the results obtained by implantation of 5-FU at different times after pupation as listed in the left column. It also shows the number of animals the scales of which have reached the stage indicated on the 14th day. Treatment during the 48 h following pupation completely suppresses any outgrowth. The situation changes abruptly after treatment at 54–78 h. All animals investigated possessed grown-out scales, slightly smaller than normal size, but very soft, without rigid trabeculae and of abnormal shape. The stems at the base are extraordinarily long, the distal parts frequently compressed and the teeth irregularly arranged and smaller and sharper than normal. Many of these scales show vacuolated cytoplasm. Scales of this type are illustrated in Fig. 1 without a stage number, between stages III and IV. In Table 2 they are designated by 'abnormal'. After treatment at 78–84 h, these abnormal scales are found together with normal scales of stage VI in the same preparations. Application of 5-FU to pupae older than 84 h allows the scales to differentiate further, up to stage VI or VII, depending on the time of treatment.

Table 2. DEVELOPMENT OF THE WING SCALES OF *Ephestia* AFTER TREATMENT WITH 5-FLUOROURACIL AT DIFFERENT TIMES (Ten animals in each experiment)

Time after pupation (h)	No. of animals showing different stages of scale development
42–48	10: stage 0
54–60	10: abnormal
66–72	10: abnormal
78–84	5: abnormal 5: stage VI
102–108	10: stage VI
126–132	10: stage VI
150–156	8: stage VI 2: stage VII
174–180	3: stage VI 7: stage VII
198–204	10: stage VII
222–228	10: stage VII
246–252	10: stage VII
270–276	10: stage VII

It is noteworthy that, as Table 2 shows, the reaction of the scale is an all-or-none reaction; intermediate types are absent. This seems to indicate that 5-FU interferes with a few distinct steps in the development of the scale. These steps must be assumed to initiate the proper growth and differentiation processes, since these processes themselves are not affected by 5-FU and they proceed or may even start in the presence of 5-FU.

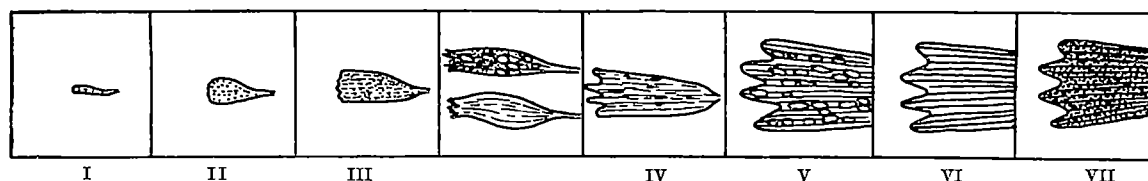


Fig. 1. Stages in the development of scales on the fore wing of *Ephestia*. (The unnumbered sketch indicates the appearance of 'abnormal scales' induced by 5-FU at 54–84 h)

Thus, treatment with 5-FU reveals at least three clear-cut phases in the development of the scale, which initiate the successful completion of specific developmental processes: (1) The preparation for the outgrowth of the scale. This phase ends between 48 and 54 h. In the following time the growing scale may still be subjected to modification, but outgrowth cannot be suppressed by 5-FU. (2) The preparation for enlargement to full size and for the differentiation of the normal trabecular structure; this phase ends at 78–84 h. (3) The preparation for the scale pigmentation; this is completed after 180 h.

It appears, therefore, that treatment with 5-FU may have three possible results, depending on the time of treatment: it may stop the outgrowth of the scales, it may result in the formation of a characteristic type of abnormal scale, or it may permit development up to stages VI or VII. The occurrence of abnormal scales is of interest for an understanding of the possible mechanisms underlying the action of 5-FU on a developing system. It shows that the action of 5-FU does not consist only in the blocking of certain developmental steps, but also that this substance is capable of modifying the determinative processes in such a way that an abnormal structure results. It could be assumed that the normal shape of the scale, known to be under genic control, is the outcome of the synthesis of specific proteins, the formation of which is mediated by RNA. Since 5-FU has been shown to be incorporated instead of uracil in the synthesis of RNA, it is quite conceivable that, if this holds true for *Ephestia*, an abnormal RNA is being synthesized which is responsible for the synthesis of altered proteins, thus furnishing an abnormal scale. This view is consistent with the findings of Gros and Naono<sup>7</sup>, who observed the appearance of altered enzymes in *E. coli* after the addition of 5-FU. But the possibility that in the *Ephestia* system 5-FU acts by inhibition of thymidylate synthetase, and consequently affects DNA synthesis, cannot be excluded by the present experiments<sup>4,8</sup>.

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## Acetylcholine as a Growth Factor in Early Larval Development of the Silkworm

ACETYLCHOLINE is required in the diet of silkworm larvae for normal moulting to occur. On a semi-synthetic basal diet (Table 1, No. 4) the newly hatched larvae die after about 14 days without further moult. In tests each using twenty larvae, it was found that the diet containing mulberry leaves (diet 1, ref. 1) yielded 100 per cent successful moult to second instar with a larval weight of 5.4 mg each; the diet with defatted soybean<sup>2,3</sup> and no other important adjuvants (diet 2) yielded 80 per cent moult (4.5 mg); the diet with royal jelly<sup>4</sup> (diet 3) yielded 100 per cent moult (5.85 mg); the basal diet without adjuvants (diet 4) yielded no moult; the basal diet with royal jelly (diet 5) yielded 70 per cent moult (4.8 mg); and basal diet plus acetylcholine (diet 6) yielded 80 per cent moult

Table 1. COMPOSITION OF ARTIFICIAL SILKWORM LARVAL DIETS

	1	2	3	4	5	6
	3.0 g	5.0 g	5.0 g	5.5 g	5.5 g	5.5 g
Cellulose powder	3.0 g	5.0 g	5.0 g	5.5 g	5.5 g	5.5 g
Casein purified	0	0	0	1.0	1.0	1.0
Soybean defatted	2.0	2.0	2.0	0	0	0
Starch	1.5	1.5	1.5	2.0	2.0	2.0
Sucrose	1.0	1.0	1.0	1.0	1.0	1.0
Minerals (Wesson's)	90 mg	90 mg	90 mg	90 mg	90 mg	90 mg
* Vitamins	40 mg	40 mg	40 mg	40 mg	40 mg	40 mg
† Stimulators	170 mg	170 mg	170 mg	170 mg	170 mg	170 mg
Mulberry powder	2.0 g	0	0	0	0	0
Royal jelly	0	0	1.5 g	0	1.5 g	0
Acetylcholine	0	0	0	0	0	5 mg
Water	15 c.c.	15 c.c.	15 c.c.	15 c.c.	15 c.c.	15 c.c.

\* A vitamin mixture composed of the following at the designated concentration in mg/10 g of diet: biotin, 0.02; Ca-pantothenate, 0.2; choline chloride, 2.0; folic acid, 0.02; niacinamide, 0.2; VB<sub>6</sub>—HCL, 0.1; VB<sub>12</sub> (FMN—Na), 0.1; VB<sub>3</sub>—HCL, 0.1; VB<sub>7</sub>, 0.1, and VC, 37.16.

† As previously pointed out<sup>7</sup>, the following compounds (especially the first) greatly stimulate the feeding reaction. The rates are identical to those listed for vitamins:  $\beta$ -sitosterol, 50; morin, 20; inositol, 50; and K<sub>2</sub>HPO<sub>4</sub>, 50.

(4.0 mg). From these results one observes that royal jelly increases moulting and larval weight. Royal jelly contains large amounts of acetylcholine<sup>5,6</sup>. A water-soluble, low-molecular-weight, dialysable fraction of royal jelly also provided similar growth stimulation. We therefore added acetylcholine to the diet<sup>7</sup> expecting the same results as with royal jelly (diet 6). As described here, acetylcholine can replace royal jelly in stimulating moulting of the larvae.

The next question concerns the optimal quantity of acetylcholine for diets, and the period during the instar when it is required. The following experiments were undertaken. (1) Feeding with the basal diet (diet 4) for all the first instar. (2) Feeding with basal diet for the first four days and then with diet 6 containing acetylcholine for the succeeding days. (3) Feeding with diet 6 for all the first instar.

Table 2. TIMING OF ACETYLCHOLINE REQUIREMENT FOR SILKWORM LARVAE

	Proportion of larvae reaching second instar (%)
No. 4 diet throughout	0
No. 4 diet for first 4 days then	
No. 6 diet for successive days	93
No. 6 diet throughout	80

Table 2 indicates that larvae do not require acetylcholine throughout the first instar, but rather addition of acetylcholine after four days of early development will provide a satisfactory growth response.

Fig. 1 shows that the larvae require more than 3,000 $\gamma$ /10 g diet for optimal growth and the threshold moulting response is between 2,000 $\gamma$  and 3,000 $\gamma$ . Acetylcholine chloride at 5 mg was used in the basal diet for the foregoing experiments, but the bromide and iodide were also

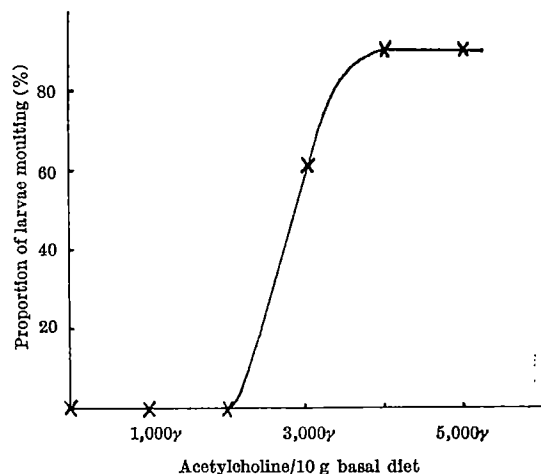


Fig. 1. Relation between dietary acetylcholine and larval moulting of first instar larvae



effective, yielding in comparable order and concentration 76, 51 and 27 per cent moult.

As summarized by Gilmour<sup>8</sup>, most insects require choline for growth. We conclude that acetylcholine or possibly other choline salts must be added in sufficient amount to semisynthetic basal diets for satisfactory growth. Insufficient levels are evidenced by inability to moult. By the addition of sufficient acetylcholine we have succeeded in rearing silkworms on an artificial diet from hatching to spinning without mulberry leaves or their extracts.

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## MICROBIOLOGY

### Stimulation of Transformation by Thalidomide

At present congenital abnormalities caused by thalidomide are engaging the attention of a number of laboratories. However, only a few papers concerning the effect of the drug on protozoa and micro-organisms have been noticed so far<sup>1-3</sup>. In some cases it is difficult to analyse the effects of thalidomide because of its high instability. In neutral and aqueous solutions thalidomide is unstable with a tendency to open the phthalimide and glutarimide rings, with hydrolysis of the amide groups of phthalimide and glutarimide<sup>4</sup>. In the work recorded here the influence of thalidomide on transformation of the indole marker in *Bacillus subtilis* was examined.

An aqueous solution of thalidomide was prepared by dissolving the drug in 0.1 N KOH and then immediately neutralizing with diluted HCl so that concentration was 100 mg/ml. Directly after its dissolution thalidomide was added to the medium to a final concentration of 200 µg/ml. One general approach was made in this investigation. An attempt was made to examine the effect of thalidomide on recipient cells using the procedure of transformation by Spizizen<sup>5</sup>. To a series of tubes containing cultures of the recipient cells thalidomide was supplemented 120, 60 and 30 min before, together with and 60 min following the addition of transforming DNA. Aliquots of the culture were taken for determinations of transformation frequency 4 h after DNA was added.

Table 1

Time of treatment with thalidomide 200 µg/ml.	No. of recipient cells per ml.	Average No. transformants per ml.	Per cent transformation
0	5 × 10 <sup>8</sup>	0.2 × 10 <sup>2</sup>	0.004
3	4 × 10 <sup>8</sup>	2.4 × 10 <sup>2</sup>	0.6
4	4 × 10 <sup>8</sup>	0.2 × 10 <sup>2</sup>	0.005
4.5	3 × 10 <sup>8</sup>	3.1 × 10 <sup>2</sup>	0.1
5	4 × 10 <sup>8</sup>	5.3 × 10 <sup>2</sup>	0.066
6	8 × 10 <sup>8</sup>	5.5 × 10 <sup>2</sup>	1.37

Similar results were obtained in 4 repetitions of the foregoing experiment. Whereas in control tubes the frequency of transformation ranged from 0.002 to 0.005 per cent, in some aliquots with thalidomide it was higher than 2 per cent. To obtain a high percentage of transformants, it was found essential to supplement the drug 2 h before or 1 h following the addition of DNA to the recipient culture. The mechanism of the thalidomide effect is still obscure. It is not related to the mutagenic action of the drug. Significant differences in the number of transformants depending on the time of the drug addition suggest that the promotion of the transformation is not connected with the selection process either as a nutritional effect of thalidomide or its breakdown products. It may be assumed that the stimulation of the transformation is related to the specific effect of thalidomide on the competence of the recipient cells. Moreover, we have observed in our preliminary investigations the stimulating effect of thalidomide on the frequency of the colicinogenic factor transfer.

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### Conversion of DDT to DDD by *Proteus vulgaris*, a Bacterium isolated from the Intestinal Flora of a Mouse

THE dechlorination of 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT) to 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane (DDD) by various animals has been reported recently<sup>1,2</sup>. The same detoxification reaction appears to be carried out by yeasts<sup>3</sup>. DDT administered intraperitoneally to mice was dechlorinated after, but not before, the animals were killed<sup>4</sup>. We undertook to test the hypothesis that the degradation of DDT to DDD in animals is due, at least in part, to the activity of the microflora of the gut.

Several types of micro-organism were isolated from the gut of a DDT-resistant female mouse<sup>5</sup> by serial dilution and streakplate methods on agar-brain-heart-infusion media. An ethanol solution of pure *p,p'*-DDT was placed in sterile test-tubes and evaporated over a hot-water bath. Sterile brain-heart infusion medium was then introduced into the cooled test-tubes. These preparations were inoculated with several isolates obtained from the mouse gut and the cultures were incubated for 5 days at 30° C. The contents of the tubes were extracted with methanol and chloroform<sup>6</sup>. The mixture was permitted to stand long enough to give a very clear chloroform layer. The extract was sufficiently clean to produce very clearly defined spots on paper chromatography. Paper chromatographic analysis was performed quantitatively for DDT on these extracts<sup>7</sup>.

One isolate, which was identified by morphological and biochemical tests<sup>8,9</sup> as *Proteus vulgaris*, was thus shown to dechlorinate DDT to DDD.

A culture of *P. vulgaris* (Macdonald College Culture No. 261) was obtained from the Department of Bacteriology and the experiment with the DDT-containing medium was repeated with this organism. Morphological and biochemical tests showed no significant differences between the gut isolate which produced the DDD and *P. vulgaris* from the laboratory culture. This species is associated with some diseases of animals and is one of the primary invaders of animal tissues after death<sup>10</sup>. Analysis of extracts of the media after incubation with the laboratory culture showed DDD to be the main metabolite present.

Total DDD production in the gut isolate cultures decreased in inverse proportion to the logarithm of the time of incubation, as shown in Table 1. The decrease in the total quantity of DDD produced suggests that this material is further metabolized to another product which has not yet been adequately identified.

Table 1. THE QUANTITY OF DDD PRODUCED FROM 5.45 MG OF DDT AFTER DIFFERENT PERIODS OF INCUBATION AT TWO TEMPERATURES

Time of incubation	DDD in mg 30° C	37° C
6 days	0.355	—
10 days	—	0.395
15 days	0.270	0.355
20 days	0.243	0.300

*P. vulgaris* was also grown on a medium containing 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene (DDE) but no DDD was found in extracts of these preparations. Thus as in DDT detoxification by yeasts<sup>3</sup>, DDE does not appear to be an intermediate in the production of DDD from DDT.

We conclude that the bacterium *P. vulgaris*, commonly found in the gut of mice and other animals, has the capacity of metabolizing DDT to DDD and can be assumed to be an agent, if not the sole one, in the conversion of DDT to DDD in animals which have been killed by DDT poisoning.

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### Isolation of the Crystalline Parasporal Bodies of *Bacillus thuringiensis*

THE method of Angus<sup>1</sup> for the isolation of parasporal bodies (crystals) from a mixture of crystals and spores has been modified so that only two treatments instead of five with fluorocarbon are necessary, and the separation can be completed in approximately 6 h. It dispenses also with the need for preliminary germination of spores.

The crystal-spore suspension is obtained by washing a crude sample as described by Angus<sup>2</sup> and is adjusted to contain 0.05 g wet wt./ml. 10 ml. of the suspension is

lightly shaken for 15 min in a 1-oz. McCartney bottle containing five drops of tri-*n*-butyl citrate and 5-g glass beads (2.0–2.5 mm diameter) by means of a Microid flask shaker (Griffin and George). The shaken suspension is transferred to the blender bottle of an MSE homogenizer containing 80 ml. 1 per cent Na<sub>2</sub>SO<sub>4</sub> solution, 1 ml. tri-*n*-butyl citrate, and 10 ml. trifluorotrichloroethane and stirred for 2.5 min at 7,700–8,000 r.p.m. The mixture is immediately transferred to a cylindrical separating funnel and allowed to stand for 15 min, when the organic phase separates out. The organic phase is discarded and the aqueous phase is centrifuged at 3,000 r.p.m. for 20 min in an MSE bench centrifuge. The deposit is taken up in a minimum of water and re-subjected to treatment with the fluorocarbon, including the shaking with glass beads. After centrifuging the separated aqueous phase, the deposit contains more than 99 per cent crystals and less than 1 per cent spores.

The deposit can be stored as an aqueous suspension or evaporated to dryness over silica gel under vacuum at room temperature, when a yield of approximately 0.2 mg/ml. of original suspension is obtained.

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### Invertase Content of Yeast Protoplasts

IN 1958 three of us reported<sup>1</sup> that a large part of the invertase is liberated in a soluble state from living yeast cells (*Saccharomyces cerevisiae*) by the action of snail digestive juice. This was observed independently by Friis and Ottolenghi<sup>2</sup> and later confirmed by a number of other authors<sup>3,4</sup> although there remained some disagreement as to the extent to which protoplasts retain invertase activity.

Burger, Bacon and Bacon<sup>5</sup>, who were concerned primarily with establishing the soluble nature of yeast invertase in opposition to the views held at that time by Myrback<sup>6</sup>, stated without giving experimental data that "protoplasts isolated after 20 h (of snail juice action) and washed by centrifuging in 0.2 M lactose liberated only traces of invertase when lysed in water". Millbank<sup>4</sup>, on the other hand, like Friis and Ottolenghi<sup>2</sup> and Sutton and Lampen<sup>3</sup>, demonstrated that nearly a third of the invertase of the original cells was retained in the protoplasts. Furthermore, the protoplasts obtained from Millbank's yeast strains metabolized sucrose, without lag, at rates comparable with those of the intact cells.

A quantitative examination of the results on which these statements are based shows that no real discrepancy exists. The total invertase activity of the culture of yeast used by Burger *et al.* was almost ten times that used by Millbank. Variations of such magnitude are quite common<sup>7</sup>. Table 1 gives the results of several unpublished experiments by Burger *et al.* which show that the invertase activity of the protoplasts was in all cases of the same order as those of Millbank. However, in relation to the total activity they represented usually only 3–5 per cent 'in-

Table 1. INVERTASE ACTIVITY OF PROTOPLASTS PREPARED FROM *R XII* YEAST BY THE ACTION OF SNAIL CROP JUICE

Exp.	Total invertase activity* of original yeast	Invertase activity* of protoplasts produced in presence of 0.2 M lactose	Percentage of invertase activity retained in protoplasts
1	278	7	2.5
2	208	9	4.3
3	256	12	4.7
4	157	16	9.6
5	146	8	4.1

\* Expressed as units/g wet pressed yeast: one unit of enzyme liberates 1 mg reducing sugar/min at 20°. Full details of all procedures have been given previously<sup>2</sup>.

soluble' invertase; it was perhaps something of an exaggeration to refer to this amount as 'traces'.

In view of the present discussions of the place of invertase in the economy of plant cells<sup>8</sup>, and such recent observations as those of Davies and Elvin<sup>9</sup> on the effects of  $\beta$ -mercaptoethanol on protoplast formation and invertase liberation, we thought it would be useful to remove an appearance of disagreement between ourselves on this particular point. We should also like to re-emphasize the differences which may exist between yeast strains and to stress the dangers of simple generalizations when using the term 'yeast'.

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## VIROLOGY

### Stress-induced Suppression of Interferon Production in Virus-infected Mice

INCREASED susceptibility to various viruses has been observed in mice exposed to psychological stress<sup>1</sup>, in which adrenal hyperactivity is considered to be a mediator. The decrease in resistance of stressed mice to vesicular stomatitis virus (VSV) appears not to be related to impairment of antibody response<sup>2</sup>. Interferon has been proposed as an important factor in recovery from viral infections<sup>3</sup>, and interferon production in the chick embryo is inhibited by exogenous corticoid<sup>4</sup>. For these reasons, the following experiments were performed to determine whether the levels of interferon produced *in vivo* differ between stressed and unstressed mice infected with viruses.

Webster (BVR5) Swiss mice, 6-8 weeks old female, were infected intranasally with  $1 \times 10^2$ - $10^3$  plaque-forming units (P.F.U.) of VSV and divided into two groups, one of which was subjected to a high-intensity sound stress (800 c/s at an intensity-level of about 120 dB) for 3 h a day continued for 6-8 days. Under these conditions, stressed mice showed a significantly higher rate of mortality due to encephalitis caused by the VSV than had been shown earlier<sup>1,2</sup>.

At daily intervals after infection, 5 mice in each of the stressed and unstressed groups were killed by drawing blood under ether anaesthesia, and the pooled brains of each group homogenized in a glass grinder in the presence of 10 ml. Hanks's balanced salt solution without bicarbonate. The serum and brain preparations were frozen at  $-20^\circ\text{C}$  until titration for VSV-neutralizing antibody in the former, and for virus as well as interferon content in the latter. Neutralization tests and virus assays were performed on primary chick embryo fibroblasts by the plaque technique. For interferon titration, the brain homogenates were centrifuged at 32,500 r.p.m. (69,590g) for 1 h in a Spinco model L preparative ultracentrifuge. This procedure removed from the test samples all but  $1 \times 10^{-4}$  of the virus originally present before centrifuga-

tion. The residual virus in the supernate was inactivated by heating at  $56^\circ\text{C}$  for 30 min although it was found later that, unlike chick embryo interferon, which was heat-stable, this amount of heat reduced the mouse interferon activity by 30-40 per cent. The interferon preparations were serially diluted 2- to 4-fold and placed in 1.5-ml. amounts per flask (30-ml. capacity, Falcon Plastic, Los Angeles) containing primary Swiss-mouse embryo cell monolayers and incubated at  $37^\circ\text{C}$  for a period of 3 h. At the end of that time, the interferon preparations were poured off and the cells were challenged with 30-70 plaque-forming units of neurovaccinia. After 2-3 h for virus adsorption, the unadsorbed virus was removed and the cells overlaid with an agar medium containing lactalbumin hydrolysate 0.5 per cent tris 0.3 per cent, agar 0.6 per cent, and agamma calf serum 10 per cent. After further incubation at  $37^\circ\text{C}$  for 4 days, the cells were stained with neutral red and the plaques counted. The reciprocal of the highest dilution which gave 50 per cent reduction of plaque count, as compared with the control cells treated with diluent only and challenged with the same dose of virus, was reported as the titre of interferon. Overnight treatment of cells with interferon increased the titre 2-fold. Challenge with oncephalomyocarditis virus gave similar results except that the titres of interferon were lower than when neurovaccinia was used.

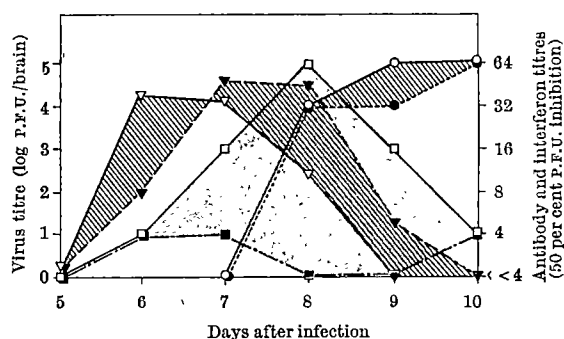


Fig. 1. Comparison of virus, interferon and antibody concentrations in stressed and unstressed mice infected with VSV. Unstressed,  $\nabla$ , virus;  $\square$ , interferon;  $\circ$ , antibody. Stressed,  $\blacktriangledown$ , virus;  $\blacksquare$ , interferon;  $\bullet$ , antibody. The shaded area represents the difference in values between unstressed and stressed mice.

Fig. 1 summarizes an experiment using a relatively small inoculum,  $1 \times 10^2$  plaque-forming units and 8-week-old mice in which the differences between stressed and unstressed groups were more clearly seen than in 6-week-old mice. As compared with the unstressed group, the stressed group showed an initial inhibition of virus multiplication followed by accelerated virus growth and prolonged presence of virus in the brains. Neutralizing antibody appeared on the 8th day post-infection and rose to titres of 64 at similar rates and remained the same throughout the later period of observation for both groups of mice. Interferon was detectable on the 6th day post-infection, and the maximum titre reached, 64 on the 8th day, was followed by a rapid decline in the unstressed group; whereas in the stressed group the interferon titres remained markedly low and erratic in level throughout the whole period of observation. It appears, therefore, that suppression of production of interferon rather than neutralizing antibody is important in increased susceptibility of mice subjected to these stress conditions. The negation of the initial suppression of viral multiplication in the stressed group is probably related to the suppression of interferon production, thus tipping the balance between virus multiplication and interferon production in favour of the virus. These results are in accord with those of Kilbourne *et al.*<sup>4</sup>, who showed that corticosteroids exerted an inhibition of interferon synthesis in chick embryo-influenza virus



system, because in the stressed mice increased secretion of corticoid hormone would be expected. The possibility remains, though, that stress-induced suppression of interferon production as well as higher susceptibility of mice to viral infections is mediated by systems other than the adrenal cortex. The question as to whether or not the activity of interferon as well as the rate of production *in vivo* is inhibited in the stressed as compared with the unstressed group also remains to be investigated.

The interfering activity of our preparation of interferon derived from mouse brain was destroyed by treatment with crystalline trypsin (50 µg/ml. at 37° C for 2 h) and not neutralized by anti-VSV serum. It was relatively heat-labile (56° C for 30 min), non-sedimentable in ultracentrifuge at a condition in which almost all the virus particles were spun down, active against heterologous viruses, and showed no detectable activity on chick embryo cells *in vitro*. These properties indicate that it is very similar to, if not identical with, mouse interferon as reported by others.

It was recently reported that mice infected with polyoma virus at the age of 2-3 weeks developed a higher incidence of tumours when exposed to sound stress for 3 h a day for 6 days, and then followed by avoidance-learning stress in a shuttle box for 6 h a day continued for 2 weeks, as compared with unstressed mice infected similarly<sup>5</sup>. Again the titres of serum neutralizing antibody against polyoma virus were not significantly different among the stressed and unstressed groups. The titres of virus and interferon in the organs of mice and the neutralizing antibody in the serum followed the same pattern as already described here for VSV, that is, in the stressed group there was an initial phase (4-7 days) of inhibition of growth of virus which was followed by a phase of accelerated virus multiplication with marked suppression of interferon production as compared with unstressed, polyoma virus-infected controls.

Thus, impairment of interferon production may be a significant factor in stress-induced increase in susceptibility to VSV and polyoma viruses in mice. These observations also provide further evidence for the general role of interferon in resistance to viral infections.

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### A Virus as the Causal Agent of Bark Cracking in *Hevea brasiliensis*

BARK cracking accompanied by the exudation of latex (Fig. 1) in young trees of certain clones of *Hevea brasiliensis* Mull.-Arg. has been observed in the Eastern rubber-growing countries for a number of years<sup>1</sup>. The symptoms of this disorder and the accompanying histological abnor-

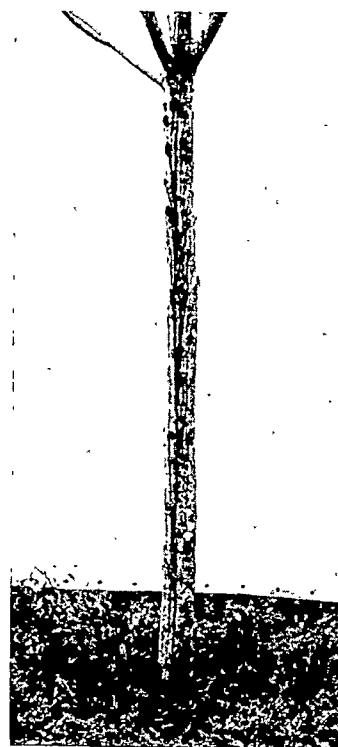


Fig. 1

malities have been described recently<sup>2</sup>. This is a brief note on the aetiology of the disease.

Detailed investigations, carried out at the Rubber Research Institute of Ceylon, proved that the symptoms were not caused by a bacterial or fungal infection. Anatomical abnormalities in affected plants, however, closely resembled those produced in other virus-infected woody plants<sup>3</sup>. Various methods of grafting were tested to find out whether the disease was transferable in this manner, but symptom expression following graft transfer of viruses is often long delayed. Under field conditions budded trees show symptoms of this disorder in the second and third years, and the experimental budgrafts, which are 7 months old at present, have shown only unspecific symptoms. Therefore, serological examinations<sup>4</sup> were undertaken to establish the nature of the disorder.

Antisera were prepared by injecting a clarified Seitz-filtered extract of rubber wood, from trees severely affected by the disease, into rabbits. The immunization schedule consisted of four paired intraperitoneal and intravenous injections given at 3-day intervals. The animals were bled out a fortnight after the last pair of injections. To remove possible antibodies to the normal constituents of rubber wood, the antisera were first absorbed with healthy rubber wood extracts, and the absorbed antisera were then tested by the precipitin and complement-fixation methods.

All antisera from rabbits inoculated with extracts from infected trees gave positive precipitin reactions in combination with diseased plant extracts up to a titre of 1:2,048. In complement fixation tests, the sera were tested against different dilutions of normal and infected rubber wood extracts. The antisera gave a titre of 1:640 with infected extracts, but no fixation at 1:40 dilution of normal extracts, which also showed no reaction with a 1:40 dilution of antiserum. A comprehensive series of controls was included for both types of test, using normal and infected extracts of rubber wood in combination with normal and immunized rabbit serum; all controls gave little or no reaction. These results indicate that bark cracking in young *Hevea* trees is caused by a virus

infection and not by wind damage, as suggested in an earlier publication<sup>4</sup>. A series of bud-grafting trials have been carried out to confirm these results in the field.

This is the first record of a virus disease of the *Hevea* rubber tree, although Taysum<sup>5</sup> referred to a natural virus infection of certain bacteria found in *Hevea* latex, and implied that this virus occurs normally in the latex. Investigations on the transmission of the disease under field conditions have been initiated, but for the present, as *Hevea* is a tree mainly propagated by bud-grafting, a word of warning must be sounded against the use of bud-wood from plantations which have had a history of bark cracking, as this is a likely method of disseminating this disease. Field records of the locations from which bud-wood has been selected have confirmed that bark cracking has been spread by the use of infected bud-wood in the past. A detailed account of the serological methods used here and the grafting trials carried out will be published elsewhere.

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### DEAE-Chromatography of Adenoviruses of Rosen's Group II

SEVERAL antigens of adenovirus type 5 have been separated from infectious virus particles by chromatography on DEAE columns<sup>1,2</sup>. They differ in their serological specificity, and some of them have been correlated with haemagglutinins<sup>3</sup>. Differences in the elution properties between adenovirus serotypes have also been demonstrated in several instances<sup>2,4,5</sup>.

We investigated prototype adenoviruses of Rosen's group II (ref. 6), which have the common property of agglutinating rat blood cells with a complete haemagglutination pattern. Our aim was to investigate the elution properties of various viral activities and to compare the serotypes of this group with one another as well as with other adenovirus types. Type 8 was omitted from the work because of its low virus titre. Virus material from infected HeLa tissue culture fluid was treated twice with a solution of freon 112 in *n*-heptane ( $d = 1.25$ ), dialysed against 0.01 M phosphate buffer of pH 7.0 and adsorbed to columns of DEAE cellulose (Whatman, London). Elution was performed with increasing concentrations of sodium chloride (0.01, 0.05, 0.1, 0.15 M, etc.), buffered with 0.01 M phosphate buffer (pH = 7.0). Eluted material was tested for infectivity in HeLa cells, for haemagglutination with rat and (for some types) with human blood cells and for complement-fixing (CF) activity against both homologous and heterologous rabbit antisera as well as against human convalescent serum. CF tests were run with dialysed, undiluted material, a constant amount of serum (4–8 antibody units) and graded amounts of complement<sup>7</sup>.

The results are summarized in Table 1. It can be seen that the elution patterns are fairly uniform for all types of this group with only minor differences. Infectivity was found in 2–3, occasionally in more successive elution fractions, while the other antigens were mostly demon-

Table 1. RESULTS OF DEAE CHROMATOGRAPHY WITH ADENOVIRUSES OF ROSEN'S GROUP II AND WITH TYPE 5

Virus type	Elution of viral activities at indicated NaCl molarity		Infectivity
	Complement-fixing antigen	Haemagglutinin for Rat blood cells Human blood cells	
9	0.15	0.2	0.25
10	0.1	0.15	0.2
13	0.05 to 0.1	0.15	0.15
19	0.15	0.15	0.25
26	0.1	0.2	0.2
27	0.1	0.15	0.2
15	0.1	0.15	0.2
17	0.1	0.15	0.25
22	0.05 to 0.25	0.15	0.25
23	0.1	0.15	0.15
24	0.05 to 0.2	0.15	0.25
5	I: 0.01 to 0.15 II: 0.2 to 0.3	0.01 to 0.1 —	0.3 to 1.0

strated in two successive fractions only, for example, in 0.15 and 0.2 M NaCl (the first active fraction is listed in Table 1). CF activity was mostly eluted at 0.1 M NaCl, and identical reactions were found with both homologous and heterologous rabbit immune sera. Next the haemagglutinins were found in 0.15–0.2 M NaCl fractions, mostly overlapping with both CF antigen and infectivity. There was no difference in the elution pattern of haemagglutinins for rat or human blood cells. At 0.2–0.25 M NaCl infectious virus was eluted, which in most instances could be clearly separated from CF antigen, but not, or only partially, from haemagglutinin. In tests with types 22 and 26 a second peak of CF activity, eluting at higher molarities, was observed, which, however, was too low to be considered as definite. The failure to isolate a type-specific CF antigen may be due to the low antigenic strength of the material. Types 22 and 24 tended to exhibit a broader elution pattern of CF antigen than the other types.

The total quantity of viral activities eluted from the DEAE columns was 75–100 per cent of the original material for haemagglutinin, approximately 50 per cent for CF antigen, and was not determined for infectious virus.

It may be seen from the last row in Table 1 that the elution behaviour of type 5 was entirely different from that of all types of group II in all three measured activities. In contrast to the three antigens observed by Klemperer and Pereira<sup>1</sup> we found only two CF antigens, the first of type, the second of group, specificity. This discrepancy is probably due to the lower pH used by the British authors<sup>1,2</sup>. The elution of infectious virus from DEAE columns, as observed for type 8 by Haruna *et al.*<sup>4</sup> and for types 9, 10 and 15 by Zhdanov and Mekler<sup>5</sup>, corresponds well with our results. On the other hand, the notes of the Russian authors<sup>5</sup> on CF antigen and haemagglutinin are too scanty to be compared with our findings.

In a separate investigation<sup>8</sup> it was shown that group II adenoviruses fall into two sub-groups: the haemagglutinin of types 13, 26, and possibly type 23 can be separated completely from the virion by density gradient centrifugation or by adsorption to red cells. In the remaining types (9, 10, 15, 17, 19, 22, 24, 27) a virus-associated and a 'soluble' haemagglutinin is present. It is noteworthy that in the chromatographic investigation no separation of haemagglutinin and infectious virus was possible for the first sub-group (types 13, 26 and 23, Table 1), in which haemagglutinin and virion appear to have similar surface properties. On the other hand, in the second sub-group the soluble haemagglutinin was always eluted first and then virus plus virus-associated haemagglutinin. In some experiments virus fractions eluting at higher molarities were obtained without haemagglutination activity, probably because haemagglutinin was below detectable level.

Disregarding this difference, the close relationship of the virus types of group II, as indicated in Table 1, is just as striking as their dissimilarity to virus types of other adenovirus sub-groups, for example, type 5.

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## ANTHROPOLOGY

### Sequence and Development of Mousterian Traditions in South-western France

THE cave and rock-shelter sites of south-western France have produced an exceptional wealth of cultural remains dating from the earlier part of the last glaciation. Much of the interest of these 'Mousterian' flint industries derives from the fact that they have frequently been found in association with neanderthal skeletal material, the evolutionary position of which is still far from settled. During the past fifteen years these assemblages have been the object of an intensive re-investigation by Prof. F. Bordes, and for this he has devised a standardized typological classification for comparing the numerous assemblages in quantitative terms. The statistical analyses provided by Bordes *et al.* have permitted the first detailed and objective comparison of French Mousterian industries, and as a result Bordes has identified five main groupings within the assemblages. These have been termed the 'Mousterian of Acheulian tradition', 'Quina', 'Ferrassie', 'Typical' and 'Denticulate' variants<sup>1</sup>.

By analogy with the Upper Palaeolithic industries of the same region one might expect that each of these variants would occur in a consistent stratigraphical position with respect to the others—in other words, occupy a specific time horizon within this region. As yet, however, no one has put forward such an interpretation and the implication is rather that the different variants represent largely contemporaneous traditions which co-existed within this area throughout much of early Würm. The purpose of the present article is to suggest that some at least of the typological patterns observed by Bordes can, in fact, be explained in more simple chronological terms and that, within the rockshelter sites of this region at least, three of the entities listed above may well be clearly separated chronologically and occur in a consistent stratigraphical order. The three variants in question are the Ferrassie, Quina and Mousterian of Acheulian tradition types, and the principal evidence for placing them in that order chronologically is discussed here briefly.

The main argument for placing the Mousterian of Acheulian tradition after the Quina variant depends on at least ten sites where characteristic cordiform hand-axes have been found stratified above Quina assemblages (Les Merveilles, La Rochette, Combe-Capelle (Bas), Pech de L'Azé, Combe-Grenal, La Quina, Roc-en-Pail, Chez-Pourré, Hauteroche, and Le Moustier (upper shelter)). Since no site appears to have produced any clear evidence of a reversal of this order, the possibility must be admitted that most—or conceivably all—of the Mousterian of Acheulian tradition assemblages are later than the Quina industries. A further argument for isolating the Mousterian of Acheulian tradition chronologically is provided by Bordes's impressive site of Combe-Grenal where, throughout five metres of rich Mousterian deposits, no trace of hand-axe industries was found<sup>2</sup>. Taken at its face

value this clearly suggests a considerable span of time during which hand-axe makers were absent from the region. These arguments, of course, apply only to the cave and rock-shelter sites of this particular area. Much earlier hand-axe/Levallois industries are well known from open sites in northern France (for example, Le Tillet) and similar sites may eventually be found farther south.

Direct stratigraphical evidence for the relationship between Quina and Ferrassie industries is more scanty but apparently consistent. At Combe-Grenal nine Quina layers were stratified above six Ferrassie levels; at the Abri Chadourne a single Quina layer was found above two Ferrassie horizons, and a similar situation probably exists at the Roc de Marsal, though full analyses for the latter site have not yet been published. The chronological separation of the Quina and Ferrassie variants is further supported by the faunal associations of the industries, which suggest much colder conditions during the time of the Quina than during the time of the Ferrassie occupation. Thus while Quina industries are generally associated with faunas in which reindeer remains are dominant (Merveilles, Hauteroche, La Quina, La Chapelle-aux-Saints, Roc-en-Pail, Roc de Marsal, Le Regourdou, Le Moustier (upper shelter)) the Ferrassie horizons of La Ferrassie (large and small abris), L'Ermitage and Les Cottés produced mainly horse and bovid remains. Relatively mild conditions at the time of the Mousterian of Acheulian tradition occupation are suggested both by the faunas (in which bovid or even red deer remains are generally dominant) and by the frequency of open air sites belonging to this tradition.

Apart from the question of the succession of the major variants discussed above, an equally interesting issue concerns the possibility of tracing any typological development which occurred within these traditions during their occupation of the Périgord region. As Bordes has pointed out, considerable typological variations exist among, for example, the various Mousterian of Acheulian tradition assemblages, and the question arises how far these variations can also be explained in chronological terms. Approaching this question initially from a purely typological point of view, a series of histograms (Fig. 1) was constructed in an attempt to arrange the various assemblages in their correct chronological order. One set of diagrams comprises the various hand-axe assemblages and in the other set the Quina and Ferrassie assemblages have been considered together. Each histogram represents one typological feature of the assemblages and in each diagram the assemblages are arranged in the same order, the suggested chronological order, from left to right.

That, with the sites arranged in this order, each diagram shows a fairly clear overall pattern or trend can scarcely be disputed and an obvious inference is that these represent the actual patterns of technological change which took place within the traditions during their occupation of the region. With regard to the Quina and Ferrassie assemblages the implication is that one develops directly into the other, thus supporting the stratigraphical and climatic evidence for the chronological separation of these two variants discussed earlier.

On the typological evidence alone, of course, the chronological interpretation of these seriation patterns is disputable. The most convincing support for such interpretations comes from sites where two or more assemblages of the same tradition have been found in a stratified succession. While fully published sites of this type are rare, the evidence from them is very satisfactory. Thus for the Ferrassie/Quina tradition the postulated trends can be checked at the Abri Chadourne and La Ferrassie (three levels each) and for the Mousterian of Acheulian tradition at Gare de Couze and Pech de L'Azé (two principal levels each). In addition to the features considered in the histograms, it is also very likely that the development of the Ferrassie/Quina tradition was characterized equally by a gradual decrease in the Levallois index. In the two three-



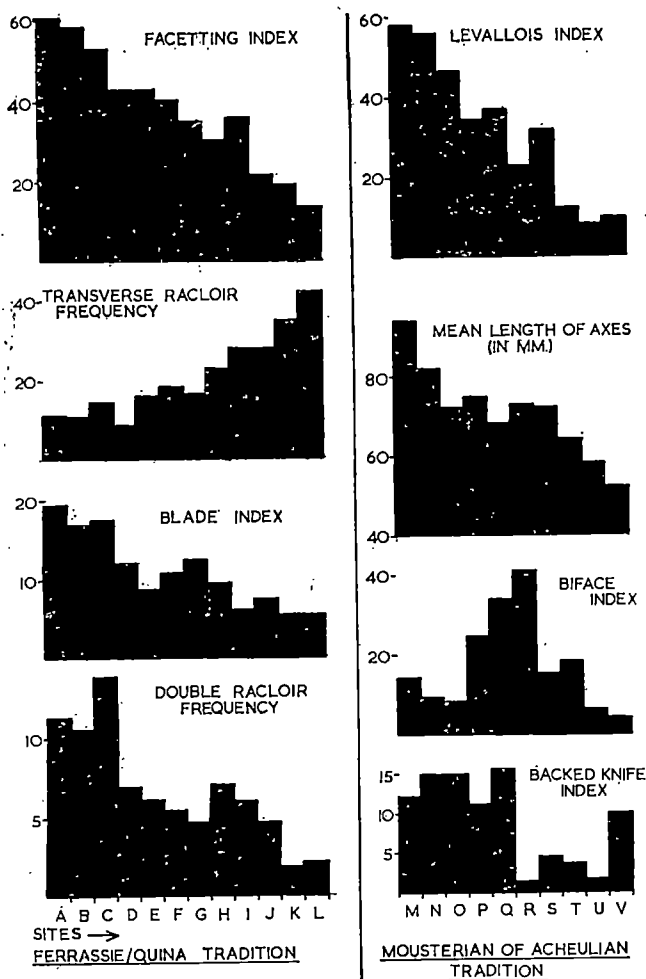


Fig. 1. Seriation diagrams for the Ferrassie/Quina tradition (left) and Mousterian of Acheulian tradition (right) in south-western France. In each histogram the assemblages are arranged in the same order—the suggested chronological order—from left to right. For the Ferrassie/Quina tradition the assemblages are: (1) L'Ermitage; (2) La Ferrassie (layer C); (3) La Ferrassie (D1); (4) Abri Chadbourne (D); (5) La Ferrassie (D2); (6) La Ferrassie, small abri; (7) Abri Chadourne (C); (8) Abri Chadourne (B); (9) Mas Viel; (10) Hauteroche; (11) La Rochette; (12) Les Merveilles. For the Mousterian of Acheulian tradition the assemblages are: (13) Abri Blanchard; (14) Gare de Couze (lower layer); (15) Gare de Couze (upper layer); (16) Combe Capelle Haute; (17) Le Mustier (layer G.); (18) Fontmaure; (19) Combe Capelle Bas; (20) Sandougnie; (21) Pech de L'Aze (lower layers); (22) Pech de L'Aze (upper layers). The tool frequencies and indices are taken from analyses according to Bordes's method. (N.B. The frequency of transverse racloirs is expressed as a percentage of all simple racloirs and not as a percentage of the total assemblage)

level sites quoted here a steady decrease in this index was clearly recorded and Bordes has stated that the same trend occurred throughout the fifteen Ferrassie and Quina layers of Combe-Grenal<sup>3</sup>. Unfortunately, the fact that this index can be considerably raised by selective collecting of unretouched flakes makes it impossible to compare directly collections from old and modern excavations on the same diagram.

The general issues of the sequence and development of early Würmian industries in south-western France discussed here are of interest from several points of view. The apparently early position of the Quina and Ferrassie industries—possibly corresponding with the post Brørup cold interval—is interesting in view of the frequency with which these assemblages have been found in association with neanderthal skeletal remains (La Quina, La Ferrassie, La Chapelle-aux-Saints, Le Regourdou, Combe-Grenal and probably Roc de Marsal). In this connexion, Brace stressed that one of the major difficulties in assessing the relationship between neanderthal and sapient forms was the lack of any clear idea of even the relative chronology

of many of the relevant neanderthal remains<sup>4</sup>. Secondly, the apparently late position of the Mousterian of Acheulian tradition lends support to Bordes's view that it was from these assemblages that the earliest Upper Palaeolithic industry of south-western France (the Chatelperronian or Perigordian 1) developed.

Of more general interest are the prospects for applying quantitative seriation methods of the type used here to flint assemblages of other periods. Similar methods—applied to collections of potsherds—have been used for some time in the New World and are useful not only for helping to construct detailed relative chronologies of sites within a limited region but also as a means of approaching the very difficult questions of the continuity or otherwise of cultural traditions in the earlier periods.

This work was supported by a Ministry of Education State studentship award.

I thank Dr. C. B. M. McBurney and Prof. J. G. D. Clark for their advice. I also thank Prof. F. Bordes, University of Bordeaux, for generous help during two visits to France.

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<sup>1</sup> Bordes, F., *L'Anthropologie*, 54, 19 (1950); *Arch. de l'Institut de Paléontologie Humaine, Mém.* 27 (1957); *Publications de l'Institut de Préhistoire de l'Université de Bordeaux, Mém.* 1 (1961); *Science*, 134, 803 (1961).

<sup>2</sup> Bordes, F., *Gallia-Préhistoire*, 4, 233 (1961).

<sup>3</sup> Bordes, F., Fitte, P., and Blanc, S., *Bull. Soc. Préhistorique Française*, 51, 229 (1954).

<sup>4</sup> Brace, C. Loring, *Current Anthropol.*, 5, 3 (1964).

## PSYCHOLOGY

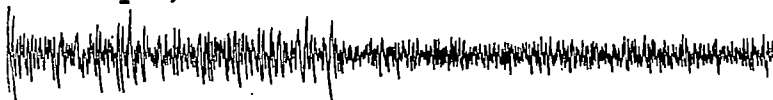
### Role of the Olfactory System in Arousal to X-ray

It has been reliably demonstrated that rats can discriminate between the presence or absence of X-rays. Responses with latencies of less than 0.2 sec have been observed on X-rays delivered at 0.05 r./sec, where the X-ray served as the conditioned stimulus. X-rays have also served as arousing stimuli, waking sleeping or drowsy animals within 2 sec of the onset of exposure. Since these responses have been observed in ophthalmectomized animals, they are not the result of the well-known effect of ionizing radiation on the retina<sup>1-3</sup>.

The process by which X-rays elicit arousal and orienting reactions in mammals has not yet been determined. However, for simplicity we assume this mechanism operates via a 'radiation receptor'. Attempts to locate this hypothetical radiation receptor have yielded conflicting results. Some recent investigations of behavioural arousal and electroencephalographic desynchronization to X-ray have indicated a rather diffuse receptor system dispersed throughout the entire body, as well as the head of the animal<sup>4,5</sup>.

In our laboratory, on the other hand, investigations of instrumental conditioned responses to X-ray have indicated that the radiation receptor is located in the anterior region of the head and that the body of the animal is relatively insensitive to the immediate stimulating effect of X-rays<sup>6</sup>. We used a narrow 3/16-in. X-ray beam as a signal or conditioned stimulus to warn the animal of a subsequent shock to the paws. The beam was most effective when it was directed at the olfactory region of the head.

One difference between the conflicting investigations so far cited here is in the response measure used. In our work, localizing the receptor in the olfactory region, we used X-ray as a conditioned stimulus. In the work reporting more diffuse reception, the X-ray was used as an arousing stimulus. In an attempt to clarify this issue, we conducted a study of the effectiveness of X-ray as an arousing stimulus in rats the olfactory bulbs of which had been removed. De-synchronization of a previously syn-

EEG - [50  $\mu$ V

MOTOR ACTIVITY



X-RAY

2 sec

Fig. 1

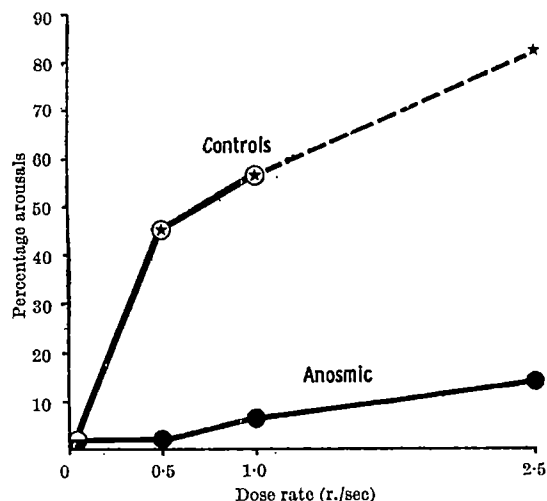


Fig. 2. Percentage of arousal as a function of dose rate. Points are based on groups of 10-14 rats each receiving 4 (5-sec) exposures. The extrapolated control point is based on similar tests of 2 rats

chronized electroencephalogram (EEG) within 2 sec following the onset of the X-ray was the criterion of effective arousal. The EEG was recorded from epidural silver-silver chloride electrodes permanently implanted on the cortex of albino rats. The olfactory bulbs were removed by aspiration. Two types of control animals were prepared. In one, the cerebrum extending from bregma to the anterior tip of the hemispheres, approximately 4 mm deep block, was removed bilaterally. A small continuous portion of the midline was left intact. A second control group consisted of intact animals. Both groups were implanted with recording electrodes.

One to three weeks after surgery, all animals were first tested for sensitivity to X-ray. For radiation exposure, the animals were confined to a small plastic chamber enclosed in a larger acoustically and electrically shielded wooden box. The floor of the box was mounted on springs. The movements of the animal were converted into an electrical signal by a crystal pickup and recorded on an EEG channel. The interior of the box was well lighted and equipped with a speaker emitting a continuous wide-band masking noise. The box was placed so that the plastic chamber was directly under the X-ray machine (250 kV; 0.5 mm copper filter, 1.7 mm copper half-value layer). Output was measured by a Victoreen chamber placed in the plastic chamber. The technique for exposure of the animal to X-ray has been previously described<sup>2</sup>. It permits all other potential stimuli to be held constant during exposure to X-rays. In addition, all animals were tested with sham exposures identical to X-ray exposures except that a lead plate, inserted in place of the copper filter in the X-ray machine, reduced the radiation dose to zero. All sham and X-ray exposures were of 5-sec duration. Each animal was given 4 sham

and 4 X-ray exposures at each dose rate. Some experimental and control animals were tested with more than one dose rate. In such cases more than a week elapsed between the two test series.

X-ray exposure which produced a prompt arousal (Fig. 1) in control animals had little effect on the animals without olfactory bulbs. Fig. 2 summarizes the results. The frequency of animals exhibiting one or more arousals was statistically greater ( $P < 0.01$ ) for control groups than for the comparable experimental groups at the 0.5 r. and 1.0 r./sec dose rates. Since the lower dose rates (0.5, 1.0 r./sec) produced a high proportion of arousals in the control animals, a control group at the highest

dose rate (2.5 r./sec) appeared superfluous. Two animals tested at 2.5 r./sec exhibited intense reactions to the increased dose rate as was expected. The highest dose rate (2.5 r./sec) appeared to produce a slightly greater number of arousals in the anosmic animals as well (Fig. 2).

The results indicate a distinct loss of sensitivity when the olfactory bulbs are removed. This agrees with our previous work<sup>4</sup> in which a collimated X-ray beam was used as a conditioned stimulus to demonstrate a maximum sensitivity in the region of the olfactory bulbs. The two methods agree in locating the radiation receptor in the olfactory system. At present we are unable to reconcile our results with reports that both the head and body are sensitive to X-ray at the dose-levels we have reported here. Our control animals with relatively large cerebral lesions were all able to detect X-rays. On the other hand, animals with olfactory lobectomies, while insensitive to X-rays, appeared to arouse as readily to auditory stimuli as did controls. This makes it unlikely that a generally decreased sensitivity due to damage to the nervous system can explain the data.

While the 'specifics' of X-ray detection remain to be determined, the effect does not appear to depend on the smelling of ozone. When the collimated beam was directed through the nasal and oral passages anterior to the olfactory bulb, it was relatively ineffective. (Note added in proof. Five rats with chronic tracheotomies, which prevented nasal inhalation of ozone or any other radiation by-product, all aroused to X-ray.)

In the work recorded here the number of arousals in the anosmic animals was small, but there is an apparent correlation with intensity or dose rate. This may indicate that our animals were not completely anosmic, or it may mean that other receptor systems were stimulated. It seems plausible that other receptor systems could be activated if higher dose rates and longer durations were used.

This work was supported by U.S. Public Health Service grant RH-00068.

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\* Recipient of a U.S. Public Health Service award (GMK 3509).

<sup>1</sup> Garcia, J., Buchwald, N. A., Feder, B. H., and Koelling, R. A., *Nature*, 196, 1014 (1962).

<sup>2</sup> Garcia, J., Buchwald, N. A., Bach-y-Rita, B., Feder, B. H., and Koelling, R. A., *Science*, 140, 289 (1963).

<sup>3</sup> Garcia, J., Buchwald, N. A., Feder, B. H., Koelling, R. A., and Tedrow, L. H., *Second Intern. Symp. Response of the Nervous System to Ionizing Radiation*, edit. by Haley, T. J., and Snyder, R. S. (in the press).

<sup>4</sup> Hunt, E. L., and Kimeldorf, D. J., *Nature*, 197, 536 (1963).

<sup>5</sup> Cooper, G. P., and Kimeldorf, D. J., *Science*, 141, 1040 (1964).

<sup>6</sup> Garcia, J., Buchwald, N. A., Feder, B. H., Koelling, R. A., and Tedrow, L. H., *Science*, 141, 1470 (1964).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 8

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. B. Ackner: "Organic Mental Syndromes".\*

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. Bondi, F.R.S.: "The Disproof of Theories".

UNIVERSITY OF LONDON (in the Physiology Department Theatre, St. Bartholomew's Hospital Medical College, West Smithfield, London, E.C.1), at 5.30 p.m.—Prof. K. J. Ullrich: "Characteristics of Renal NaCl-transport, and its Modification by Aldosterone and Saluretic Sulphonamides".\*

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. B. W. Leyland: "Earth Return Applied to High Voltage Systems".

ROYAL AERONAUTICAL SOCIETY, HISTORICAL GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Sqn./Ldr. S. C. Winfield Smith: "British Aviation Fifty Years Ago".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. J. B. Ward Perkins, C.B.E.: "The Changing Historical Landscape of Italy".

## Monday, February 8—Tuesday, February 9

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2)—Conference on "Electronics Design".

## Tuesday, February 9

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Effect of Variations in Supply Voltages in Industrial Processes" opened by Mr. J. A. Tatchell and Mr. H. J. Sheppard.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Society of Tropical Medicine, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. A. P. Riding: "Rubber Products used in the Printing Industry". 7 p.m.—Mr. V. N. Chatterton: "New Application Developments with Viton Fluoroelastomers".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. W. Harris: "Central Nervous Regulation of the Pituitary Gland". (Eighth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. J. E. Robinson: "The Design and Development of Pistons for Automobile Engines".

ROYAL AERONAUTICAL SOCIETY, ASTRONAUTICS AND GUIDED FLIGHT SECTION (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. J. G. Walker: "Communications Satellites".

ROYAL SOCIETY OF MEDICINE, PSYCHIATRY SECTION (at 1 Wimpole Street, London, W.1), at 8 p.m.—Meeting on "The Assessment of Methods of Psychological Treatment". Speakers: Dr. D. H. Malan and Dr. M. G. Gelder.

## Wednesday, February 10

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Prof. G. V. R. Born: "Platelet Aggregation".\*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. E. V. D. Glazier: "Radar—Present Position and Future Trends".

UNIVERSITY OF LONDON (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 5.30 p.m.—Prof. D. J. Anderson: "Science in Dentistry". (First of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)\*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E./I.E.R.E. MEDICAL ELECTRONICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Discussion on "Safety of Operating Theatre Equipment".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. K. Saxl: "The Pendulum Mill—a New Method of Rolling Metals".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. R. W. G. Hunt: "Recent Developments in Colour Photography" (Peter Le Neve Foster Lecture).

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Mr. F. I. L. Knowles and Mr. D. N. Gascoyne: "Measurement of Transient Events in Armaments Research".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP AND NUTRITION PANEL (Food Group) (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. G. D. Rosen: "The Microbiological Assay of Protein Quality".

OFFICE OF HEALTH ECONOMICS (at the Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1), at 8.30 p.m.—Mr. G. Teeling-Smith: "The Role of Marketing in Scientific Progress".\*

## Thursday, February 11

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion Meeting on "Pion-Nucleon Scattering and Excited Nucleon States" organized by Prof. R. H. Dalitz, F.R.S.

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. K. Vickerman: "Developing Africa; the Biologist's Dilemma".\*

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Operational Research as Applied to Transportation" introduced by Mr. P. I. Welding.

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Electrometer Amplifier—Its Design and Applications" opened by Mr. G. I. Hitchcox and Mr. G. R. Taylor.

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. W. Feldberg, F.R.S.: "A New Concept of Temperature Regulation in the Hypothalamus".\*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. S. Shaldon: "Long-Term Haemodialysis as a Substitute for Kidney Function". (Ninth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

UNIVERSITY OF LONDON (in the Physiology Department Theatre, St. Bartholomew's Hospital Medical College, West Smithfield, London, E.C.1), at 5.30 p.m.—Prof. K. J. Ullrich: "Micropuncture Studies of Salivary and Sweat Glands".\*

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Should there be Less Specialization in University Courses?".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at "The Feathers" Restaurant, Tudor Street, London, E.C.4), at 6.45 p.m.—Mr. B. Collier: "Methods of Estimating Activities of Commercial Enzymes".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION, KENT SUB-SECTION (at the Bull Hotel, Rochester, Kent), at 7 p.m.—Mr. K. C. Bryant: "Polymers from Petroleum".

## Friday, February 12

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.50 a.m.—Symposium on "Soil Sterilization".

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Captain C. J. Wennink: "Problems of Ice Navigation with Modern Tankers".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Use and Abuse of Ultra-High-Strength Steels".

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at "The Feathers Inn", 34 Tudor Street, London, E.C.4), at 7 p.m.—Discussion Meeting devoted to Professional Affairs.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Sir John Cass College Chemical Society, at Sir John Cass College, Jewry Street, London, E.C.3), at 7 p.m.—Prof. J. Chatt, F.R.S.: "Stabilization of Low Valent States of the Transition Metals".

ROYAL INSTITUTE (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. A. F. Huxley, F.R.S.: "Muscle Fibres Under the Microscope".

## Monday, February 15

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 3 p.m.—Colloquium on "Design of Solid State Power Supplies".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. A. J. Clarke: "Hatching Agents for Cyst-Forming Nematodes".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER in PURE or APPLIED MATHEMATICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (February 13).

SENIOR LECTURER in MAMMALIAN PHYSIOLOGY in the DEPARTMENT of BIOLOGICAL STUDIES—The Principal, Lanchester College of Technology, Priory Street, Coventry (February 15).

LECTURER (specializing in statistical or solid state theory) in THEORETICAL PHYSICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (February 19).

ASSISTANT LECTURER (with a good honours degree in electrical engineering or physics, with special interests in control, materials or communications) in the DEPARTMENT of ELECTRICAL ENGINEERING—The Secretary, The University, Edinburgh (February 20).

LECTURER in ASTRONOMY—The Registrar, The University, Leicester (February 20).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT of APPLIED MATHEMATICS—The Registrar, The University, Liverpool, quoting Ref. CV/376 (February 20).

LECTURER or ASSISTANT LECTURER in EXPERIMENTAL PHYSICS, and a LECTURER or ASSISTANT LECTURER in THEORETICAL PHYSICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (February 22).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT of MICROBIOLOGY, and an ASSISTANT LECTURER (preferably with experience in spectroscopy, X-ray diffraction or electron microscopy) in the DEPARTMENT of PHYSICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (February 22).

LECTURER (physicist or physical chemist) in the DEPARTMENT of PHYSICS to assist with the teaching of the degree course in the physical properties of materials—The Registrar (Room 22, O.R.B.), The University, Reading (February 22).

ASSISTANT LECTURER in the DEPARTMENT of GEOGRAPHY—The Assistant Secretary, London School of Economics and Political Science, Houghton Street, London, W.C.2 (February 25).

ASSISTANT LECTURER or LECTURER in PSYCHOLOGY—The Registrar (Room 22, O.R.B.), The University, Reading (February 27).



**ELECTRON MICROSCOPIST** (with a first- or good second-class honours degree in chemistry or physics with post-graduate research experience in X-ray or electron diffraction, a knowledge of diffraction theory as applied to structural crystallography, and preferably some familiarity with biological applications of electron microscopy) IN THE DEPARTMENT OF PEDOLOGY—The Secretary, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (February 27).

**GEORGE HOLT CHAIR OF PATHOLOGY**—The Registrar, The University, Liverpool (February 27).

**LECTURER** (with field work experience) IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY—The Secretary, The University, Edinburgh (February 28).

**CHAIR OF APPLIED MATHEMATICS**—The Registrar, The University, Sheffield (March 1).

**CHAIR OF MATHEMATICS**—The Registrar, The College of Aeronautics, Cranfield, Bedford (March 1).

**CHAIR OF PSYCHOLOGY**—The Registrar, University College of Swansea, Singleton Park, Swansea (March 1).

**LECTURER IN THE DEPARTMENT OF BIOLOGICAL CHEMISTRY**—The Secretary, The University, Aberdeen (March 1).

**RESEARCH FELLOW** (with a degree in soil science or agriculture with some postgraduate experience, preferably in tropical soils) IN THE DEPARTMENT OF CROP HUSBANDRY AND HORTICULTURE, University of Ghana—The Assistant Registrar, University of Ghana Overseas Office, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box 25, Legon, Accra, Ghana (March 3).

**LECTURER IN ORGANIC CHEMISTRY**—The Secretary, The University, Aberdeen (March 6).

**READER IN ENGINEERING GEOLOGY**—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 7).

**CHAIR OF STRUCTURAL ENGINEERING WITHIN THE DEPARTMENT OF CIVIL ENGINEERING**—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (March 12).

**CHAIR OF STATISTICS**—The Registrar, University of Kent at Canterbury, Westgate House, Canterbury (March 15).

**LECTURER IN ZOOLOGY**—The Secretary, The University, Aberdeen, Scotland (March 15).

**LECTURER** (preferably with a knowledge of physiology) IN PHYSIOLOGICAL CHEMISTRY with special reference to Biochemistry; and a LECTURER IN PHYSIOLOGICAL CHEMISTRY with special reference to Physiology—The Registrar (Room 22, O.R.B.), The University, Reading (March 16).

**ASSISTANT LECTURER** (with a good honours degree) IN THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1.

**LIBRARIAN** (Chartered Librarian, with a working knowledge of at least one foreign language, and preferably experience of scientific literature) at Long Ashton Research Station—The Secretary, Research Station (University of Bristol), Long Ashton, Bristol.

**MASTER** (able to teach to university scholarship standard) FOR MATHEMATICS—The Rector, The Edinburgh Academy, Edinburgh, 3.

**MATHEMATICIAN** (qualified to teach to university scholarship level)—The Head Master, Bedford School, Bedford.

**RADCLIFFE-HENRY SKYNNER FELLOW IN ASTRONOMY**—The College Secretary, Balliol College, Oxford.

**SENIOR RESEARCH ASSOCIATE IN INORGANIC CHEMISTRY** to work on reaction mechanisms in boron chemistry under the direction of Dr. J. C. Lockhart—The Registrar, The University, Newcastle upon Tyne.

**TAXONOMIC BOTANIST** (national of the United Kingdom or the Republic of Ireland, with a B.Sc. degree, preferably with some postgraduate practical experience) with the East African Common Services Organization, for duties at the East African Herbarium—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/214/02.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Proceedings of the University of Newcastle upon Tyne Philosophical Society. Vol. 1, No. 1: Musical Sand—The Singing Sands of the Seashore, Part 2. By A. E. Brown, W. A. Campbell, J. M. Jones and E. R. Thomas. Pp. 1-17. Vol. 1, No. 2: Some Demonstrations with Polarised Light. By W. E. Curtis. Pp. 18-23. (Newcastle upon Tyne: The University Philosophical Society, 1964.) [2511]

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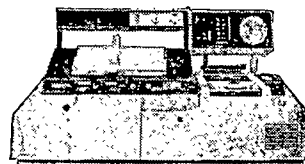
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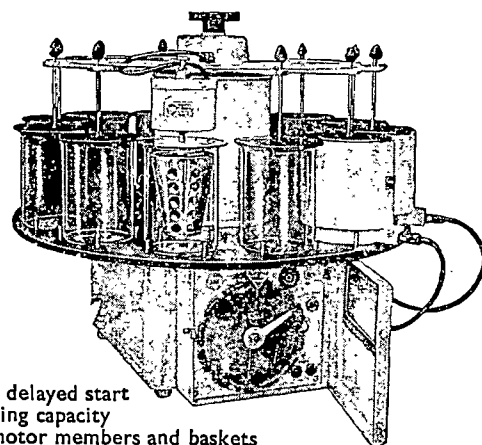


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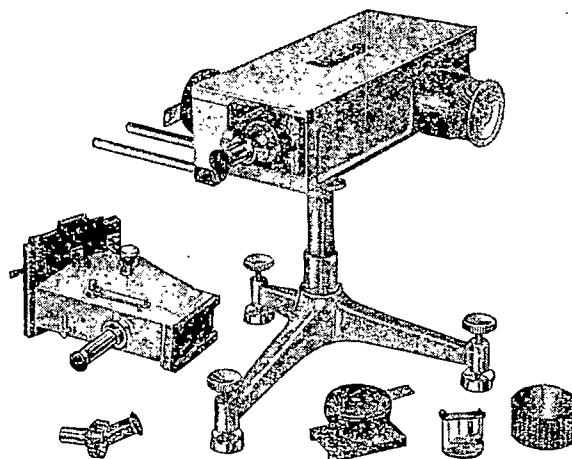
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## EFFECTIVE NATURE CONSERVATION IN BRITAIN

THE fifteenth annual report of the Nature Conservancy\*, covering the year ended September 30, 1964, like the fourteenth annual report, displays not merely the increasing range of scientific research for which the Conservancy is now responsible, but also the growing extent to which, through its conservation work and also through its scientific advisory service, the Conservancy contributes to the development and utilization of Britain's natural resources. The report, in fact, demonstrates even more clearly how these three sections of the Conservancy's work are related, and for this reason alone the report is of special significance in relation to the proposals which the present Government has formulated for a Ministry of Land and Natural Resources and a Natural Environment Research Council. The Conservancy has always recognized that conservation is too large a task to be undertaken by itself alone, and the present report welcomes the creation of a wider Natural Environment Research Council as the logical outcome of the Conservancy's pioneer work, expressing the hope that the essentially scientific problems arising from the impact of modern man on his natural environment may now be tackled before it is too late.

The Conservancy, however, expressed these views in the light of the statement made to the House of Commons on July 28, 1964, by the Secretary of State for Education and Science, then Mr. Quintin Hogg, regarding the formation of a Natural Environment Research Council with responsibility for the Geological Survey and Museums, the Soil Surveys, the Nature Conservancy, the Hydrology Research Unit and the Committee on Hydrological Research. It would take over, from the Admiralty and the Development Commission, responsibility for the National Institute of Oceanography. It would also establish a Fisheries Advisory Committee and would support research in geophysics, geology, oceanography, fisheries, hydrology, forestry, terrestrial ecology, and Nature conservation as well as research in geomagnetism and seismology, now undertaken by the Meteorological Office. The Nature Conservancy recognizes that years of patient and statesmanlike effort would be required to generate a sense of common purpose and mutual aid among the diverse elements in the life and earth sciences, both terrestrial and aquatic, which were designated for inclusion in the Natural Environment Research Council. That is no less true of the Natural Environment Research Council as proposed by the present Government, which is to take over the functions of the Nature Conservancy and of the National Oceanographic Council and will be responsible for research in the Earth sciences and ecology, including the establishment, maintenance and management of Nature reserves.

What is not yet clear under the new arrangements is where the responsibility lies for conservation—the importance of which is pre-eminent in the Conservancy's present report. The Minister of Land and Natural Resources will now be responsible for the National Parks Commission, for Commons land and the Forestry Commission as well as for the conservation of water, with which the Nature Conservancy has been increasingly

concerned. It is difficult to see how the Conservancy can effectively discharge these duties under the proposed arrangements, and its conservation work is scarcely distinguishable from that required generally for the availability and utilization of natural resources for which the Minister of Land and Natural Resources is now to be responsible.

When the Nature Conservancy writes in the present report that the Natural Resources Research Council would face very real difficulties, it could scarcely have visualized difficulties arising out of such divided responsibilities, nor would it have been so confident that those in charge would pay sufficient attention to the lessons to be learned from past experience to overcome those difficulties and succeed. By and large, it seems that those lessons have been deliberately ignored. It is difficult to share the confidence which led the Conservancy to give full support to the proposal formulated last July and expect a more rapid advance on a much broader front under the new Research Council. On the contrary, unless extreme care is taken in working out the details of the new arrangement, and unless they are administered with vision and clear understanding of the needs of science and conservancy, and those interests are safeguarded with real determination against sectional intrusions, frustration, waste and disappointment are all too probable.

The Conservancy's own anxieties on such matters are reflected in the report without altogether offsetting the confidence with which, at that time, it looked to the future. Once the transitional difficulties were over it hoped that the enlarged teams, setting aside all sectional prejudices, would settle down to their task so that the larger conception, which has now emerged, will prevail. The Conservancy has no illusions about the difficulties and dangers, and it is to be hoped that the Secretary of State for Education and Science, to whom the report is presented, as well as the Minister of Land and Natural Resources, will take due notice of the warning so clearly and reasonably expressed.

The details of the report are summarized on p. 658 of this issue of *Nature*, but there are some general features calling for special comment. First, the Conservancy refers to the initiation by the International Council of Scientific Unions in Vienna in November 1963 of an International Biological Programme. This Programme, which was launched at a General Assembly in Paris in July 1964, is characterized by the Conservancy, which played a leading part in its initiation, as an outstanding event of the year. It brings the support and endorsement of world science to fundamental ecological studies and to the conservation of sites for the use of future scientists—the two principal activities of the Conservancy which it has fostered in Britain on a scale now surpassing anything of the sort elsewhere in the world. Seven sectional committees have now been set up under the Programme and it is expected that during the next two years emphasis will be placed on studying the design and feasibility of projects which will be operated, so far as they prove practicable, during the following five years.

Next, the report refers particularly to important advances made in co-ordinating the efforts and even integrating the policies of the Conservancy, the Forestry

\* Report of the Nature Conservancy for the year ended September 30, 1964. Pp. vii + 173 + 10 plates. (London: H.M.S.O., 1964.) 13s. net.



Commission and the National Parks Commission. The first two of these now manage about 0.5 per cent and 4.5 per cent, respectively, of the total acreage of Great Britain, while a further 9 per cent of the area of England and Wales is included in the National Parks. They both have important research interests, and while the Conservancy and the Parks Commission are free of responsibilities for production they have a special duty of trusteeship for the land from the point of view of amenity and of science. All three are concerned with land and with the impact of human activities on it.

New ways of acting and thinking together have been opened up by meetings between the Chairman and Chief Officers, while liaison with the Agricultural and Education Departments and with numerous semi-official or unofficial interests has also been improved. All this has severely taxed the Conservancy's small staff, but more is still needed in this direction. It remains true that the main threats still come from major Government Departments, such as the Ministry of Power, the Ministry of Transport, the Armed Forces, and the Ministry of Housing and Local Government. It is therefore disconcerting to find that the responsibility for land now rests with a Minister who does not enjoy Cabinet status. Nor is this the only complication, for it is understood that the Parliamentary Secretary to the Ministry of Public Building and Works has a special responsibility for parks and amenities.

The shortage of staff is particularly acute in conservation work, on which, in September 1964, 87 of the Conservancy's staff of 355 were engaged, 44 of these being warden naturalists or wardens. Of the total, 165 are scientifically qualified and carrying out scientific duties. The pressure on conservation work is due in part to the increase in the number of declared national Nature reserves (from 19 to 111 over the past ten years), to the wider application of the system of management under an approved scientific plan, and to the demarcation and recording of sites of special scientific interest. The establishment of the Council for Nature and the county Naturalists' Trusts has also greatly increased conservation activities, though it has sometimes been possible to hand over to the Trusts some non-statutory functions. In consequence, however, of the increasing public and Parliamentary recognition of conservation, Bills coming before Parliament dealing with land-use frequently include specific clauses placing special statutory duties on the Conservancy, as in the case of the Water Resources Act, 1963. The pressures on Britain's remaining natural and semi-natural areas of land and water have greatly increased, and apart from the conflicting claims for natural resources like gravel and peat which are changing the use of the countryside, as already noted, the increase in leisure and wealth, and ease of transport, have made much unspoilt country vulnerable.

The past year has seen a major advance in the general acceptance and application of the outlook, policies and practices of conservation. The inter-relation between the positive management of land and water and the zoning for special uses has been emphasized, notably in the study conference "The Countryside in 1970" and in the Conservancy's co-operative studies on the Broads, Purbeck, Snowdonia and the Cairngorms, which have stressed the importance of enabling Britain's natural resources of land, water and wild-life to sustain their status through suitable management. Pressures are sometimes so intense that new ideas and fresh measures are required to safeguard

the natural resources, including the features of scientific interest, which form part of the national heritage. While in general the conservation and management of a resource have much in common with preservation of amenity, conflict does sometimes arise, and the Conservancy is finding that with so much pressure it is not always easy to maintain the objective approach and cool scientific appraisal of the facts which are essential to the function of a research council.

Already the Conservancy has met the danger by ensuring not only that the regional officers and sufficient supporting staff are well-qualified and experienced in the appropriate sciences, but also that there are close relations between this small number of conservation scientists and the larger number of research workers in the Conservancy's own institutes and in the universities. One outstanding feature of the whole report is the way in which it displays the intimate inter-relation between the conservation work, the scientific advisory services, and the research work which might be regarded as the prime function of a research council. The fear that this delicate, but vital, inter-relation may be disturbed is the prime reason for regarding with misgivings the new situation created by the establishment of a Ministry of Land and Natural Resources separate from the Natural Resources Research Council.

Before leaving this question of conservation, reference should be made to the concern expressed here and in other recent reports over the increasing impact which man is having on his environment and particularly on the countryside. This derives largely from the growth and movement of population, recreation and holiday needs. In coastal areas the competition for land by holiday and outdoor recreational interests constitutes the greatest problem for conservationists, as indeed it does for the National Park authorities. For example, many areas of high scientific interest and special conservation value lie within the undeveloped parts of the East Anglian coastline or in the areas of low-density development. Moreover, at many places, such as Blakeney Point, Winterton Dunes and Minsmere, this increasing public pressure has already led to increasing disturbance to breeding birds, erosion of sand dunes and vandalism. Effective wardening is becoming more difficult and a comprehensive approach to the future use of coastal areas in Britain as a whole is urgently needed.

The Conservancy accordingly notes with approval that the National Trust is to launch a nation-wide coastal campaign to last until December 1966, entitled "Operation Neptune". This is also welcomed by the National Parks Commission in its report (see p. 659 of this issue of *Nature*), which discusses at some length the question of recreational facilities in National Parks and the problem of striking a reasonable balance. It believes with John Dower that while access and facilities for holiday-making should be amply provided, and available to the public at large, National Parks are not the place for those who want to spend their holidays gregariously. The Commission recognizes that in National Parks, in areas of outstanding natural beauty and in the countryside generally, and especially on the coast, some restrictions or prohibitions on motor traffic, the siting of caravans and camping sites, or on the establishment of holiday resorts and of marinas must be accepted. The Conservancy believes that the designation of well-chosen recreation areas and their careful development and management near towns or in the countryside away

from the National Parks might relieve pressure on the Parks.

One reason the Commission desires wider powers is to enable it to discharge such functions better, and the same policy might well assist to relieve pressure on some of the Nature reserves. What is apparent above all is that the problem is one problem for all its wide implications and demand for co-operative effort, and that there must be a single, but adequate, authority to enforce an agreed policy. The hope of the National Parks Commission that in the present Parliament new measures, framed on imaginative and forward-looking lines, to ensure the due protection and proper use of the countryside in the face of mounting demands, will receive high priority in the legislative programme is one that the Nature Conservancy may well endorse.

Two other features of the Conservancy's report call for comment, and both illustrate the close inter-relation of its research work and the scientific advisory services and the importance of close co-operation with other interests. First, the Conservancy returns at some length to the risks to wild-life arising from the use of persistent organochlorine pesticides. The evidence submitted on behalf of the Conservancy to the Advisory Committee on Poisonous Substances used in Agriculture and Food Storage was based on investigations carried out by the Toxic Chemicals and Wild Life Section on organochlorine insecticides residues in wild birds, eggs and mammals and of the peregrine falcon and golden eagle.

The main conclusions of the Conservancy's evidence were that contamination is widespread and is not confined to areas sprayed, freshwater and marine habitats being particularly affected. Birds which feed on vertebrate prey contain higher residues than do insectivores, omnivores and herbivores, and the eggs of predatory birds contain larger amounts of insecticides than do the eggs of other species. The persistent chemicals now in use appear to offer a much more serious threat to wild-life than do less persistent chemicals, both because they build up in natural systems and because they have sub-lethal effects on reproduction. However carefully applied, even at rates well below those likely to cause direct poisoning, they may endanger wild-life. Total contamination of an environment by persistent chemicals from all sources is a factor which must be considered in the control of chemicals. Because their build-up in the field cannot be predicted adequately in the laboratory, persistent chemicals, in the existing state of knowledge, cannot be adequately controlled. The hypothesis that the declines in population and reproductive capacity of the peregrine falcon and of the reproductive capacity of the golden eagle are due to lethal and/or sub-lethal poisoning by organochlorine insecticides is well supported.

Many of the conclusions and recommendations of the Conservancy were accepted by the Advisory Committee, and after publication of the first part of the Committee's report in March 1964, the Minister of Agriculture announced the Government's decision to give full effect to the recommendations and that assurances of co-operation in curtailing the use of these chemicals had been given by all the interests concerned. The Government had also asked the Committee to examine the present voluntary safety measures and to consider whether any improvement or extensions of these would be desirable. Its terms of reference have also been extended to include the use of these organochlorine chemicals for wood preservation and mothproofing. In future, the Committee will primarily be

responsible to the Ministry of Education and Science. The Frazer Committee, which dealt with toxic chemical research, also reported in March 1964, and the report included specific recommendations directing the attention of the Conservancy to the need for further ecological investigations on whole populations of chosen species; for examining the practicability of controlling a land area of about 1,000 acres so as to permit quantitative studies on the effects of pesticides on wild bird populations; and for collaboration with other bodies in investigating contamination of fresh waters by pesticides, their accumulation in animals and the faunal and floral changes in inland waters.

These recommendations are being implemented by the Conservancy "as fully as present resources permit", but it is obvious that all these developments call for greater resources and more staff. That same remark appears in connexion with the pollution of air and fresh water, for although some cases of pollution of rivers and of land from aerial discharges of industrial effluvia affecting the scientific interest were brought to the attention of the Conservancy during the year, these aspects of environmental contamination await further resources before they can be examined fully. The second matter is that of oil pollution of the sea, and while this will remain serious until the recent amendments to the 1954 International Convention for the Prevention of Pollution of the Sea by Oil are accepted and followed by the 24 countries which had ratified the Convention by the end of 1963, the Conservancy's report notes a striking development in this field announced in June 1964.

Shell International Marine, Ltd., and the British Petroleum Tanker Co., Ltd., have devised and tested new techniques which, by obviating the need for ships to clean their tanks at sea, will reduce oil pollution of the sea and beaches considerably. Steps are already being taken to persuade oil carriers throughout the world to adopt this new technique. While it may be several years before oil already floating in the sea becomes wholly dispersed, it is anticipated that the new technique will bring a rapid improvement. Meanwhile, the present report records no large-scale spillages during the year in British waters. However, it is obvious that here, as elsewhere, there can be no relaxation of vigilance, and that the present expenditure of the Conservancy of some £692,000, of which only some £104,000 is allocated to research studentships, training and special surveys, is all too small for the tasks that it is called on to undertake. Together with a fresh look at the appropriateness of the new organization in the light of the facts disclosed in this report, the Ministers concerned might well reconsider the adequacy of the grant-in-aid of no more than £674,000.

## INSECT PHYSIOLOGY

### Advances in Insect Physiology

Vol. 2. Edited by J. W. L. Beament, J. E. Treherne and Sir Vincent Wigglesworth. Pp. xi+364. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 70s.

VOLUME 2 of *Advances in Insect Physiology* maintains the high standard set by the first volume. Its editors have again drawn heavily on the experience of people who are, or were until recently, members of the Agricultural Research Council Unit of Insect Physiology at Cambridge, but the wide range of subjects covered is evidence of the breadth of interest of this distinguished

group. The articles illustrate well the way in which problems of wide biological interest are now being tackled using insect material, and how necessary it is for anyone who calls himself a general physiologist to be aware of the opportunities offered by animals other than those which are usually found in pre-clinical departments.

Prof. Weis-Fogh leads off with the first full review of the distribution, biochemistry and mechanical properties of the rubber-like protein 'resilin' which he himself discovered only four years ago. This is clearly a progress report, since the researches are being actively pursued in his laboratory in Copenhagen. We may expect soon to hear more about the nature of the covalent links which bind the peptide chains into a three-dimensional framework, and about the way in which the molecular structure is laid down during development.

Dr. Beament has collected together all the evidence, both direct and circumstantial, which has led him to the conviction that active transport of water occurs through various regions of the insect cuticle. The slightly emotional tone of parts of this article can perhaps be understood in view of the difficulties which have attended previous postulations of such a mechanism. Particularly valuable is the discussion of the physico-chemical properties of the polar lipid monolayer which is not in thermodynamic equilibrium with identical solutions on the two sides. This is a good example of the way in which biologists have to be prepared sometimes to re-examine widely held physical principles. The final fourteen pages of this article should be read by everyone interested in the nature and properties of the cell surface.

Dr. Burkhardt discusses colour discrimination in insects and gives a particularly clear account of the misconceptions which can arise when behavioural evidence is used to draw conclusions about physiological mechanisms or *vice versa*. Micro-electrode investigations on single reticular cells, together with training experiments with intact insects, have, however, combined to establish that the hive bee, like man, has a trichromatic visual sense, but shifted 100 m $\mu$  into the ultra-violet. Less is known about other insects.

A major part of Dr. Cottrell's article on ecdysis is biochemical; he includes a full review both of the tanning of the cuticle and also of the nature of the tyrosinases of the blood on the grounds that "it is impossible to know how much of the available information is relevant to sclerotization". It is a pity that the title did not indicate this. Other components of the complex process of shedding and renewing the cuticle are listed and discussed.

Dr. Davey catalogues and discusses the rather inconclusive information which exists about the nature and functions of the visceral nervous system and about endocrine control of the viscera. This is evidently a field of investigation in which much more could be done; it is clear that, though they are striated, the visceral muscles differ in many of their properties from those classed as somatic.

The final chapter by Prof. Wigglesworth on the hormonal control of growth and reproduction brings up to date his earlier surveys. Sixty-nine pages of text and twenty pages of references are needed to cover the work of five years. Although "the subject has reached the point where the comparatively consistent hypotheses which were earlier accepted are being obscured by a multitude of apparent exceptions", this period has seen two of the most important hormones chemically characterized and a beginning made with the understanding of their action on the genetic expression of epidermal and other cells. Further clarification is likely to come, as the author suggests, through experiments which separate "immediate effects" at the cellular level from "the secondary consequences" of hormone action.

The editors' comprehensive author and subject indexes add considerably to the usefulness of the whole book. This ought to be a firm rule for publishers of annual reviews.

J. W. S. PRINGLE

## ADVANCES IN MICROBIOLOGY

### Recent Progress in Microbiology

Symposia held at the eighth International Congress for Microbiology, Montreal 1962. Edited by N. E. Gibbons. Pp. xi + 720. (London: Oxford University Press, 1963.) 172s.

THIS is a well-edited and well-presented account of the eighth International Congress for Microbiology. Papers from thirteen symposia are included and the collection is preceded by the opening addresses of the President of the International Association of Microbiological Societies and the President of the congress. Each symposium opens with its chairman's remarks, some of which serve the useful purpose of giving a broad overall view of the field covered by the symposium.

As might be expected at an international congress, the symposia range over a fascinatingly wide field of interests. In addition, there are reports of three panel discussions on industrial microbiology. The symposia fall under five main headings; the first, "Structure and Function", comprises four papers on membrane permeation and four on the properties of isolated cellular particles. These papers survey important recent advances in molecular biology, even including a paper on "The Biosynthesis of Haemoglobin in Reticulocyte Ribosomes". Many theories are put forward and it is interesting to compare the evidence gained from electron micrography and from biophysical and biochemical techniques. No doubt future symposia will resolve the relative merits of the various theories.

The editor states that "some of the Symposia were planned to cover aspects of Microbiology not covered in recent Congresses for Microbiology", into which category fall the four symposia on agricultural microbiology. Some of the work less familiar to most microbiologists appears in the symposium on insect microbiology under such interesting titles as "Crystalliferous Bacteria", "Entomogenous Fungi" and "The Entomophilic Microsporidians". Two further symposia in this section deal with "Enzymes in Soils" and "Effect of Chemical and Biological Control Measures on Soil Micro-organisms". Some of these papers are a very wholesome reminder to the pure culturists in the ivory towers of their laboratories that we still have a long way to go before we reach a clear understanding of the important and complex problems that abound in the microbial environments of Nature.

It is of interest, too, to read the account of the panel discussion on the "Microbial Production of Amino-Acids". We all realize that one of the great problems of the future will be the provision of food for the world's ever-expanding population, and the harnessing of micro-organisms to the task is a bright possibility. It is cheering to note that a dozen or more amino-acids have been produced by microbial means, in low yield in some cases, but in others the yield is high and in several cases the process competes favourably with the synthetic methods available from organic chemical research. It is to be hoped that further strain selection and a better understanding of genetics will enable the development of high-yielding strains which will provide still cheaper sources of the amino-acids essential to the human diet. All praise to the Japanese workers who have done so much to bring this possibility well on the way to realization.

Supplementary to this theme are the two other discussions covering aspects of the beneficial uses of microbial properties in providing for Man's needs—"Genetics Applied to Industrial Microbiology" and "Evolutionary Operation and Horizons in Industrial Microbiology". There are two symposia on virological topics, one of them on interference and interferon; another deals with the mechanisms of immunity in infectious diseases. Symposia X and XI cover aspects of pleuropneumonia-like organisms as disease agents and the virulence of *Staphylococci*.



Symposium XII once more harries the vexed question of microbial classification, almost entirely from the point of view of bacteria. The serious deficiencies in the present systems of classification as further exposed by the rapid advances in the fields of biochemistry and genetics are once more loudly voiced, but despite the discourses of five eminent scientists who lay bare the problems, there seems little hope that a classification (even of bacteria) satisfactory to all will emerge in the foreseeable future. The final symposium comprises six papers on the "Influence of Environment on the Epidemiology of Mycoses".

The volume is completed by an addendum on the statutes of the International Association of Microbiological Societies; the constituent member societies and the various committees are listed along with a report of the general meeting and plenary session of 1962. At the very end is the resolution on "The Contribution of Microbiology to World Food Supplies", which points only too clearly to the great present need for microbiologists and for work towards this end.

Experimental details are lacking from most of the papers so that they are, on the whole, quite short; any deficiency in this regard is made good by the fact that nearly 2,000 references are listed. One paper, "Mechanisms of Immunity in Virus Infections" by Jonas Salk, lists no references at all—which is a pity. Only three papers appear in French, the rest in English; the *Proceedings* at the end appear in both languages. There has been a break with tradition in omitting the discussions following symposia. It removes a little of the flavour of the book, but it is well justified for reasons of economy, since discussions tend to be reiterative. The table of contents is, thankfully, expanded to include the titles of all the papers and is a model of clarity. The range covered by this volume is quite remarkable and it makes a useful and interesting book for all microbiologists. L. B. QUESNEL

## BRAIN AND BEHAVIOUR OF OCTOPUS

### A Model of the Brain

By Prof. J. Z. Young. (The William Withering Lectures, 1960.) Pp. vii + 348. (Oxford: Clarendon Press; London: Oxford University Press, 1964.) 35s. net.

THE behaviour and neuroanatomy of *Octopus vulgaris* have been intensely investigated during the past fifteen years. This work was originated by Prof. Young and most of the more recent developments have been directly inspired by him. In *A Model of the Brain*, he summarizes the experimental data that have been collected and he reviews the theories of brain functioning to which the data have led. Young's aim has been to discover how the structure of the nervous system of the octopus gives rise to the observed behaviour of the animal: in pursuit of this goal he has made considerable use of the findings and concepts of cybernetics and the investigation of information processing by automata. The book is an outstanding example of what can be achieved when the techniques of classical biology (zoology, neuroanatomy, and neurophysiology) are used in conjunction with the methods of experimental psychology and with concepts derived from the field of automata investigations. It is a foretaste of the results that are likely to be achieved during the next few decades with other species by bringing the different techniques to bear on the same fundamental problem—the relation between brain and behaviour.

There are two reasons why the octopus is a particularly suitable species for this type of investigation. The brain and behaviour are simple enough to be investigated reasonably comprehensively, yet complex enough to be interesting. Whereas the rat brain has of the order of 2,000 different nuclei, the octopus brain has only about

50: the task of tracing out the information flow in the mammalian brain is extremely difficult, but Young has succeeded in getting at least within sight of achieving this with the octopus brain. Secondly, and more prosaically, the octopus is an extremely convenient experimental animal both for lesion work and for behavioural work.

In the early part of the book Young expounds the basic behaviour of the octopus, contrasting this species with other cephalopods and showing how it has adapted to its environmental niche. He then describes the organization of the nervous system, and discusses some of the theories that have been put forward to explain such phenomena as shape recognition in *Octopus*. The most original and important parts of the book, however, are the later chapters in which Young describes the model of learning that he has been developing during the past four years. It is impossible to do justice here to the richness of the ideas presented.

Young argues that there is a hierarchy of centres in the brain originally concerned with giving the reflex commands: "attack food", "draw in food", "bite food", "eat food". The lobes from which the first two commands are issued are much more complex than those issuing the last two. Both attacking food and drawing in food are under the control of distance receptors—respectively vision and touch (the octopus uses its suckers to explore food tactually before drawing it to the mouth). Young shows that there are startling anatomical similarities between the lobes concerned with vision and attack and those concerned with touch and drawing in. Moreover, lesions to corresponding parts of each system have similar effects on the two types of behaviour. Young suggests that in phylogeny the elaboration of these two sets of centres takes place to allow for learning: the animal learns which stimulus objects to attack and which not to attack. Such learning depends on the animal being able to switch the input from a given stimulus to give either an output corresponding to "attack" or an output corresponding to "no attack, or retreat". Such switching must be set up by events that occur after the stimulus has ceased to impinge on the receptors, since the animal is rewarded or punished only after it has attacked and it is this reward or punishment that determines whether the animal will learn to attack or not to attack a given stimulus object. Young argues that some of the elaborations of the lobes concerned, and particularly the recurrent pathways involved, have taken place to enable the animal to keep the representation of the stimulus object "addressed" during the delay between the stimulus occurring and the reward or punishment that follows attacking or drawing in.

In both the lobes concerned with vision and in those concerned with touch there are centres containing a proliferation of small cells: such small cells do not occur in the centres concerned with reflex eating and biting. Young suggests that these small cells may be responsible for setting up the switch involved in learning. Thus, in the cuttlefish, there is no elaboration of the touch system as in the octopus and there is no proliferation of small cells in that system. Presumably, there is little tactual learning in the cuttlefish since it does not use touch as a distance receptor: the lobes concerned with vision are, however, anatomically similar to the corresponding lobes in the octopus.

This account has necessarily oversimplified Young's ideas. Further research will doubtless fill in and complicate many of the details of the model, but the model as it stands gives a very plausible framework within which to understand the basic data on the neuroanatomy and behaviour of the octopus: the construction of such a model at this stage is a very notable achievement.

The book is beautifully written, and conveys the author's own zest for the subject in every line. Although it is mainly devoted to the octopus, it sets the subject in a broad biological perspective, and the story it has to tell

should grip the attention of anyone interested in biological phenomena. *A Model of the Brain* will itself serve as a model of how work on brain functioning should be undertaken and presented for some time to come.

N. S. SUTHERLAND

## AGRICULTURAL RESEARCH

**The Principles and Practice of Agricultural Research**  
By Dr. S. C. Salmon and Dr. A. A. Hanson. Pp. ix + 384.  
(London: Leonard Hill (Books), Ltd., 1964.) 75s. net.

ANY book concerned with the principles and practice of agricultural research is particularly welcome at this time when there is such a need for increased food production in many of the developing countries, and that by Salmon and Hanson is a very good introduction to the subject. Although it does not discuss any particular aspects in sufficient depth to make it a manual for the research worker, it is well-worth reading, not only by agriculturists but also by everyone who is concerned about the world's food supply and methods of increasing it.

The first part gives a brief sketch of the history of agricultural improvements, tracing the development of some of the more important aspects such as plant breeding improvements, and directing attention to the methods used by some of the scientists whose work later became important in agriculture, men such as Liebig, Darwin and Pasteur. This discourse leads to the conclusion that many of the improvements in agriculture have been made without the aid of formal research but that such advances have been painfully slow, indicating that a better understanding of research methods might enable us to proceed more quickly in the future. To this end, the second part is devoted to the 'Philosophy of Research', a very useful section which is pertinent to all research workers, not just those applying their talents to agriculture. The various basic methods of research are discussed briefly and a chapter is devoted to the possible reasons why erroneous conclusions are sometimes drawn. Agriculture, which is so affected by uncontrollable variation, is particularly suitable for directing attention to such difficulties in research and for considering the meaning of 'proof' which the authors discuss in the last chapter of this section.

Part 3 is devoted to statistical methods, a subject which is already very well covered by standard textbooks. This section does not attempt any new explanation but simply shows, mainly by example, how various statistical computations are made, without attempting to show much basic theory. The reader is simply asked to accept that each method is correct; for example, the reason for using standard deviations instead of average deviation "is that many statistical deductions and practically all statistical tables... are based on standard deviations". This approach is no doubt admirable for those who wish to use statistics as a research tool without involving themselves in mathematical formalities, provided they wish to use only those methods detailed in the book; but, if they try to extrapolate the ideas to slightly different designs or situations, lack of fundamental understanding is liable to lead to inefficient experimentation or even erroneous results. However, the impression given of the absolute simplicity of the methods may well encourage those who have been avoiding statistical techniques to examine statistical textbooks and so adopt better designs and analyses. The aspects covered include standard deviation and variance, significance tests using  $t$ ,  $\chi^2$ ,  $F$  and Duncan's multiple-range test, analysis of variance for randomized block designs and other factorial arrangements, Bartlett's test of homogeneity of variance, the use of simple transformations, correlation, regression and co-variance. There is a chapter on sampling which sets out in quite simple terms

the effects of randomization and is a good reminder of the sound views of Fisher which are so often forgotten in these days of more sophisticated statistical techniques. The section ends with a discussion of the uses and limitations of statistical methods which very wisely produces the conclusion that they are no substitute for critical observation and thought, but should be used, where appropriate, for the purposes for which they are designed.

This appreciation of statistics is followed by an examination of the techniques of agricultural research, which first deals with problems found in all kinds of field research, such as differential responses from place to place and year to year, and then goes on to deal with choice of experimental material, size, shape, replication and management of plots in field trials. Another chapter in this section is devoted to experiments with farm animals in which most experimental aspects are mentioned, though none receives very exhaustive consideration, which is to be expected in a book which attempts to cover such a wide subject in so small a space. There is also a chapter on experimental design which demonstrates the possibilities of Latin squares, cross-over trials, split-plot and incomplete plot designs, without attempting to show how these are analysed, and the book ends with some thoughts on the methods of research in agricultural economics including a reference to linear programming.

W. J. RIDGMAN

## MONOSACCHARIDES

### The Monosaccharides

By Jaroslav Staněk, Miloslav Černý, Jan Kocourek and Josef Pačák. Pp. 1006. (New York and London: Academic Press; Prague: Publishing House of the Czechoslovak Academy of Science, 1963.) 228s. 6d.

*The Monosaccharides*, by Jaroslav Staněk, Miloslav Černý, Jan Kocourek and Josef Pačák, is an advanced text and reference work. It was originally published in Czech, but the present volume is an entirely new edition, considerably supplemented and partly rearranged in comparison with the first Czech edition: the literature survey and addenda inserted in the form of notes directly into the text cover the period to mid-1962.

The scope of the volume is so vast that it will best be apparent from its chapters, that deal with: (1) introduction; (2) structure of the monosaccharides; (3) isolation of the monosaccharides from natural material, their properties and industrial utilization; (4) preparation of the monosaccharides by synthetic methods; (5) esters of cyclic monosaccharide forms and organic acids; (6) the glycosyl halides and their derivatives; (7) glycosyl derivatives of aromatic hydrocarbons; (8) esters of monosaccharides and inorganic acids; (9) glycosides and thioglycosides; (10) monosaccharide orthoesters; (11) monosaccharide ethers; (12) condensation products of monosaccharides and carbonyl compounds; (13) sugar anhydrides (glycosans) and anhydro sugars; (14) unsaturated derivatives of monosaccharides; (15) deoxy sugars; (16) glycosylamines; (17) nucleosides; (18) amino sugars; (19) monosaccharide oximes; (20) reaction products of monosaccharides and aromatic hydrazine derivatives; (21) glycosyl azides; (22) oxo derivatives of monosaccharides; (23) the reaction products of monosaccharides and hydrazine, urea, thiourea, guanidine, semicarbazide, thiosemicarbazide and similar compounds; (24) acyclic derivatives of monosaccharides; (25) other derivatives of monosaccharides containing sulphur; (26) alditols; (27) aldonic acids; (28) branched-chain aldonic acids; (29) the saccharinic acids; (30) oxo derivatives of aldonic acids; (31) ascorbic acids; (32) monosaccharide dicarboxylic acids; (33) amino-acids from the monosaccharide series; (34) polyhydroxyalkylbenzimidazoles; (35) inositols and related compounds; (36) synthesis and transformations of sugars in living systems; (37) sugar analysis.

Thus the entire field of monosaccharide chemistry is covered. I thoroughly enjoyed reading the book and was particularly impressed by Chapters 2, 4, 6, 12, 15, 18, 20, 31, 35 and 36. The standard of treatment is uniformly high: reaction mechanisms are given where appropriate and a comprehensive bibliography (including references to the patent literature) is given at the end of each chapter. It is noted that recrystallization of some monosaccharides from boiling solvents may lead to traces of new substances, for example, D-fructose from ethanol.

There can be little doubt that Dr. Staněk's book will be regarded as the standard reference work on the monosaccharides and no carbohydrate chemist can afford to be without it either in his personal library or in his laboratory. I suggest that all libraries should acquire a copy: most organic chemists will wish to own or have access to this excellent book. The price is high, but not unduly so by modern standards having regard to its length and content.

The translation is generally good, but a few isolated clumsy expressions will be found (see, for example, pp. 35, 37, 117, 539): only two misprints were found (pp. 563 and 869). The author and subject indexes are very satisfactory. The book was printed in Czechoslovakia: the production and format are very creditable by any criteria.

A. I. VOGEL

## PEROXIDASE AND RELATED CATALYSTS

### Peroxidase

*The Properties and Uses of a Versatile Enzyme and of some Related Catalysts.* By Dr. B. G. Saunders, Dr. A. G. Holmes-Siedle and Dr. B. P. Stark. Pp. x+271. (London: Butterworth and Co. (Publishers), Ltd., 1964.) 60s.

SINCE the publication in 1948 of Lemberg and Legge's comprehensive *Hematin Compounds and Bile Pigments* no comparable survey of so vast a field has appeared. This is scarcely surprising in view of the explosive growth of knowledge since that time. The present volume, dealing with one group of haemoproteins, is an inevitable development. It provides very broad cover: it deals with sources of enzyme, isolation and purification, chemical and physical properties, spectroscopy and reaction kinetics, possible biological roles, and the chemistry of oxidation products of hydrogen donors in peroxidatic reactions. There is also a chapter on "Contributions to the Problem from Inorganic Chemistry" and an extremely useful atlas of haemoprotein spectra. The sequence of subject-matter does not follow a very logical course: thus a complete account of the Chance mechanism for the reaction of horse-radish peroxidase with peroxides is scattered in four chapters: under general survey, properties of peroxidase, rapid reaction mechanisms and chemistry of peroxide compounds. Other subjects, such as George's concept of higher oxidation states of iron, are similarly fragmented. Although Chapter 8 is entitled "The Spectra and Function of the Haemoproteins", questions of biological function are first considered in Chapter 9. However, a very full index enables such difficulties to be surmounted.

The authors write in two very distinct styles: (1) comprehensive reporting of the literature, devoid of critical evaluation and often highly compressed; (2) authoritative summaries of certain fields to which the authors have themselves made valuable contributions. While it is inevitable that some emphasis of the authors' interests should occur, the imbalance can become excessive as in the introductory chapter "General Survey" in which 28 out of 37 pages are occupied by an account—and an excellent one—of the authors' work on the peroxidation products of phenols and aromatic amines.

The enzyme peroxidase is widely distributed in plants, but many references to animal peroxidases must be

treated with reserve since all haemoproteins, and other haematin derivatives, can catalyse reactions which, at least superficially, are similar to those that characterize peroxidase. However, the activity of such derivatives is lower, by a factor of at least 10,000, than that of the enzyme peroxidase and is at the level of denatured peroxidase. Failure to appreciate this point has been frequent and it is unfortunate that the authors of this book have not driven the point home. They define true, atypical, artificial and model peroxidases and state that, failing indication to the contrary, the term peroxidase will be used to mean true peroxidase, that is, a haemoprotein enzyme. In Chapter 13, however, they make the surprising statement that "most workers agree that true peroxidase activity seems to be largely restricted to iron-porphyrin compounds and to iron phthalocyanines". They then ascribe peroxidase activity to such diverse materials as heavy metal compounds, granite, charcoal, aldehydes, organophosphorus compounds, nucleic acids and vitamin A. Traces of heavy metals can accelerate the peroxidation of typical hydrogen donors for peroxidase, and the list of materials with thermostable peroxidase-like activity can be extended indefinitely (to include even the dust on the laboratory floor) so long as no standard criteria of reaction rates or specific activities are applied.

Chapter 9, on catalase, gives emphasis to the peroxidatic activity of the enzyme. Under "Biological Role" (and again, for no obvious reason, in Chapter 13) are described *in vitro* reactions which the enzyme is known to catalyse. However, we do not know whether such reactions are related to its biological function. A more serious error is the statement, made more than once, that in the functioning of the respiratory chain cytochrome reduces oxygen to hydrogen peroxide and, by inference, that the decomposition of such peroxide is a biological role of catalase. The brief comments (p. 91) on the use of the spectroscope by MacMunn and by Keilin are quite inaccurate and the statement that cytochrome c peroxidase is associated with the respiratory chain will be disputed. Otherwise errors of fact are very few. It is not clear why almost identical tables appear on pages 35 and 147, and the thirteen pages occupied by structural formulae in Appendix A might have been put to better use since most of the formulae appear in Chapter 1.

The authors no doubt felt that considerations of non-enzymatic peroxidation would widen the interest in their book. But to those whose attention is caught by the title, to the enzymologist, these considerations will be seen as intrusions on limited space. While this book contains snares for the newcomer there is no doubt that it will become an essential work of reference for all who wish to keep abreast with developments in haemoprotein catalysis.

E. F. HARTREE

## POSITRONIUM—THE CHEMISTRY OF A MINIAURE HYDROGEN

### Positronium Chemistry

By James Green and John Lee. Pp. xii+105. (New York: Academic Books, Inc.; London: Academic Books, Inc. (London), Ltd., 1964.) 44s.

DESPITE the short history of positronium chemistry and the comparatively few workers actively involved, there is a great amount of recorded data, as is evidenced by the extensive and thorough bibliography provided in this book. The authors have obviously decided to cope with this situation by selecting certain aspects which interest them most. Most readers, however, will require to be 'put in the picture' and this is attempted in an opening chapter of eight pages. This is condensation at its worst and the result is most unsatisfactory. The authors hop



from one aspect of the subject to another without warning and the effect is confusing. In such a situation it is difficult to understand the use of one page of the eight for a largely irrelevant discussion of mesonic atoms.

Chapter 2, "Experimental Methods", is excellent and, together with the references, will be invaluable to any research worker entering the field. The introduction of a sentence on copper-64 into the middle of an account on sodium-22, however, is surprising. So also is the absence of any consideration of the effect of fourteen-stage multipliers on techniques. Two chapters are devoted to interesting summaries of positrons in gases and in solids. A very clear description is given of the behaviour of positrons and positronium in different experimental circumstances and of the various theoretical and experimental investigations up to a very recent date. The effects of electric and magnetic fields, pressure, type of gas or solid are all well described and discussed and make interesting reading.

Chapter 5 deals with the theoretical chemistry of positronium, and the authors from this point onwards are increasingly concerned with positronium as a chemical element. The final two chapters are about positronium chemistry in gases, liquids and solids. It soon becomes apparent that the subject is as yet in a very unsatisfactory state. Experimental results are still unreliable and incomplete. This is perhaps the most useful aspect of this book: it shows clearly where inconsistencies exist, what readings must be repeated and improved, what auxiliary measurements must be made, and where there are serious gaps in the published data. It is manifest that, even when all this is done, interpretation will be difficult, but as things stand interpretation is impossible. Because of the present chaotic state these final chapters make difficult reading—but rewarding reading.

There is much in *Positronium Chemistry* which could be improved. Chapter 1 needs rewriting and expanding considerably, as has been indicated; for the time being, the reader should read one or more of the reviews listed in the introduction. Some re-ordering of material would be helpful. For example, in Chapter 3 (on gases) it is assumed that the reader is familiar with the use of 3 per cent nitric oxide to quench all 3-photon annihilation in gases, which is not explained until Chapter 4 (on solids). In addition there is the usual crop of small confusions and printer's errors. Most of these are trivial, but some are disconcerting. The definition of quenching, for example: "quenching means the conversion in collision of triplet to singlet at a rate much greater than the triplet annihilation..."—surely this should be "at any rate"?  $\tau$ , paralysis time, is used on p. 10 where the resolving time of the coincidence circuit is intended. Most confusing of all, ten lines from the bottom of p. 4,  $n$  factor should be  $N$  factor. Despite these criticisms this is a useful book; it maps positronium country with the uncharted areas clearly shown. It is an important and worth-while addition to the literature.

W. F. WILLIAMS

## THE SCIENCE OF SCIENCE

### The Science of Science

Society in the Technological Age. Edited by Maurice Goldsmith and Alan Mackay. Pp. 235. (London: Souvenir Press, 1964.) 30s. net.

**J.** D. BERNAL'S important book *The Social Function of Science* was published in 1939. It had considerable influence and many of its conclusions are now part of the assumptions of everyday life of those who have any broad social awareness. Its principal theme was that science could, and should, be planned and that this planning was susceptible to rational analysis. The work now under review, twenty-five years after the publication

of the former book, offers a perceptive assessment of the present position with regard to the social uses of science.

A considerable number of Bernal's recommendations and prophecies have become fact. The contributors include some of the foremost names in science to-day: Blackett, Haldane, Kapitsa, Needham, Powell, de Solla Price, Sir Charles Snow and Synge—three Nobel prize winners, nine Fellows of the Royal Society. They develop Bernal's view that an affluent, prosperous, developing society free from war, hunger, unemployment and tyranny is possible. The book is also intended to do public honour to Prof. Bernal and commences with a spirited biographical account of him by Sir Charles Snow.

The royalties from the sale of *The Science of Science* are to be used to help set up a Science of Science Foundation, the object of which will be to promote the application of scientific methods to the understanding of science itself, especially in its relations with society (see *Nature*, 205, 10; 1965).

Each of the chapters, written by a scientist of distinction, could have been expanded without superfluity to a large book. The whole publication is a masterpiece of compression. It is packed with good topics for critical and creative discussion in sixth-form, university and extra-mural classes. Not only science teachers but those who have at heart the condition of Man and his future, anywhere in the world, should know the problems with which it deals. If prospective Members of Parliament ever have to pass an elementary examination before being elected, this should be one of the half-dozen set books.

To some of the chapters there is appended a bibliography or reading list. This might have been extended to all of them. Other chapters of equal interest include a version of Blackett's 1957 address to the British Association for the Advancement of Science; Herbert Coblan's chapter on the problems of scientific communication, in particular, those of the enormous bulk and number of new scientific papers every year; Kapitsa on future problems of science; Needham on eastern and western science; Haldane on human genetics; Pirie on the maldistribution of research effort; Korach on the science of industry; Price on the science of science; and Bernal's own reflexions on the applications of science in the past quarter of a century.

Naturally, the political ideas of some of the writers do not show the same objectivity as those of their scientific statements, but the discerning reader can consider them in conjunction with one another. Thus we have "One must admit some truth to the platitude that the scientist outside his chosen discipline reverts to the status of layman" (Price) and "At your schools they taught you the laws of Newton—but why did they not teach you Marx's sociological laws in the capitalist countries? Indeed, there are laws on society which are valid for the whole globe, wherever society exists, they are as valid in all human societies, just as the law of attraction—Newton's discoveries are valid for any body" (Kapitsa). Nevertheless, capitalism, or individual effort, does not seem, by implication, to be so bankrupt: "In addition to likely commercial and government short-term lending, an additional £1,000 million a year is needed as a free gift or as long-term loans from the 400 million rich Westerners to the 1,000 million Asians, Africans and South Americans in the underdeveloped countries outside the society orbit" (Blackett), and "Over the past decade one of the principal measures for sustaining U.S. agricultural economy against the crushing burden of its surpluses has consisted in shipping those surpluses overseas to feed the hungry. Since 1954 more than Dollar 9,000 million worth, 75 million tons of agricultural commodities have been delivered to forty-four developing countries. Shipments of wheat alone have amounted to two entire bumper crops. India has been the principal recipient of these shipments, more than Dollar 2,000 million worth" (Piel).

W. L. SUMNER

**Imperfections and Active Centres in Semiconductors**

By R. G. Rhodes. (International Series of Monographs on Semiconductors, Vol. 6.) Pp. xii+373. (London and New York: Pergamon Press, 1964.) 90s. net.

**A**LTHOUGH the word "semiconductors" is used in the title, this book is in reality a review of certain properties of germanium and silicon, and mention of other materials is extremely brief. The topics covered are the detection, geometry and motion of dislocations including plastic deformation and twinning, crystal growing and the behaviour of impurities incorporated during growth and by diffusion techniques. A final chapter deals with the chemistry of etching of germanium and silicon and a discussion of the formation of etch pits produced by defects. The general theme is the interactions between various defects including dislocations, vacancies and impurities and the effects of such reactions on the electrical properties of the bulk material; there is no discussion of such effects in thin films prepared by vapour phase growth.

R. G. Rhodes states that the book should be useful in teaching, as well as of practical help to the specialist, and for this reason brief elementary discussions are given as introductions to some of the chapters. The teaching value, however, appears to be rather limited, since the main subject-matter is quite definitely specialized. The space devoted to the elementary preambles which are covered more generally and in more detail in other texts could have been used to amplify other sections which are treated in only a cursory manner. For example, although considerable space is devoted to the detection of dislocations by X-ray methods, the technique of electron microscopy is dealt with in one page; the wealth of information on the behaviour of impurities and their interactions with one another and defects, such as vacancies as determined by electron spin resonance, is scarcely mentioned. In addition there is virtually no mathematical formulation of the various elastic interactions and kinetics of diffusion and precipitation which are discussed.

*Imperfections and Active Centres in Semiconductors* is, therefore, rather disappointing because of its incompleteness. It should, however, be of value to technologists and new research workers entering this field, since it gives a factual survey of the literature up to and including 1962 with adequate references apart from the deficiencies noted here.

R. C. NEWMAN

**Trace Analysis of Semiconductor Materials**

Edited by J. Paul Cali. (International Series of Monographs on Analytical Chemistry, Vol. 11.) Pp. ix+282. (Oxford, London, New York and Paris: Pergamon Press, 1964.) 70s.

**T**RACE analysis of semiconductor materials presents problems of exceptional severity because of the extremely low levels at which impurities can have a significant effect on the properties of these materials. *Trace Analysis of Semiconductor Materials* aims to give an account, up to 1961, of the battle being fought by the semiconductor analyst to achieve the necessary sensitivities.

The book essentially consists of four chapters, each by specialist authors. The first, and perhaps appropriately the longest, chapter is on activation analysis, by J. P. Cali. A short second chapter, by P. E. Lightly and E. W. Currier, is concerned with emission spectroscopy, and Chapter 3, by R. E. Honig, deals with mass spectrometry. Chapter 4, by C. A. Parker and W. T. Rees, comprises three sections on absorptiometric, fluorimetric and polarographic methods.

The book therefore in effect consists of a series of monographs on the six particular analytical techniques which have been found most rewarding in semiconductor trace analysis. It is perhaps a pity that the coverage was not extended to consider a number of matters common

to the various techniques of trace analysis, for example, the vexed problem of sample preparation, and the difficulties of preparing ultra-pure reagents to permit the analysis of ultra-pure materials. On the other hand, there is much to commend. The emphasis throughout is on information of practical value; in particular the radiochemical separations and the table of published analyses in Chapter 1, and the analytical methods detailed in Chapter 4, are most useful compilations. The book could also be of value to many analysts not concerned with semiconductor materials.

H. J. CLULEY

**Genetics of Forest Tree Improvement**

By J. W. Wright. Pp. xvi+399. (Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1964.) 20s.; 4 dollars.

**I**T is some two hundred years since Duhamel du Monceau published his observations on hereditary behaviour in forest trees, and although later workers have interested themselves in freak forms of trees, in hybrids and in provenance trials, no serious attempt was made to bring about improvement in forest trees until the present century. In fact, credit must be given to the Americans for first adopting "Tree Breeding" in the name of a forest research station. This was in 1925. Many developments have taken place since then and the silviculturist realizes the useful tool he has in the subject of forest genetics. The recent publication, *Genetics of Forest Tree Improvement*, prepared by J. W. Wright and issued by the Food and Agriculture Organization of the United Nations, deals with the subject in a most comprehensive way. It is said in the foreword that the book is aimed at three types of readers—experienced professional tree breeders, students and practising foresters. There is no doubt that these aims have been achieved in the subject-matter and its lay-out.

For obvious reasons much of the genetic improvement work must be expected in species which are commonly planted rather than in those in which natural reproduction is the rule. The rate of improvement is usually more spectacular in species planted outside their natural range, such as *Pinus sylvestris* in the United States, *P. radiata* in the southern hemisphere and eucalypts in the Mediterranean countries. But genetic gains are also being demonstrated in species which are planted within their native ranges, such as Douglas fir in the Pacific north-west. All this is a very different story from what has happened when trees have been raised from unknown provenances and planted in the wrong sites. The result has been a waste of time and money.

After giving illustrations of basic genetic principles, the author deals with population genetics, pollen migration rates, tree selection and selective tree breeding. There are general remarks on geographic variation in forest trees and examples are quoted for a number of species. The professional breeder will find much interest in the chapter on species and racial hybridization. The importance of exotic trees for afforestation and reafforestation schemes is well known and the author claims that species introduction is best considered as a part of forest genetics because differences between species and ecotypes are a matter of degree and therefore tests of new species and new ecotypes should be organized as part of a tree-breeding programme. He adds that the present generation of foresters is largely dependent on tree breeders for most of their detailed knowledge of exotics. There are also fascinating chapters on polyploidy, experimental design and analysis, controlled pollination and vegetative propagation. Finally, there is a most useful, and essential, glossary.

Much more could be said about this book, but it can be summed up by saying that it is ably presented and is a very valuable contribution to forestry literature.

C. J. TAYLOR

## HYBRID CELLS DERIVED FROM MOUSE AND MAN: ARTIFICIAL HETEROKARYONS OF MAMMALIAN CELLS FROM DIFFERENT SPECIES

By PROF. HENRY HARRIS and DR. J. F. WATKINS

Sir William Dunn School of Pathology, University of Oxford

THE ability of certain animal viruses to induce the formation of multinucleate cells by fusing together single cells suggested the possibility that these viruses might be used to amalgamate different cell types and thus produce artificial animal cell heterokaryons. In this article we describe some properties of heterokaryons which we have produced by fusing together cells of human and murine origin.

The two cell types used in the present experiments were HeLa cells (originally derived from a human carcinoma) and mouse Ehrlich ascites tumour cells. These cells were chosen because they could be obtained in quantity as suspensions of single cells: the HeLa cells were grown *in vitro* in suspension culture<sup>1</sup>; the Ehrlich tumour was maintained in the ascitic form in the peritoneal cavity of Swiss mice. Some experiments we have made with other cell types indicate, however, that the technique can be applied to a variety of cells, including differentiated somatic cells, provided that they are susceptible to the strain of virus used to produce fusion. Of the large number of viruses which are known to induce the formation of multinucleate cells, the para-influenza I group of myxoviruses seemed the most promising, because it had been shown by Okada<sup>2</sup> that one strain of these viruses (H<sub>1</sub>VJ) could induce rapid fusion in suspensions of Ehrlich ascites cells *in vitro*. The virus used in the present experiments was a strain of Sendai virus supplied by Dr. H. G. Pereira of the National Institute for Medical Research, Mill Hill.

The virus was propagated in the following way. Infected allantoic fluid with a titre of 8,000 haemagglutinating units/ml. was diluted 1 in 10<sup>4</sup> with phosphate-buffered saline: 0.1 ml. of this preparation was injected into the allantoic cavity of 10- or 11-day-old fertile hens' eggs, which were incubated for 3 days at 37° C. The eggs were then maintained at 4° C overnight and the allantoic fluid collected. The pooled allantoic fluid was centrifuged at 400g for 10 min and the haemagglutination titre of the supernatant determined. The supernatant was then centrifuged at 30,000g for 30 min and the deposit resuspended in one-tenth of the original volume in Hanks's solution<sup>3</sup> (used throughout the present experiments without glucose). The haemagglutination titre was again determined and the concentrated virus suspension stored in 1-ml. lots at -70° C. This suspension, suitably diluted in Hanks's solution, was used for the experiments. Haemagglutination titrations were performed in Salk-pattern haemagglutination trays. Doubling dilutions of virus were made in 0.5 ml. of phosphate-buffered saline, and approximately 2.5 × 10<sup>7</sup> guinea-pig erythrocytes suspended in 0.05 ml. of this saline were added to each cup. The smallest amount of virus which produced complete haemagglutination after 2 h at room temperature was defined as one haemagglutination unit (HAU).

Since any investigation of the physiology of the heterokaryons would be greatly complicated if these cells were engaged in virus production, the virus used to produce cell fusion was inactivated by ultra-violet light. One ml. of the concentrated suspension of virus in a watch-glass was exposed for 3 min to ultra-violet light emanating from a Philips 15-W 18-in. germicidal tube, type 'T.U.V.'. The intensity of the radiation incident on the surface of the fluid was 3,000 ergs/cm<sup>2</sup>/sec. The suspension of virus was mixed by pipetting at the end of the first and second

minutes. Infectivity titrations on the irradiated virus were carried out by a modification of Fulton's method<sup>4</sup> in which pieces of chorio-allantoic membrane were incubated with the virus in Medium 199 (ref. 5) in a haemagglutination tray. It was found that after 3-min exposure to ultra-violet light under the present conditions, the infectivity of the virus, as measured by the production of haemagglutinin, was reduced to levels at which the measurements were obscured by the haemagglutination produced by the initial viral inoculum. This represented a reduction in infectivity of at least 3 logs. Although the ability of the virus to multiply in the chorio-allantoic membrane had thus been drastically reduced, its ability to induce cell fusion *in vitro* remained unimpaired. Evidence will be presented to show that the virus inactivated by ultra-violet light was not reactivated in the vast majority of the heterokaryons.

The technique used for inducing cell fusion was essentially similar to that described by Okada<sup>2</sup>. HeLa cells from a suspension culture were spun down and resuspended at a concentration of 2 × 10<sup>7</sup> cells/ml. in Hanks's solution. Ehrlich ascites cells, withdrawn from the peritoneal cavity, were washed once by centrifugation in this solution and then resuspended in it at a concentration of 2 × 10<sup>7</sup> cells/ml.: 0.5 ml. of each cell suspension was pipetted into a chilled inverted T-tube together with 1.0 ml. of the suspension of virus, diluted, if necessary, in Hanks's solution. The cells clumped immediately, and the size of the clumps was roughly proportional to the amount of virus added. The T-tube was kept at 4° C for 15 min and then shaken in a water bath at 37° C for 20 min at a rate of 100 excursions/min. During this time the cells in the clumps underwent varying degrees of fusion. Fig. 1 shows an electron micrograph of a section through a pellet of cells obtained by centrifuging a preparation at 200g for 10 min immediately after the period of shaking at 37° C. A trinucleate cell is shown, formed by the fusion of three discrete cells; and a fourth cell is seen in the process of fusing with this trinucleate cell.

One ml. of the cell suspension and 5 ml. of culture medium were pipetted into a 6-cm-diameter Petri dish containing 15 coverslips 1 cm in diameter. The culture medium consisted of 20 per cent calf serum and 1 per cent tryptose broth in Medium 199, to which penicillin at a concentration of 100 international units/ml. and streptomycin at a concentration of 100 µg/ml. were added. The dishes were incubated at 37° C in a gas mixture of 5 per cent carbon dioxide in air. The coverslips were transferred to fresh medium after 24 h and again after 4 days.

Four h after the cell suspension had been introduced into the Petri dishes multinucleate cells were found to have adhered to the coverslips; and within 24 h most of these had flattened out on the glass. From cell counts it could be calculated that the multinucleate cells adherent to the coverslips accounted for about 10 per cent of the single cells originally present in the suspension. Each of these multinucleate cells contained from 2 to about 20 nuclei. The nuclei were of two easily distinguishable morphological types. With May-Grünwald-Giemsa or Leishman stain one type of nucleus stained more deeply than the other. The more deeply staining nucleus contained numerous small nucleoli or coarse condensations of chromatin, while the less deeply staining nucleus con-



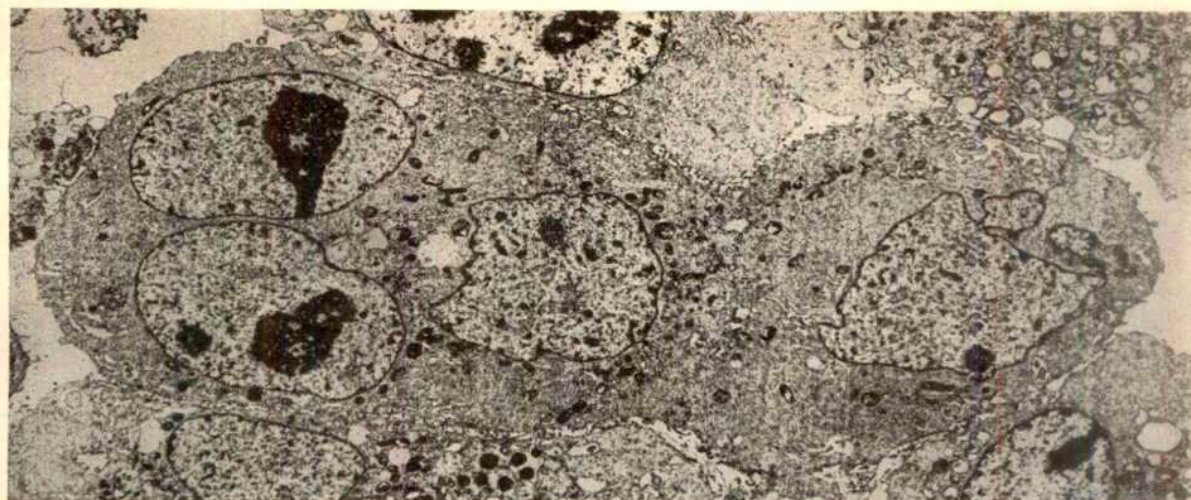


Fig. 1. An electron micrograph of a section through a preparation in which HeLa cells and Ehrlich ascites cells were fused together. A trinucleate cell is shown, formed by the fusion of three discrete cells. A fourth cell, on the right, is seen in the process of fusion with the trinucleate cell. (By courtesy of Dr. G. I. Schoeff)

tained one to three large nucleoli. The two types of nucleus are illustrated in Fig. 2, which shows a tetranucleate cell containing two nuclei of each type. Since the deeply staining pachychromatic nuclei clearly resembled the nuclei of Ehrlich ascites cells, and the less deeply staining nuclei resembled the nuclei of HeLa cells, it was difficult to avoid the conclusion that the multinucleate cells were formed by fusion of the two cell types. This was confirmed in the following way: 100  $\mu$ c. of tritiated thymidine was added to a suspension culture of HeLa cells at 10 a.m., 2 p.m., 6 p.m. and 10 p.m. on one day, and again at 7 a.m. and 10 a.m. on the following day. Since the generation time of the cells under these conditions was about 20 h, it was thought that this procedure would succeed in labelling the nuclei of virtually all the cells in the culture. Autoradiographs of smears of the cells, made 2 h after the final addition of tritiated thymidine, confirmed that this was so. When a mixture of labelled HeLa cells and unlabelled Ehrlich ascites cells was treated with virus, autoradiographs of the resulting multinucleate cells revealed that the deeply staining pachychromatic nuclei were not labelled, whereas the less deeply staining nuclei with the large nucleoli were. This is illustrated in Figs. 3-5, which show a series of heterokaryons containing labelled and unlabelled nuclei in varying proportions. When the reciprocal experiment was made with labelled Ehrlich ascites cells and

unlabelled HeLa cells, it was found that the deeply staining pachychromatic nuclei were labelled and the others were not. It is thus clear that the multinucleate cells were indeed formed by the fusion of HeLa cells with Ehrlich ascites cells. At least 98 per cent of the multinucleate cells were heterokaryons when virus concentrations of 8,000 HAU/ml. or more were used to induce the cell fusion (Table 1).

Table 1. EFFECT OF VIRUS CONCENTRATION ON THE CHARACTER OF THE HETEROKARYONS PRODUCED

Counts made 24 h after formation of heterokaryons	Concentration of virus used			
	Virus inactivated by ultra-violet light	800 HAU	8,000 HAU	Infective virus 8,000 HAU
Percentage of multinucleate cells which were heterokaryons	77	99	98	97
	84	98	97	99
	85	97	97	99
Mean	82	98	98	98
No. of nuclei per heterokaryon	4.0	(18.4)	10.0	6.3
	4.3	5.9	8.5	5.1
	4.4	9.4		7.4
Mean	4.2	(11.2)	9.3	6.3
Ratio of HeLa nuclei to Ehrlich ascites nuclei	2.8	1.9	3.2	2.3
	1.9	2.1	2.7	2.6
	2.5	2.0		2.6
Mean	2.4	2.0	3.0	2.5
Ratio of multinucleate to mononucleate cells	1.0	3.5	8.4	3.1
	0.6	2.0	7.5	9.0
	0.8	3.1		8.4
Mean	0.8	2.9	8.0	6.8

The average number of nuclei per heterokaryon and the ratio of multinucleate to mononucleate cells could be varied by changing the concentration of virus used. Table 1 shows that at lower concentrations of virus the number of nuclei per heterokaryon and the ratio of multinucleate to mononucleate cells fell. Since the proportion of the original cell suspension recovered as multinucleate cells was unaffected by the concentration of virus used (Table 2), the increase in the ratio of multinucleate to mononucleate cells at higher concentrations of virus must have resulted from preferential elimination of mononucleate cells. This was apparently due to the fact that the multinucleate cells were more resistant to the cytotoxic effects of the virus than the mononucleate cells, a conclusion supported by the observation that infective virus eliminated a greater proportion of the mononucleate cells than inactivated virus at the same concentration (Table 1). With high concentrations of virus, however, and especially with infective virus, some of the multinucleate cells showed marked vacuolation. Table 1 also shows that the ratio of HeLa nuclei to Ehrlich ascites nuclei in the heterokaryons was between 2 and 3 irrespective of virus concentration. This preponderance of HeLa nuclei might be due either to the fact that HeLa cells fuse more easily than Ehrlich ascites cells or, more probably, to the fact that cells with

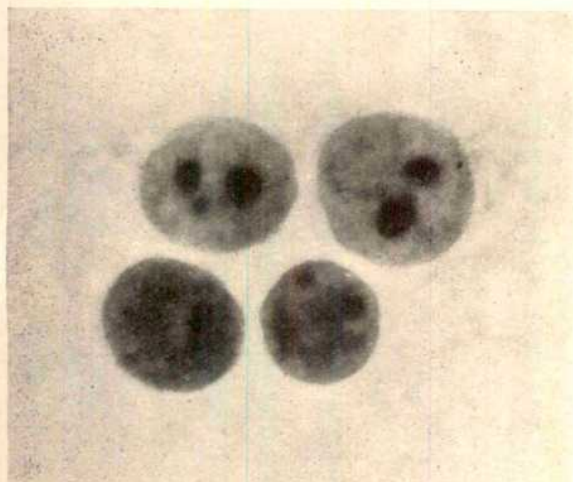


Fig. 2. A tetranucleate cell in which the two upper nuclei are derived from HeLa cells and the two lower ones from Ehrlich ascites cells ( $\times 1,300$ )



Table 2. SURVIVAL TIME OF HETEROKARYONS MADE WITH DIFFERENT CONCENTRATIONS OF VIRUS

Days after production of heterokaryons	No. heterokaryons per coverslip			Infective virus
	Virus inactivated by ultra-violet light 80,000 HAU	8,000 HAU	800 HAU	
1	4.2 × 10 <sup>3</sup>	2.7 × 10 <sup>3</sup>	4.9 × 10 <sup>3</sup>	4.5 × 10 <sup>3</sup>
	4.3 × 10 <sup>3</sup>	3.2 × 10 <sup>3</sup>	7.1 × 10 <sup>3</sup>	5.3 × 10 <sup>3</sup>
2	Medium changed			4.8 × 10 <sup>3</sup>
	3.3 × 10 <sup>3</sup>	3.3 × 10 <sup>3</sup>	5.5 × 10 <sup>3</sup>	
3	2.9 × 10 <sup>3</sup>	3.6 × 10 <sup>3</sup>	6.2 × 10 <sup>3</sup>	6.3 × 10 <sup>3</sup>
	2.1 × 10 <sup>3</sup>	3.2 × 10 <sup>3</sup>	5.0 × 10 <sup>3</sup>	8.5 × 10 <sup>3</sup>
4	2.0 × 10 <sup>3</sup>	3.9 × 10 <sup>3</sup>	4.4 × 10 <sup>3</sup>	4.3 × 10 <sup>3</sup>
	2.2 × 10 <sup>3</sup>	5.0 × 10 <sup>3</sup>	5.1 × 10 <sup>3</sup>	2.3 × 10 <sup>3</sup>
5	1.1 × 10 <sup>3</sup>	3.7 × 10 <sup>3</sup>	5.2 × 10 <sup>3</sup>	3.5 × 10 <sup>3</sup>
	Medium changed			3.3 × 10 <sup>3</sup>
7	1.8 × 10 <sup>3</sup>	2.3 × 10 <sup>3</sup>	3.2 × 10 <sup>3</sup>	
	1.2 × 10 <sup>3</sup>	2.8 × 10 <sup>3</sup>	3.1 × 10 <sup>3</sup>	3.9 × 10 <sup>3</sup>
	—	1.4 × 10 <sup>3</sup>	1.9 × 10 <sup>3</sup>	5.6 × 10 <sup>3</sup>
			2.2 × 10 <sup>3</sup>	2.5 × 10 <sup>3</sup>

surfaces containing a large HeLa contribution adhere more readily to glass. Ehrlich ascites cells adhere poorly to glass, so that heterokaryons containing a preponderance of Ehrlich ascites components might also adhere poorly. In this way, adhesion to glass might select heterokaryons with predominantly HeLa surface characteristics.

While they remained multinucleate the heterokaryons did not multiply, but, under suitable conditions, they remained alive on the coverslips for at least five days. After this time some of them began to degenerate and to lose their attachment to the glass, but others survived for

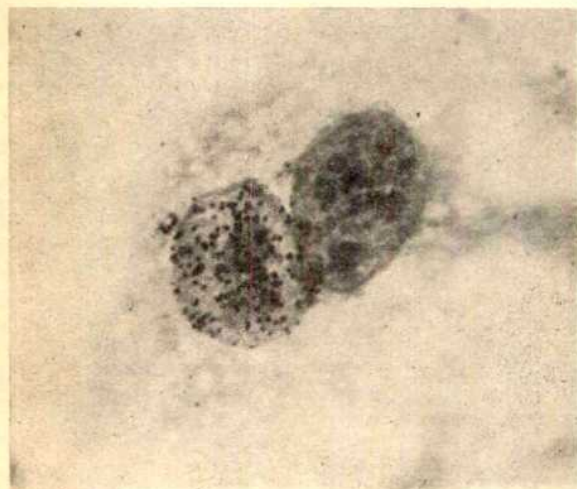


Fig. 3. Autoradiograph of a binucleate cell containing one HeLa nucleus and one Ehrlich ascites nucleus. The HeLa cells had been grown in tritiated thymidine before the heterokaryons were produced. The HeLa nucleus is labelled and the Ehrlich ascites nucleus is not. ( $\times 1,300$ )

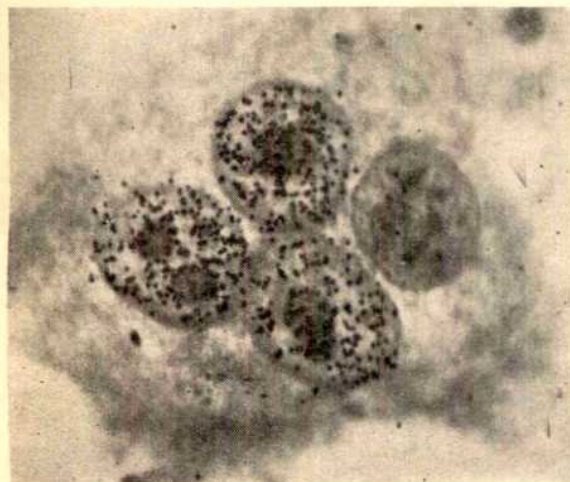


Fig. 4. A tetranucleate cell from the same population as the cell shown in Fig. 3. Three labelled HeLa nuclei and one unlabelled Ehrlich ascites nucleus are shown. ( $\times 1,300$ )

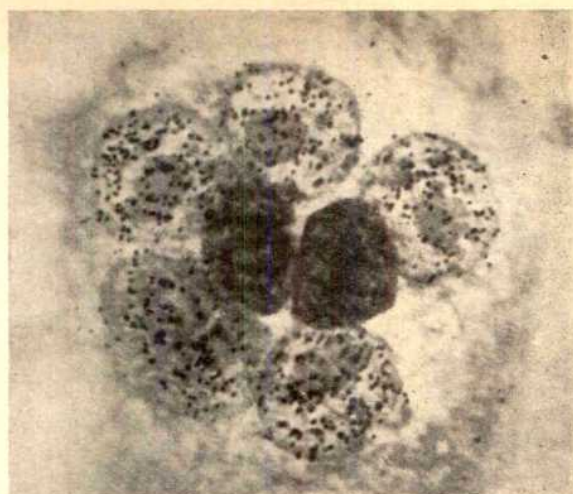


Fig. 5. An octonucleate cell from the same population as the cells shown in Figs. 3 and 4. Six labelled HeLa nuclei and two unlabelled Ehrlich ascites nuclei are shown. ( $\times 1,300$ )

as long as 15 days. Time-lapse cinemicrography revealed that some of the multinucleate cells exhibited sluggish locomotion: they moved a distance approximately equal to their own length in 4 days. The survival times of the heterokaryons produced with different concentrations of inactivated virus and with infective virus are shown in Table 2. The essential reason for the failure of the heterokaryons to multiply appeared to be the fact that the cells contained more than one nucleus. Multinucleate cells containing only HeLa nuclei also failed to multiply, thus indicating that it was the multinucleate state rather than heterokaryosis which was responsible for the deficiency. In mixed cultures containing both mononucleate and multinucleate cells, the mononucleate cells did multiply. It was therefore unlikely that failure of multiplication was due to the injury produced by the virus.

Since each heterokaryon received a large number of virus particles, the possibility existed that the virus inactivated by ultra-violet light might undergo multiplicity reactivation. In order to examine this possibility the production of infective virus and haemadsorption<sup>6</sup> were investigated. For haemadsorption studies two coverslips were examined each day. They were washed once in Hanks's solution and placed in the cups of a haemagglutination tray: 0.5 ml. of a 3 per cent (v/v) suspension of guinea-pig erythrocytes in Medium 199 was added to each cup and the tray was left at room temperature for 2 h, during which time the erythrocytes settled evenly over the coverslips. These were then removed, washed four times in Hanks's solution, fixed in methanol and stained with May-Grünwald-Giemsa. The percentage of heterokaryons showing adsorption of one or more erythrocytes was then determined. The results are summarized in Fig. 6. This shows that when infective virus was used to make the heterokaryons, all of them exhibited the presence of surface haemagglutinin for at least 5 days. But when the heterokaryons were made with inactivated virus, surface haemagglutinin disappeared from the cells at a rate which depended on the initial virus concentration. Moreover, with infective virus, the haemadsorption was massive and involved the whole of the cell surface; with inactivated virus, haemadsorption, in the decreasing number of cells which showed it, involved a progressively smaller part of the individual cell surface. At the lowest concentration of virus (800 HAU/ml.) less than 0.05 per cent of the heterokaryons exhibited haemadsorption at 24 h. On subsequent days occasional heterokaryons (between 0.5 and 1 per cent) did show haemadsorption, but it is clear that at least 99 per cent of the cells did not produce any viral haemagglutinin during the 5-day period. The



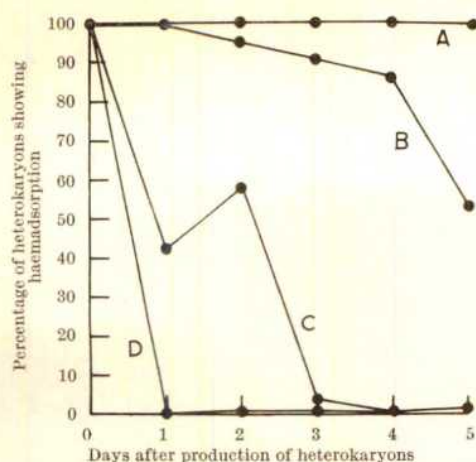


Fig. 6. Rate of loss of surface haemagglutinin in heterokaryons produced by infective virus and virus inactivated by ultra-violet light. A, Infective virus at a concentration of 8,000 HAU/ml. B, C, D, Inactivated virus at concentrations of 80,000, 8,000 and 800 HAU/ml., respectively.

loss of haemagglutinin from the surface of the heterokaryons produced by inactivated virus no doubt reflects the gradual destruction or elution of the initial inoculum.

In order to examine whether heterokaryons produced by inactivated virus released any infectious virus into the medium, 10 coverslips bearing such heterokaryons were transferred to fresh medium after 24-h cultivation and were then incubated in this medium for a further 48 h. At the end of this period 10-fold dilutions of the medium were made in Hanks's solution and 0.1 ml. of each dilution inoculated into the allantoic cavity of two 10- or 11-day-old fertile hens' eggs. After incubation for 48 h at 37°C the allantoic fluids were collected and their haemagglutinin content titrated. The results of this experiment are set out in Table 3, in which the calculated amount of infectious virus produced per heterokaryon is shown. One unit of infectious virus is defined as the amount which produced 2 HAU/ml. of allantoic fluid in 48 h. It will be seen that, when the heterokaryons were made with inactivated virus at a concentration of 800 or 8,000 HAU/ml., less than one heterokaryon in a thousand produced a single unit of infectious virus. With 80,000 HAU of inactivated virus a yield of 1-10 units of infectious virus per heterokaryon was obtained. This does not, however, necessarily mean that multiplicity reactivation had occurred. Small amounts of virus probably escaped inactivation by ultra-violet light, and these might have been responsible for the yield of infectious virus. When the heterokaryons were made with 8,000 HAU/ml. of virus which had not been inactivated, the yield of infectious virus was also only 1-10 units per heterokaryon. This suggests either that auto-interference had occurred or that the virus, even when it is not inactivated, grows poorly in this system. It is, in any event, clear that with moderate or low doses of inactivated virus at least 99 per cent of the heterokaryons produce neither infectious virus nor viral haemagglutinin.

The usefulness of heterokaryons for the analysis of nucleo-cytoplasmic relationships depends to a large extent on the ability of the two sets of nuclei to synthesize RNA

and on the ability of the hybrid cell to synthesize protein. These two functions were therefore examined by studying the incorporation of tritiated uridine into RNA and tritiated leucine into protein. Heterokaryons, which had been maintained on coverslips for periods up to 5 days, were incubated for 2-6 h with tritiated uridine. The cells were then fixed, digested for 30 min at 37°C with deoxyribonuclease and extracted with 0.3 N trichloroacetic acid at 4°C. Autoradiographs of such preparations revealed that all the nuclei in the heterokaryons were labelled. The cytoplasm became labelled also, but more slowly than the nuclei, as in other nucleated cells. Fig. 7 shows an autoradiograph of a heterokaryon from a 24-h culture exposed for 2 h to tritiated uridine. The cell contains a HeLa nucleus and an Ehrlich ascites nucleus: both are labelled, and there is also slight labelling over the cytoplasm. Fig. 8 shows a similar heterokaryon exposed for 6 h to tritiated uridine: the cytoplasm of the cell is now heavily labelled. There was no marked disparity between the HeLa nuclei and the Ehrlich ascites nuclei in their ability to incorporate tritiated uridine into RNA, or, during the first 5 days, between the amount of nuclear labelling in the heterokaryons and the amount in neighbouring mononucleate cells. It thus appears that synthesis of RNA takes place in the heterokaryons in a manner comparable with that seen in mononucleate cells, and that both sets of nuclei

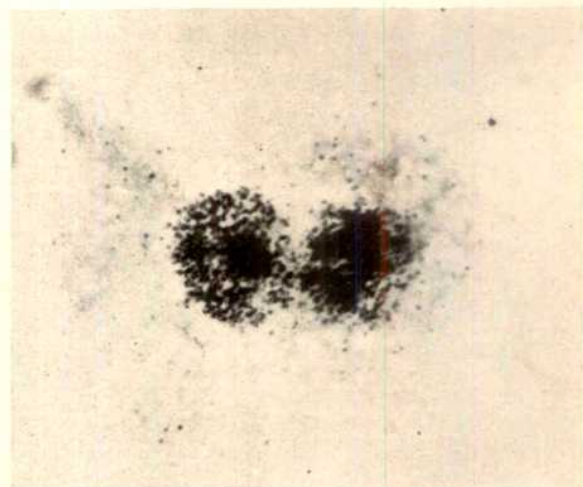


Fig. 7. Autoradiograph of a heterokaryon from a 24-h culture exposed for 2 h to tritiated uridine. The cell contains a HeLa nucleus and an Ehrlich ascites nucleus. Both are labelled, and there is also slight labelling over the cytoplasm. ( $\times 1,100$ )

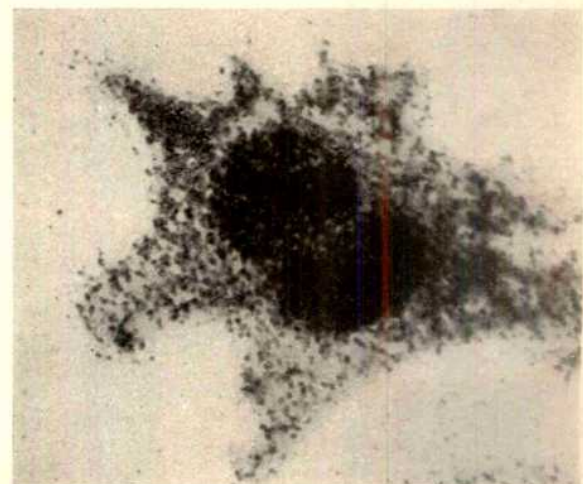


Fig. 8. A similar heterokaryon from the same population as the cell shown in Fig. 7, but exposed for 6 h to tritiated uridine. The cytoplasm of the cell is now heavily labelled. ( $\times 1,100$ )

Table 3. PRODUCTION OF INFECTIOUS VIRUS BY HETEROKARYONS

Concentration of virus used to produce heterokaryons	Haemagglutinin titre (HAU/ml. allantoic fluid) produced in 48 h by 0.1 ml. of culture medium diluted as shown						Units of infectious virus produced per heterokaryon
	$10^0$	$10^{-1}$	$10^{-2}$	$10^{-3}$	$10^{-4}$	$10^{-5}$	
Virus inactivated by ultra-violet light							
800 HAU/ml.	<2	<2	<2	—	—	—	<10 <sup>-3</sup>
8,000 HAU/ml.	<2	<2	<2	—	—	—	<10 <sup>-3</sup>
80,000 HAU/ml.	2,048	512	512	<2	<2	<2	1-10
Infective virus							
8,000 HAU/ml.	512	2,048	<2	<2	<2	<2	1-10
	512	256	256	<2	<2	<2	



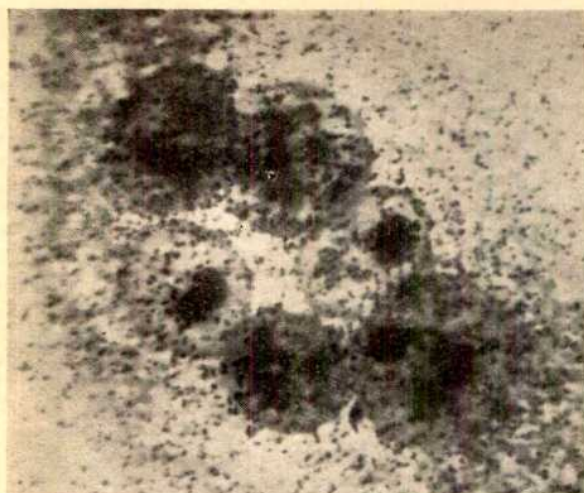


Fig. 9. Autoradiograph of a heterokaryon from a 24-h culture exposed for 6 h to tritiated leucine. There is generalized labelling over nuclei and cytoplasm. ( $\times 1,100$ )

are involved in this synthesis. The genes of both mouse and man are therefore transcribed.

Autoradiographs of heterokaryons, maintained on coverslips for periods up to 5 days and then exposed for 6 h to tritiated leucine, revealed generalized labelling over nuclei and cytoplasm (Fig. 9). Failure to extract this label with 0.3 N trichloroacetic acid at 90°C confirmed that the tritiated leucine had been incorporated into protein. It is therefore clear that the heterokaryons synthesize protein as well as RNA.

Although heterokaryons in the multinucleate state did not multiply, they did synthesize DNA. When cultures which had been maintained on coverslips for 24 h were exposed for 2 h to tritiated thymidine, autoradiographs showed that about 70 per cent of the heterokaryons contained labelled nuclei. In 5 per cent of these labelled heterokaryons all the nuclei in the cell incorporated the precursor, but in the rest labelled and unlabelled nuclei were present in varying proportions. In any one cell some nuclei of each type might be labelled and others not. Fig. 10 shows a multinucleate cell in which all the nuclei are synthesizing DNA, and Fig. 11 shows a multinucleate cell in which only two of the Ehrlich ascites nuclei are synthesizing DNA. In these labelled heterokaryons about 80 per cent of the Ehrlich ascites nuclei were synthesizing DNA, but only about 30 per cent of the HeLa nuclei. The percentage of heterokaryons showing labelling after 2 h exposure to tritiated thymidine fell from 70 at 24 h to 55 at 48 h, 44 at 4 days and 31 at 7 days. But in those heterokaryons which did show some form of nuclear labelling, the proportion of Ehrlich ascites nuclei and of HeLa nuclei which were synthesizing DNA underwent little change over 7 days. An exponentially growing population of Ehrlich ascites or HeLa cells exposed to tritiated thymidine for 2 h would show labelling in about a third of the nuclei, an expression of the duration of

the phase of DNA synthesis relative to the total intermitotic time. The very high proportion of labelled Ehrlich ascites nuclei in the heterokaryons, therefore, means that these nuclei have become partially synchronized in the phase of DNA synthesis. This partial synchronization might have resulted from selection during cell fusion of those Ehrlich ascites cells in which synthesis of DNA was taking place. But the fact that 80 per cent or so of the Ehrlich ascites nuclei in labelled heterokaryons were synthesizing DNA not only at 24 h, when 70 per cent of the heterokaryons were labelled, but also at 48 h, when 55 per cent of the heterokaryons were labelled, suggests another explanation. It seems more probable that many of the Ehrlich ascites nuclei which were not in the phase of DNA synthesis at the time of formation of the heterokaryons passed into this phase during the first 24 h, and that DNA synthesis was not terminated abruptly after a few hours, as occurs in normal cells, but continued for much longer periods. Any alternative explanation would seem to require that at least some of these nuclei underwent two or more normal cycles of DNA synthesis without mitosis. Why the percentage of HeLa nuclei synthesizing DNA at 24 h should be so much lower is obscure, but, since this percentage also shows little change on subsequent days, it is likely that the phase of DNA synthesis was prolonged in at least some of the HeLa nuclei also. These findings, in any event, make one point clear. Whether or not a mammalian cell nucleus synthesizes DNA cannot be solely determined by events in the cytoplasm. Even in a common cytoplasm DNA synthesis may, at any one time, be taking place in some nuclei and not in others; and the

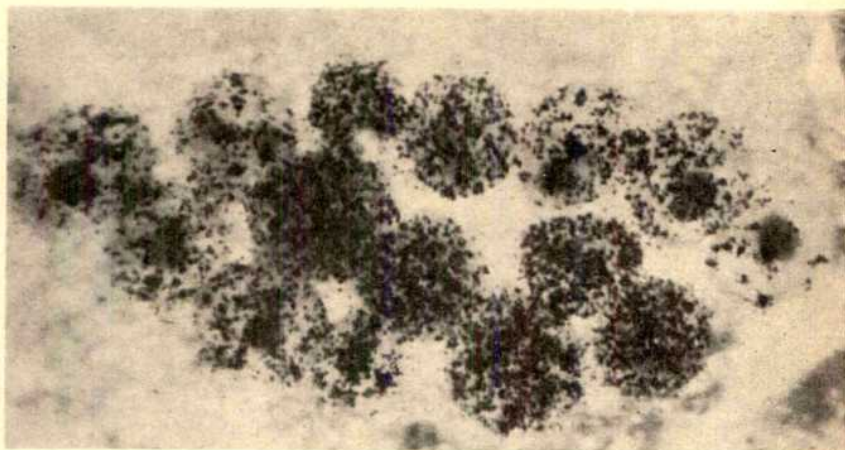


Fig. 10. Autoradiograph of a heterokaryon from a 24-h culture exposed for 2 h to tritiated thymidine. All the nuclei are synthesizing DNA. ( $\times 1,100$ )

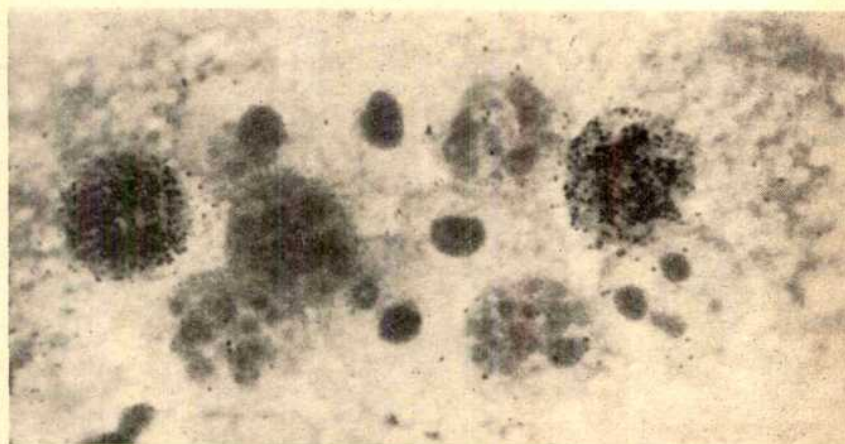


Fig. 11. A heterokaryon from the same population as the cell shown in Fig. 10. Only two of the Ehrlich ascites nuclei are synthesizing DNA. ( $\times 1,300$ )



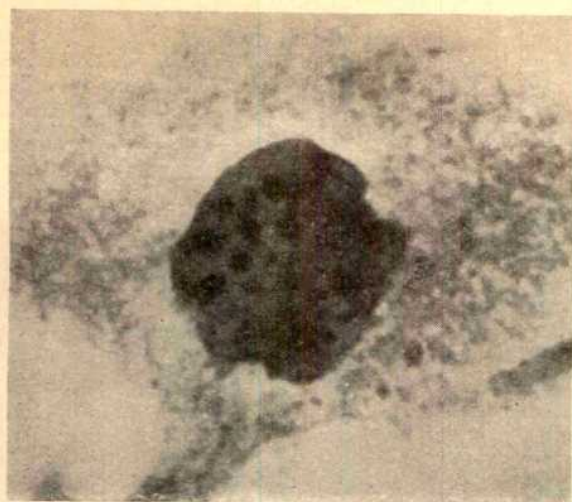


Fig. 12. A cell containing a giant nucleus produced by fusion of a number of discrete nuclei. Several nucleoli or condensations of chromatin are to be seen. ( $\times 1,100$ )

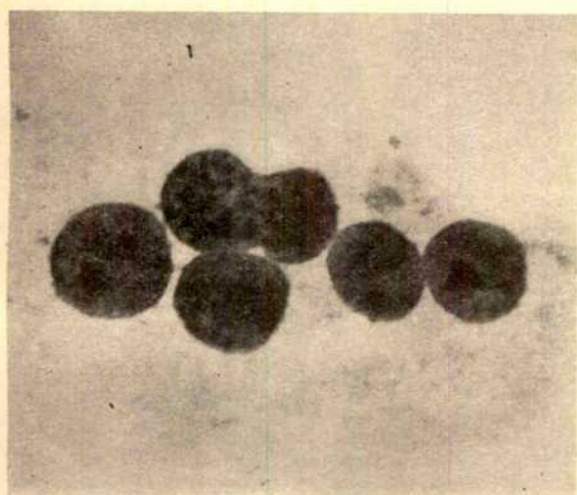


Fig. 13. A heterokaryon in which two of the Ehrlich ascites nuclei are in the process of fusion. ( $\times 1,100$ )

had been grown in tritiated thymidine with unlabelled Ehrlich ascites cells, and vice versa. Autoradiographs of such preparations showed that many of the large nuclei were labelled over only one part of the nucleus. Fig. 13 shows a heterokaryon in which two of the Ehrlich ascites nuclei are in the process of fusion. Fig. 14 shows a giant nucleus in a preparation made by fusing labelled Ehrlich ascites cells with unlabelled HeLa cells: only the right-hand half of the nucleus is labelled.

It might be thought that nuclear fusion represented an extension of the activity of the virus to the nuclear membrane. But experiments on the relationship between the concentration of virus used and the amount and rate of nuclear fusion revealed that this was not the case. Fig. 15 demonstrates that the percentage of cells showing nuclear fusion on successive days is inversely related to the concentration of virus used. With inactivated virus at a concentration of 800 HAU/ml., about 25 per cent of the heterokaryons showed some degree of nuclear fusion at 24 h, and by 5 days nuclear fusion had occurred in almost all the cells. But Fig. 6 demonstrates that at least 99 per cent of the heterokaryons produced by this concentration of inactivated virus had lost all trace of virus haemagglutinin from their surface within 24 h. It therefore appeared unlikely that nuclear fusion, which progressed throughout

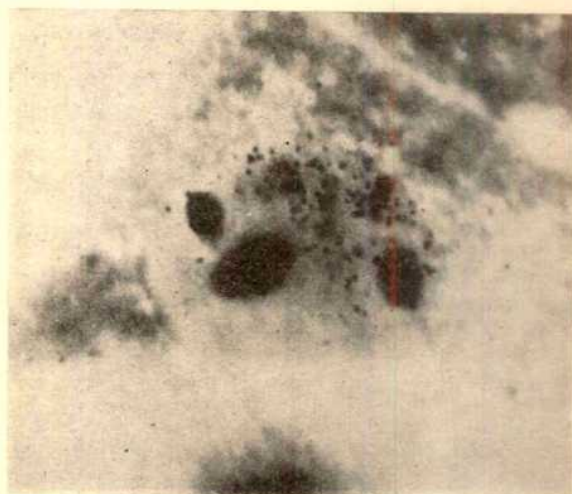


Fig. 14. Autoradiograph of a large nucleus produced by fusion of labelled Ehrlich ascites nuclei with unlabelled HeLa nuclei. The Ehrlich ascites cells were exposed to tritiated thymidine before the heterokaryons were produced. Only the right-hand half of the nucleus is labelled. ( $\times 1,300$ )

nuclei from one species may be more active in the heterokaryon than the nuclei from another. The ability of the nucleus to synthesize DNA must therefore depend, at least in part, on factors operating within the nucleus.

Over a 7-day period there was a progressive fall not only in the proportion of heterokaryons showing labelling after exposure to tritiated thymidine, but also in the intensity of the labelling. At 24 h there was no great difference between the intensity of nuclear labelling in the heterokaryons and that found in neighbouring mononucleate cells; but at 7 days the intensity of labelling in the heterokaryons was greatly reduced relative to that found in mononucleate cells. It thus appears that although the phase of DNA synthesis is prolonged in the nuclei of heterokaryons, the rate of synthesis progressively falls, and eventually synthesis stops.

Under certain conditions the nuclei within the heterokaryons underwent fusion. The fused nuclei could usually be recognized by their greater size and by the fact that they contained many large nucleoli or condensations of chromatin. In many of the multinucleate cells only some of the nuclei underwent fusion, but in others all the nuclei in the cell fused together to produce one giant nucleus which sometimes had a bizarre shape (Fig. 12). Fusion took place not only between nuclei of the same kind but also between HeLa nuclei and Ehrlich ascites nuclei. This was demonstrated by fusing HeLa cells which

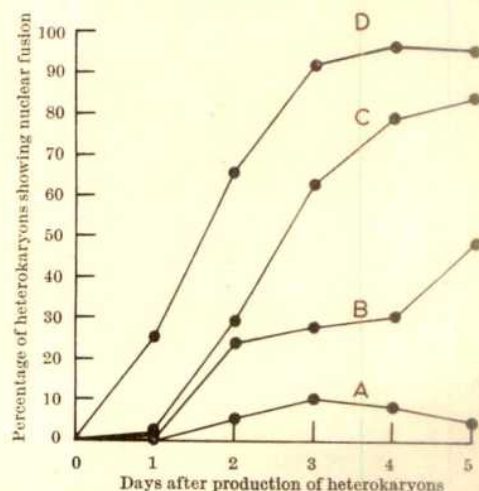


Fig. 15. Relationship between the proportion of heterokaryons showing nuclear fusion and the concentration of virus used to produce the heterokaryons. A, Infective virus at a concentration of 8,000 HAU/ml. B, C, D, Inactivated virus at concentrations of 80,000, 8,000 and 800 HAU/ml. respectively

the 5-day period, was caused by the continued activity of the virus. Indeed, the results indicated that the presence of large amounts of virus inhibited nuclear fusion. With increasing concentrations of virus the proportion of heterokaryons showing nuclear fusion on successive days was reduced; and, at the same dose, infective virus inhibited nuclear fusion more drastically than inactivated virus. It seems as if progressive nuclear fusion is the normal course of events in the heterokaryon, and that the inhibition produced by high doses of virus is a non-specific toxic effect. Preliminary experiments indicate that other toxic substances may also inhibit nuclear fusion. Since the term heterokaryon, as it is generally used, implies a cell with more than one nucleus, multinucleate cells in which the nuclei have fused together to form a single nucleus will be called synkaryons: homosynkaryons where nuclei of the same type have fused together, and heterosynkaryons where nuclei of different types have fused together.

The synkaryons continued to incorporate tritiated uridine into both nuclear and cytoplasmic RNA for at least 5 days at a rate not greatly different from that found in single HeLa cells in the same culture. Some of them also incorporated tritiated thymidine into DNA, although, over a 7-day period, the proportion showing labelling, and the intensity of the labelling relative to single HeLa cells in the same culture, progressively fell. There was, however, reason to believe that some synkaryons, including heterosynkaryons, were able to undergo at least one mitosis. In cultures containing both multinucleate and mononucleate cells it was observed that some of the cells rounded up in mitosis were very much larger than the rest. The cultures were therefore exposed for 18 h to colchicine at a concentration of 0.0025 per cent (w/v), and the karyotypes of the accumulated metaphases examined by the method of Rothfels and Siminovich<sup>7</sup>. It was found that some of the metaphase figures had up to 300 or more chromosomes, whereas the modal chromosome number for the HeLa and Ehrlich ascites cells was about 80. Moreover, some of these giant metaphases showed large numbers of both metacentric and telocentric chromosomes, including many long telocentrics. HeLa cells normally contain not more than one long telocentric chromosome, and frequently none at all<sup>8</sup>; and Ehrlich ascites cells normally contain very few metacentric chromosomes<sup>9</sup>. The presence of large numbers of both

metacentric and long telocentric chromosomes in the one cell therefore suggests that the cell originally contained both HeLa and Ehrlich ascites nuclei. Whether heterosynkaryons can produce clones of cells containing both human and murine chromosomes is the subject of current investigation.

In the experiments which we have described, an inactivated virus has been used to impose a form of artificial sexuality on mammalian tissue cells. Since the resulting hybrid cells synthesize protein, and both sets of nuclei synthesize RNA, these hybrids can be used to investigate problems of nucleocytoplasmic relationship which could hitherto be studied only in the heterokaryons of fungi or yeasts. Some features of the mammalian cell system perhaps warrant special comment. The remarkable fact that viable heterokaryons can be made with cells originating from different animal species means that a very wide range of genetic markers can be used to distinguish the two cell types. Indeed, the choice is limited only by the susceptibility of the cells to the virus used to produce fusion. The average number of nuclei per heterokaryon and the proportion of nuclei of each type can, within certain limits, be controlled. Preparations can be made in which 85 per cent or more of the cells are heterokaryons, and the ratio of multinucleate to mononucleate cells can be varied. Unlike fungi or yeasts, mammalian cells use thymidine as a specific precursor of DNA. This property makes mammalian cell heterokaryons eminently suitable for investigations on the regulation of DNA synthesis, as some of the present experiments show. Finally, autoradiographic and chemical techniques for the study of RNA metabolism in mammalian cells have now reached a moderate degree of sophistication: these procedures can be applied without modification to mammalian cell heterokaryons. There is, therefore, reason to hope that these heterokaryons will lend themselves to experiments which have hitherto not been possible in animal cells.

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## PROGRESS OF PALAEOONTOLOGY IN CHINA

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MODERN scientific investigation of fossils in China dates back to the 1920's. The few palaeontologists made a good start in spite of the difficult working conditions. Many valuable monographic papers were published in *Palaeontologia Sinica*. The memoirs on Fusulinidae (Protozoa), Productidae (Brachiopoda) and others published by the journal are still among the classics of palaeontological literature.

But the investigations were confined to only a few groups of organisms including the fossils of plants, foraminifera, corals, brachiopods, trilobites and graptolites of the Palaeozoic era, Mesozoic reptiles and Pleistocene mammals. The materials were collected in a few districts with many regions of palaeontological interest unexplored.

Palaeontology has made considerable progress since New China was established in 1949. The number engaged in palaeontological investigations has increased from about 40 in 1949 to the present 400 to meet the growing demand in the fields of geological surveying, exploration, science and education.

In addition to *Palaeontologia Sinica*, two new periodicals, *Acta Palaeontologia Sinica* and *Vertebrate Palaeoasiatica*, have been published.

At present the Institute of Geology and Palaeontology of the Academia Sinica at Nanking is mainly concerned with the examination of plant and invertebrate fossils and biostratigraphy. The Institute of Vertebrate Palaeontology and Palaeoanthropology of the Academia Sinica



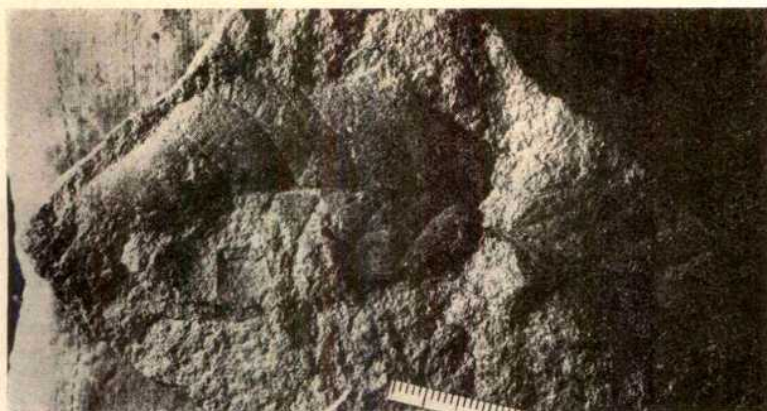


Fig. 1. Fossil of *Quercus semicarpifolia* Sm. of the Upper Pliocene Period of the Neogene Age found on the northern slope of Mt. Shisha Pangma in Tibet



Fig. 2. Fossil of *Quercus pannos*—H.-M. of the Upper Pliocene Period of the Neogene Age found at the northern slope of Mt. Shisha Pangma in Tibet

at Peking deals chiefly with the problems of vertebrate and human palaeontology and the closely related subjects of palaeolithic archaeology and vertebrate-bearing Caino-stratigraphy. Palaeontological laboratories are found in the Ministry of Geology, Ministry of Petroleum Industry, Ministry of Coal Industry, regional geological institutes, universities, colleges and museums.

Undergraduate and postgraduate courses in palaeontology are offered in the University of Peking, University of Nanking, Peking Geological College and other institutions of higher learning. The steady flow of trained personnel ensures the further development of this science.

What follows is a brief account of the work in the four main subdivisions of palaeontology: palaeobotany, invertebrate palaeontology, vertebrate palaeontology and palaeo-anthropology.

### Palaeobotany

The examination of plant fossils has an important bearing on the dating and correlation of the coal series and other continental or alternately marine and continental sediments, and on palaeogeographical and palaeoclimatological researches. In the past fifteen years progress has been made in the examination of megascopic plant fossils. Pollen and spore analysis, a valuable tool in dating the so-called 'dumb' or non-fossiliferous beds, has achieved notable results.

The Sinian and Cambrian spores discovered at Chihhsien County, Hopei Province, and Linhsien County, Shansi Province, indicate that some may belong to certain primitive land plants.

Sinian algae and post-Devonian species of *Chara* have been found in large quantity and are being investigated.

Devonian was the age when land plants began to flourish. Early Devonian plant fossils are known only in the province of Yunnan in China, and those of the Middle Devonian in Hunan, Szechuan, Kweichow and Kwangsi Provinces. Rapid progress has been made in the examination of the Late Devonian floras in the past few years. Their fossils are known in more than thirty localities in China; most were discovered after 1949. The presence of the Namurian rocks in South China and the Chilian

Mountains is chiefly demonstrated through examining the plant fossils. Great progress has also been made in the investigation of Late Palaeozoic floras, which comprises most of the palaeobotanical work done before 1949. Through detailed and comprehensive investigations of the large amount of new materials, our knowledge concerning the floral distribution, evolution and correlation of the Late Palaeozoic floras has increased.

From Triassic times, most of the sedimentary rocks in China are of continental origin and among them are many coal-bearing beds. Many problems in connexion with the subdividing and dating of these beds remain to be tackled, but examination of the microfossils and pollens and spores have yielded important preliminary results.

Attention has also been directed to the Quaternary pollens and spores. They are significant in elucidating the history of changes in the climate and vegetation of China during this period.

### Invertebrate Palaeontology

This is by far the largest and most varied of the fossil groups and has the most extensive application in geology. The examination of megascopic and microscopic fossils plays an important part in geological survey and the exploration of coal, petroleum and the ore deposits of iron manganese, phosphorus and aluminium.

Apart from providing material for stratigraphical correlations, invertebrate fossils also furnish important data for solving problems in palaeogeography, palaeoecology, evolution and migration. For example, the fossils of the fusulinids are abundant in China. In the early Carboniferous time they had already shown that the inland seas of China were connected with the Uralian and Mississippian seas.

In the middle Carboniferous time freer communication between them, especially between those of China and the boreal region, was maintained. Later in this period the fusulinid faunas of China showed close affinity with those of the Urals. At the same time they were quite close to those of southern Europe, central Asia and Japan, and also related to those of North America. In the early Permian time the fusulinids were well developed and widely distributed in the Tethys of South China and other inland seas connected with it, and some of the forms also made their appearance in the north-western part of North America. In the late Permian, at the time of world-wide regression, many parts of China were still experiencing transgression with small-sized fusulinids and other foraminiferas.

While the Devonian corals of South China are close to those of Western Europe and the Urals, those of inner Mongolia and North-east China are near to the faunas of eastern North America. The Carboniferous coral faunas of South China in the main succeeded those of the Devon-



ian, and the same relationship was retained. The Permian corals of China belong to the Tethys province.

The Palaeozoic brachiopods of China are quite characteristic and show strong local aspect. In certain places they are similar to the North American forms; in others, to those of Europe.

Much research work has been carried out on fossil molluscs and bryozoans. The formerly much neglected groups of freshwater gastropods, pelecypods and ostracods have received particular attention, and investigations in this connexion have made considerable headway. As to the trilobites and graptolites, in addition to much work on systematic description and biostratigraphy, attention has been directed to the zoogeography, ontogeny, phylogeny and microscopic structure of many of these groups, and significant results are being achieved.

Recently, under the direction and auspices of the Institute of Geology and Palaeontology, a book entitled *Fossil Invertebrata of China* has been published. It comprises the systematic revision, description and illustration of nearly all the more-than-6,000 species and sub-species of fossil invertebrates known so far in China. This work will no doubt contribute greatly to the development of palaeontological and biostratigraphical researches in China.

The Palaeozoic transgression of China, which was of wide extent and long duration, brought with it a vast array of rich marine invertebrate faunas. The Chinese palaeontologists have contributed a great service to geology in establishing stratigraphical columns and in subdividing and correlating the various Palaeozoic rock systems, series and stages. For example, results have been achieved in defining boundaries between Carboniferous and Permian, and between the Lower and Upper Permian.

### Vertebrate Palaeontology

Continentially deposited rocks, ranging in age from Devonian to Quaternary, were extensively developed in many parts of China with rich remains of fossil vertebrates. The streams and seas of the Devonian period were the centres of radiation of many groups of archaic fishes or fish-like lower vertebrates.

In southern China, Devonian redbeds corresponding to the Old Red Sandstones of northern Europe have yielded abundant remains of agnathans, placoderms, primitive sharks and bony fishes, many of which are of the same type as those of Europe or North America.

During the late Palaeozoic and Triassic times when the earlier reptiles and the reptilian ancestors of the mammals (or mammal-like reptiles) dominated the land, vast tracts of land emerged in China and were inhabited by these earlier tetrapods (Fig. 3).

Recently several expeditions have been organized to explore the richly fossiliferous beds in Sinkiang, Shansi and Yunnan, and large quantities of fossils have been excavated. The characters of faunas are in many respects common to those found in the southern parts of Africa. Fossils of Permian reptiles and Triassic amphibians (stegocephalians), both of which were unknown in China before, have been recovered. In almost every province various groups of Mesozoic reptiles have been found.

The widely distributed fossil remains of dinosaurs have been found in eastern China along the coasts as well as in the western gobis in northern Sinkiang. The Triassic dinosaurian faunas of Yunnan are nearly the

same as those of South Africa and western Europe; but those of the late Mesozoic are similar to North American forms. In Shantung, Kwangtung, Kiangsi, Hunan and Sinkiang, numerous reptilian eggs, many of them of dinosaurs, were collected.

In recent years many good specimens of marine reptiles and some Cretaceous flying reptiles have also been found. Remains of the former have been located recently in the Himalayas in Tibet. Of the Cainozoic or 'age of mammals', progress has been made in recent years with the discovery of many fossils of archaic mammals. Palaeocene mammals are known in Kwangtung, Hunan, Kiangsi and Sinkiang. Some of these early Cainozoic mammalian faunas (Eocene) are similar to those of Korea, Burma and Pakistan, and some quite similar to those of North America (Palaeocene and earlier Eocene), and the fish fauna of that time included many forms now living in south-eastern Asia, such as some of the islands of Indonesia. The general aspects of late Tertiary mammalian faunas of China resemble those now found in Africa rather than the modern Chinese faunas.

Examination of Quaternary mammals has made rapid progress as a result of its intimate connexion with engineering geology and anthropology. Since the middle Pleistocene the mammalian faunas of South China have been essentially the same as those of the countries in south-east Asia.

### Palaeoanthropology

Palaeoanthropology deals with the origin and evolution of man. All the earliest human fossils so far known in the world were found in Asia and Africa, such as *Atlanthropus* (Algeria), *Pithecanthropus* (Java) and *Sinanthropus* (China). The many important palaeoanthropological discoveries made in Africa in recent years have attracted world-wide attention and have aroused keen interest among Chinese scientists.

China is one of the few countries in the world particularly rich in human fossil remains (Figs. 4 and 5). In the past fifteen years many important new discoveries have been made in various parts of the country. Among these new findings the Tzeyang Man, found at Tzeyang County in Szechuan, and the Liukiang Man of Liukiang, Kwangsi,

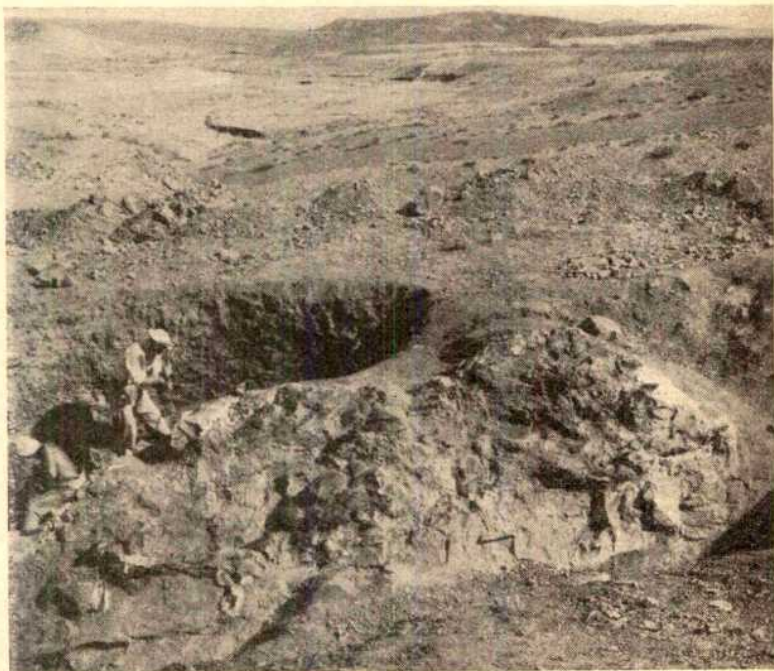


Fig. 3. A huge block with ten skeletons of mammal-like reptiles of the Late Triassic Age is being excavated at the Dzungaria Basin in Sinkiang during the 1964 palaeontological expedition of the Academia Sinica





Fig. 4. Side view of the mandible of *Sinanthropus lantienensis* ( $\times c. 1$ )

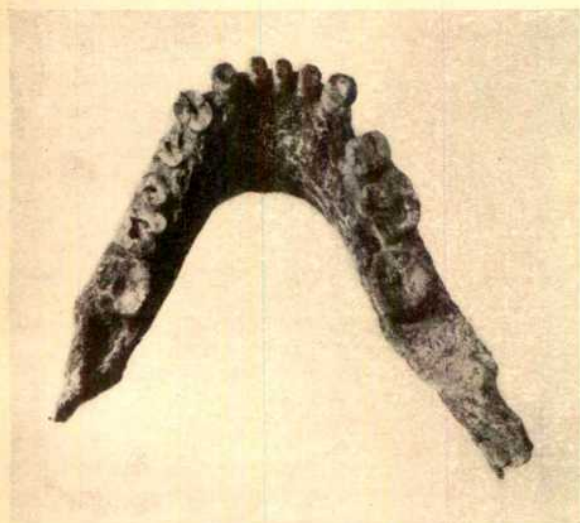


Fig. 5. Dorsal view of the mandible of *Sinanthropus lantienensis* ( $\times c. 0.75$ )

are of *Homo sapiens* type, corresponding to the Cromagnon type man of Europe. At Mapa in Kwangtung, and Tingtsun in Shansi, remains of the fossil man of the Neanderthal type were unearthed. In the well-known locality at Choukoutien new materials of *Sinanthropus pekinensis* or

Peking Man have also been excavated. In 1963 and 1964, at Lantien, near Sian in Shensi, a new type of *Sinanthropus*, including a well-preserved skull cap, was discovered. At many of these and other localities, a large number of cultural remains of early man, mainly palaeolithic implements, were found.

In close connexion with the examination of the origin of man is the examination of fossils of other higher primates. Some years ago at Liucheng in Kwangsi Province, a great number of fossils of *Gigantopithecus*, including three well-preserved mandibles and thousands of teeth, were excavated from the Lower Pleistocene cave deposits. These discoveries have aroused great interest in the scientific world. Palaeontologists and anthropologists still differ greatly as to whether *Gigantopithecus* was a kind of giant man or giant ape. The Chinese scientists who have examined the material also hold varying views. Some think that *Gigantopithecus* is a hominid of pre-homotype; others maintain that it is a member

of the Pongid family.

The discovery of the remains of *Dryopithecus* in Yunnan some years ago has supplied an important link in the evolution of man in China.

### Conclusion

Palaeontological investigation calls for a high degree of international co-operation. Constant exchange of information and experience is indispensable for the palaeontologist wherever he may be. Many of the fossils found in China are not only similar to those of her neighbouring countries, but also show close affinity with those recovered in other continents.

In some cases, fossils of constituent parts of the same kind of organisms are found separately in different countries. Here the need for international co-operation is obvious.

Besides, there are many problems in palaeontology, such as the origin and dispersal of many a group of organisms, the correlation of biota of the different regions of the world, and the investigations of fundamental principles of organic evolution—problems on which we cannot obtain a comprehensive view or reach a sound conclusion without access to relevant information gathered from other parts of the world. Frequent contact between palaeontologists of various countries will not only promote progress in science but also further strengthen the friendship and solidarity of the world's people.

## OBSERVATION OF RADIOACTIVE FALL-OUT IN NIGERIA UP TO 1961

By DR. B. N. C. AGU

Physics Department, University of Ibadan, Nigeria, and Director of Federal Radiation Protection Service

THE deposition of fission products from nuclear test explosions has been measured since 1954 by R. N. Crooks *et al.*<sup>1</sup> for 25 stations in many parts of the world. The measurements assessed the quarterly deposition of strontium-90 in rain-water and the ratio of caesium-137 to strontium-90 in the samples. Their results for Lagos, Nigeria, up to the middle of 1959 indicated low levels of fall-out, consistent with the fall-out pattern expected for an equatorial region from the world-wide fall-out distribution<sup>2</sup>. However, because of the close proximity of the French Nuclear Test Site at Reganne to the densely populated countries of tropical Africa the possibility of

local fall-out occurring in these countries became a matter of concern and it was decided to make specific measurements.

Systematic fall-out sampling was started in Nigeria in October 1959, four months ahead of the first nuclear explosion at Reganne. Six sampling stations were set up with the aid of the United Kingdom Atomic Energy Authority at airport meteorological stations situated at Maiduguri, Kano, Kaduna, Sokoto, Lagos and Port-Harcourt. These stations were chosen to provide a fairly good coverage for the whole territory of the Nigerian Federation of about 800,000 km<sup>2</sup> in area.



Altogether four nuclear explosions above ground were recorded during the Sahara test series, on February 13, April 1 and December 27, 1960, and on April 25, 1961. Only the first of these explosions resulted in significant fall-out in Ghana<sup>3</sup>, Nigeria, Israel<sup>4</sup>, Czechoslovakia<sup>5</sup>, Panama Zone<sup>6</sup>, United Kingdom<sup>7</sup> and a few other places, while the third and fourth explosions gave rise to only limited fall-out in Nigeria. The nearest Nigerian sampling station to the Sahara Test Site was at Sokoto, situated 1,300 km south-south-west of Reganne, and the farthest was at Port-Harcourt, 2,200 km to the south-south-west. However, the highest fall-out observed during the French test was at Maiduguri in Northern Nigeria, about 1,900 km south-west of Reganne.

**Meteorological factors.** The Nigerian Meteorological Services produced daily upper air charts for the region during the period of the Sahara nuclear tests. The trajectories showing the positions at various times of air parcels from Reganne on February 13 at 0600 G.M.T.—the time of the first explosion—were drawn for heights of 3,000 m, 6,000 m, 8,000 m, 10,000 m and 12,000 m above sea-level. For mid-day of February 13, 1960, 6 h after the first explosion, the charts showed an unusual wind pattern (Fig. 1). At Reganne the upper winds were mainly strong westerly, but farther to the east there was a change to north-westerly winds in layers around 10,000 m above sea-level. From the observed wind patterns and the dates of arrival of the radioactive debris at the various sampling stations, most of the radioactive clouds which left Reganne in the layer between 6,000 and 12,000 m reached Northern Nigeria in about 30 h.

Thus, the changes in direction with height, and the high wind-speeds, had the effect of carrying radioactive clouds relatively quickly to places in West Africa where, based on the more usual wind patterns, little fall-out had been expected.

**Fall-out measurements.** The three sampling methods used by the United Kingdom Atomic Energy Authority for obtaining fall-out samples from specific test explosions were adopted for Nigeria. The first method, which provides a sample of deposited particulate fall-out (often referred to as a 'sticky-paper' sample), consists of exposing

horizontally a 30 cm × 15 cm sheet of adhesive cellulose tape for a period of 24 h. In the second method, air is drawn continuously through a filter-paper of about 5 cm diameter, so that all particles in a known volume of air are trapped on the filter-paper. In the third, a daily rainfall sample is collected.

After installation, test runs were made with the apparatus to check procedures and establish background levels, and the stations were then held in readiness. On February 13, 1960, the date of the first nuclear explosion at Reganne, all stations were started and 24-h samples were flown to Kano where a preliminary assessment was made with an Ericsson type 1320B monitor. The samples were then sent by air to the United Kingdom for measurement at the Atomic Weapons Research Establishment, Aldermaston.

For the deposition samples of moderate activity, the standard counter was a 6 in. × 6 in. anthracene scintillator, which could detect a minimum activity of 920  $\mu\text{c./m}^2$  at 10 per cent counting efficiency. For low-activity samples, a gas-flow counter with anti-coincidence ring was available which had 40 per cent counting efficiency. A 2-in. end-window Geiger counter, type 2B2, was used to count air-filter samples of moderate activity, while a low background counter with anti-coincidence ring was available for samples of low activity. The water samples were first treated to concentrate their activity into a smaller bulk and then counted with the same equipment used for the air-filter samples.

The daily dispatch of samples to Aldermaston was continued until the preliminary assessment at Kano showed no appreciable fall-out activity. In general the samples arrived at Aldermaston several days after collection, and the activity of each sample was extrapolated back to the mid-time of the collection period by means of a  $t^{-1.2}$  decay curve characteristic of mixed fission products.

**Results.** No results were obtained from rainfall samples during the week following the first explosion as no rain fell in the northern part of Nigeria. Light showers occurred in Lagos and Port-Harcourt on February 17, and again at Port-Harcourt on February 19, but the level of activity in the rain-water collected was insignificant.

Fall-out was first detected at Maiduguri in Northern Nigeria on February 14, 1960, where an activity of 12,100  $\mu\text{c./m}^2$  was recorded on the sticky-paper sample. From that time onwards, the daily arrival of radioactivity decreased rapidly to less than 0.5 per cent of this maximum value in about a week, as shown in Fig. 2. Sokoto showed a similar pattern, but this began a day later than at Maiduguri and had a lower peak-value of 1,500  $\mu\text{c./m}^2$  for the sticky-paper sample. Farther south, at Lagos and Port-Harcourt, the same pattern of a sudden start at a high activity and a steady decrease to the normal background of less than 1  $\mu\text{c./m}^2$  was followed three days after the first test explosion at Reganne.

However, at Kano and Kaduna, both also in the north, a slightly different pattern was observed. In these two places, the peak fall-out was reached on February 15, one day after the first arrival of radioactive debris at these stations. Thereafter, the decrease in activity followed the usual pattern.

No explanation is offered, but it is interesting to note that the air-filter sample histograms (apart from that for Sokoto) have profiles which are consistent with this effect.

The second Sahara test explosion, on April 1, 1960, produced no detectable fall-out in any part of Nigeria, although workers in Israel<sup>4</sup> and Ghana<sup>3</sup> observed some fall-out from this explosion. The third test, on December 27, 1960, gave rise to very low fall-out activity of the order of 4  $\mu\text{c./m}^2$  for sticky-paper samples in all parts of the country, while the highest value for filter-paper samples, namely, 0.026  $\mu\text{c./m}^2$ , was collected at Kaduna. For the fourth test, on April 25, 1961, the sticky-paper samples showed no activity; while the maximum filter-paper sample was 0.0049  $\mu\text{c./m}^2$  at Kano. During this test rain-water samples were obtained for Maiduguri

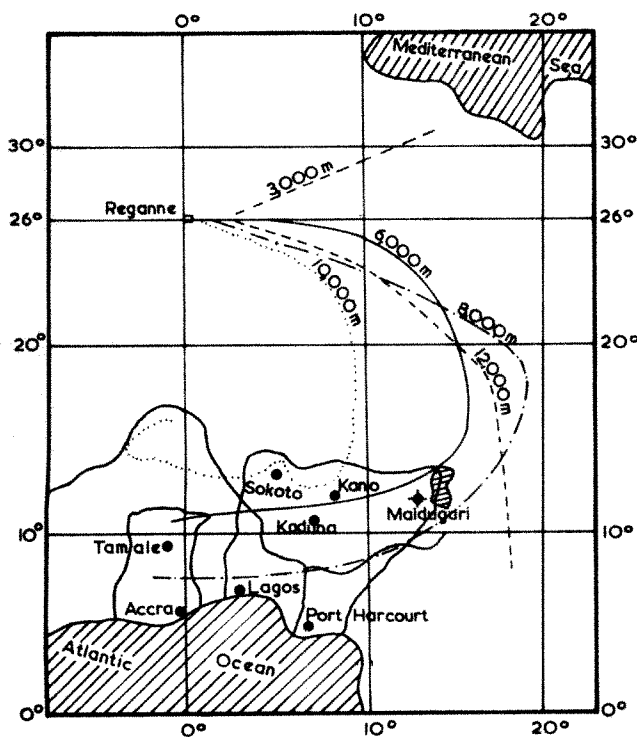


Fig. 1. Trajectories at constant height of air parcels from the test site at Reganne

and Port-Harcourt, where  $12.2 \mu\text{C}/\text{m}^2$  and  $3.1 \mu\text{C}/\text{m}^2$  respectively were recorded.

**Radiological considerations.** In estimating the dose which could be received by the gonads from fall-out deposited on the ground, conditions were assumed which would result in maximum exposure. The case was taken of a man living and sleeping in the open, who is exposed to radiations from a smooth infinite plane. In these conditions the dose to the gonads from the highest recorded levels of deposited fall-out would be only one-fifth of the average annual dose (0.1 rem) to individuals from natural causes.

When considering the possible hazard from strontium-90 it is important to remember the agricultural conditions prevailing at the time of the first test explosion. The main dietary source of strontium-90 is milk, and this element enters the food-chain principally from the grazing of cattle on contaminated pastures. Surface vegetation was very limited in Northern Nigeria during the period of the test, and hence the intake of strontium-90 by grazing cattle would be very small. Further possible sources of strontium-90 would be water from open pools and food exposed in the open. At the time of the explosions there were few standing crops which could be thus contaminated and most water is obtained from wells which are covered and so protected from contamination by fall-out.

**Discussion.** It is of interest to note that the sticky-paper method of particulate sample collection used in Nigeria was also used by the Israeli workers<sup>4</sup>. It is considered that this method is to be preferred to that of Ward and Marr<sup>3</sup>, who collected fall-out in Ghana by sweeping up the dust from a measured area of a flat, concrete roof, a technique which is liable to lead to an underestimate due to losses in the sweeping-up process. Both methods, it should be noted, will tend to overestimate the fall-out level by collecting 'secondary fall-out', that is, fall-out which has been deposited once but has been stirred up by human actions or winds. The brown color-

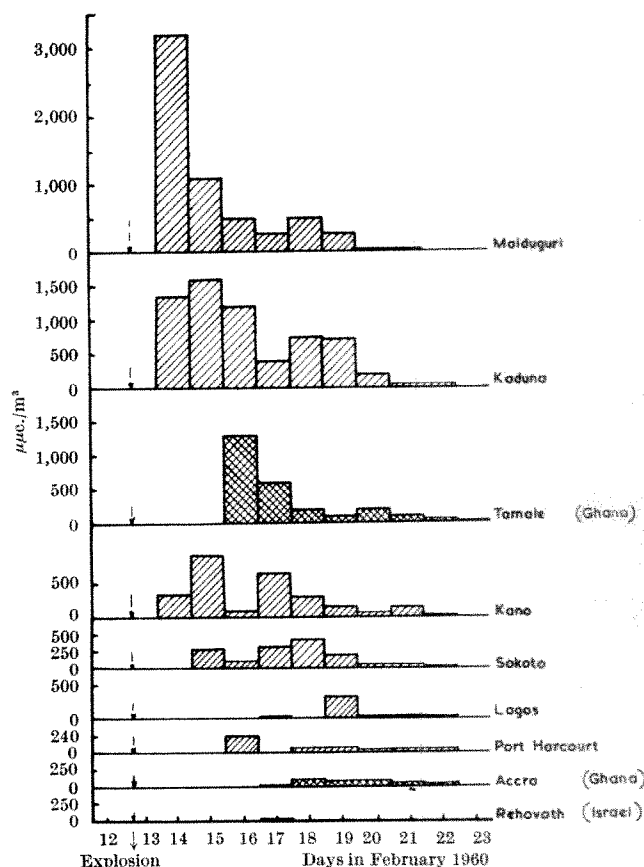


Fig. 3. Air-filter sample activities ( $\mu\text{C}/\text{m}^3$ )

tion apparent in some of the Nigerian sticky-paper samples was taken to be evidence of this phenomenon. However, this factor alone is not sufficient to account for the high fall-out activity observed in Nigeria during the Sahara tests, Fig. 2.

For the filter-paper samples, the methods used in Ghana, Nigeria, Israel, Czechoslovakia<sup>5</sup> and Panama Zone<sup>6</sup> were identical, and hence direct comparison of the results of fall-out measurements in these countries can be made. Some results are plotted in Fig. 3 which show that the filter-paper samples for Maiduguri in Nigeria gave the highest value of fall-out so far reported from the Sahara test.

In the United Kingdom, Israel and Czechoslovakia, fall-out was detected from radioactive debris which had been carried round the world. The small increases noted in some Nigerian air-filter samples of February 18, 1960, could not have originated from this source, since it would have required westerly winds of up to 320 km/h to carry the radioactive clouds round the world and back to Nigeria again in four days. This speed is far in excess of the 65 km/h estimated for the upper troposphere winds that brought the 'second-time-round' fall-out over Israel<sup>4</sup> during March 9-10, 1960.

I thank the Director of the Nigerian Meteorological Services and his staff for the collection of the samples, Mr. N. Pearce and certain members of the Radiation Measurements and Instrumentation Branch, Aldermaston, and the United Kingdom/Nigeria Scientific Committee on Fall-out Monitoring for their assistance.

<sup>1</sup> Crooks, R. N., Osmond, R. G. D., Owers, M. J., and Fisher, Miss E. M. R. *Atomic Energy Res. Est.*, R 3094 (H.M.S.O., 1959).

<sup>2</sup> Martell, E. A., *Science*, **129**, 1197 (1959).

<sup>3</sup> Ward, A. H., and Marr, J. D., *Nature*, **187**, 299 (1960).

<sup>4</sup> Feige, Y., Shalmon, E., Vardi, J., Gat, J. R., and Tokatly, Y. L., *Nature*, **189**, 90 (1961).

<sup>5</sup> Santholzer, W., Macku, G., and Podzimek, J., *Nature*, **192**, 398 (1961).

<sup>6</sup> Patterson, R. L., and Lockhart, L. B., *Science*, **132**, 474 (1960).

<sup>7</sup> Anderson, W., Bentley, R. E., Burton, L. K., and Greatorex, C. A., *Nature*, **189**, 223 (1960).

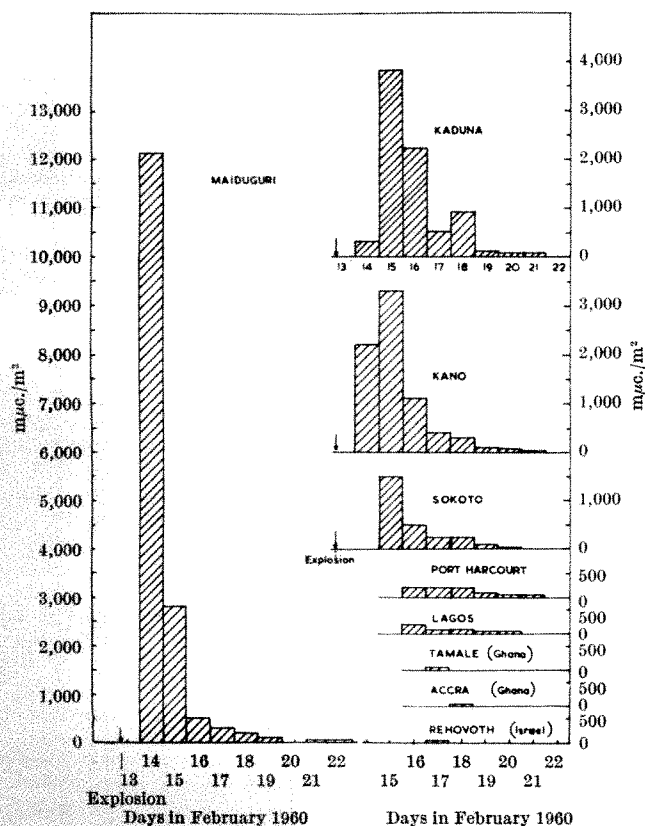


Fig. 2. Deposition sample activities ( $\mu\text{C}/\text{m}^2$ )

## OBITUARIES

## Dr. B. G. Gates

DR. BENJAMIN GEORGE GATES, who was the controller of research and development of the Australian Defence Scientific Service in the Department of Supply, died suddenly on December 1, 1964, at the age of sixty. He collapsed at a farewell retirement dinner given by his colleagues at the rooms of the Royal Commonwealth Society in Melbourne. He had a distinguished career in Scientific Research and Development, and for about thirty years was concerned with science in its application to defence.

Gates graduated from the City and Guilds College of the University of London in electrical engineering in 1924, and later was awarded a Ph.D. Between 1924 and 1935 he served with various industrial organizations, including Messrs. Johnson and Phillips, Ltd., London; Metropolitan Vickers, Ltd., Manchester; the British Electric Transformer Co., London; and the British Electrical Research Association. In 1935 he joined the Radio Department of the Royal Aircraft Establishment at Farnborough. In this establishment he first became acquainted with some of the scientific problems peculiar to defence, and soon made his own contribution to their solution.

He was concerned with the provision of reliable radio communication between aircraft and aircraft, and between aircraft and the ground; the comparative ease with which such communication is carried on to-day gives little indication of the magnitude of the problems which had to be overcome. Not the least of these was that of speech modulation of the radio transmitter in the very high noise-levels encountered in aircraft, and it was in this field that he made one of his most noteworthy contributions. Dr. Gates joined the Radio Department of the Royal Aircraft Establishment as a scientific officer, and within a decade he was head of that Department.

In 1950 he joined the Australian Defence Scientific Service as the controller concerned with the management of the Woomera Rocket Range, for which appointment he was specifically, and unequivocally, recommended by the late Sir Henry Tizard. It will be recalled that some three years earlier it had been agreed by the British and Australian Governments that a rocket range should be set up in Australia for the development of a long-range rocket, which would form the basis of a deterrent weapon system. The range at Woomera was planned originally for this purpose, and a rocket based on the German V2 of the Second World War was conceived under the name *Hammer*. Within a year, however, as a result of a change in policy of the British Government, this project was cancelled, and it was decided to proceed with the Woomera Range for the development not of a deterrent weapon system but of a series of ground-to-air and air-to-air weapons for direct defence. Dr. Gates was concerned with the planning and building of Woomera for this modified requirement, and he supervised its growth as a general purpose range capable of handling almost any project requiring space and accurate measurement. So in due course the range was readily adaptable to handle the *Blue Streak* project when, due to another reversal of policy by the British Government, the deterrent weapon project was revived. Again on cancellation of this project as a weapon, in its turn, the range was found to be readily made suitable for the launching of satellites based on the *Blue Streak* rocket, under the auspices of the European Launcher Development Organization of which Australia is a member.

Dr. Gates was concerned, as 'general manager', not only with the scientific and technical requirements for the range itself, but with all the support which such a facility

necessitates. These supporting facilities included the recruitment of some hundreds of graduate scientists and the further training of many of them overseas for periods of up to two years. The provision of a town with schools, shops, a hospital and so on was necessary to house range staff and their dependants, and a supporting air service had to be arranged in co-operation with the Royal Australian Air Force. Clearly, then, the Woomera Rocket Range owes much to the effective management by Dr. Gates in those early days.

In 1955 he was appointed 'controller of research and development' in the Australian Defence Scientific Service and deputy chief scientist (deputy chief executive), and was involved until his death with the build-up and use of resources capable of providing modern weapons and equipment for the Australian Fighting Services. Partly to train personnel for this purpose, and partly to provide a useful weapon, the British Army's anti-tank weapon *Malkara* was successfully completed.

In addition to the management of these research and development resources for the Australian Services, and the supervising of the Australian Defence Scientific Service as a whole (including the Rocket Range and associated activities) during the absences overseas of the Chief Scientist, Dr. Gates was very closely involved in managing a guided-weapon project, *Ikara*, for the Royal Australian Navy. The successful completion of this sophisticated system, which accurately delivers a self-homing torpedo to a desired location at sea, is due in large measure to Gates's conscientious supervision. Gates had a wide knowledge of those branches of science concerned with defence and, in the field of electrical engineering and electronics, this knowledge was encyclopaedic. He was a most pleasant colleague with whom to work and was unruffled in a crisis; his loss will be deeply felt. He leaves a wife, a daughter and a son.

W. A. S. BUTEMENT

## C. S. Franklin, C.B.E.

CHARLES SAMUEL FRANKLIN, one of the greatest pioneers of radio telecommunications, died on December 10, 1964, at the age of eighty-five. He will always be honoured for the prominent part he played in the small band of engineers associated with Guglielmo Marconi in the development of wireless—now radio—communications and associated valve techniques, during the first half of this century.

Franklin was born in 1879 and received his engineering and scientific training at the Finsbury Technical College under Prof. Sylvanus Thompson. After gaining some experience in electrical work, first at Manchester and afterwards with the Norwich Electricity Co., he joined the Wireless Telegraph and Signal Co. in 1899. This organization later became Marconi's Wireless Telegraph Co., with which Franklin remained associated until his retirement in 1939.

After a period in South Africa, where he helped to introduce wireless communication as a military aid during the Boer War, he sailed with Marconi across the Atlantic in 1902 to demonstrate the reception of wireless messages up to a range of some 1,550 miles from the Poldhu transmitting station in Cornwall. This was followed by a long personal association between Franklin and Marconi, during which were laid the foundations of modern world-wide radio communications.

Beginning with specially designed spark transmitters, Franklin was quick to appreciate the possibilities of the thermionic valve as a generator of radio frequency



oscillations, as well as a regenerative receiver of high sensitivity, and as early as 1913 he had filed patents covering techniques in this field. During the period 1916-20, the short-wave beam principle was developed, and one example of this was the rotating parabolic reflector station at Inchkeith, transmitting on a wave-length of 6 metres and demonstrating the possibilities of a radio beacon for navigation purposes. The Franklin master oscillator circuit of high stability formed an essential part of the subsequent development of the short-wave beam communication system.

During 1923-24, he installed a short-wave station at Poldhu, using a wave-length of nearly 100 m and directing the beam towards Marconi's yacht cruising in the South Atlantic. These experiments illustrated the practical possibilities of short-wave radio communication over long distances—by way of the ionosphere as we know to-day. In this connexion the Franklin beam aerial system was designed which, in one form or another, has formed the basis of short-wave world-wide communications, including broadcasting, for the past forty years. A necessary part of such a system was the associated development of the low-loss coaxial cable for the transmission of the very-high-frequency currents to and from the aerial arrays.

Franklin was also closely associated with broadcasting, and assisted in the design and installation of the transmitter and aerial system at London's first broadcasting station, then known as 2LO. Later he designed the transmitters and aerials for the B.B.C.'s station at Alexandra Palace which was used for the world's first regular television service. Although Franklin formally retired in 1939, he remained for some years as a research consultant to the Marconi Co.

Among the awards which came to Franklin in the course of his career were the Morris N. Liebmann Memorial Prize of the Institute of Radio Engineers (N.Y.) in 1922; the James Alfred Ewing Medal of the Institution of Civil Engineers (1936); and the Faraday Medal of the Institution of Electrical Engineers (1949) for "his distinguished work in radio engineering, and more particularly for his development of the beam aerial and other devices that made long-range high-frequency communication a practical possibility". In 1949, also, he was created a Commander of the Order of the British Empire.

Franklin was possessed of a modest and quiet but persistent manner; he was known and respected by all the scientists and engineers connected with the early history of telecommunications in the evolution of which he played such an outstanding part. R. L. SMITH-ROSE

## NEWS and VIEWS

### U.K. Atomic Energy Authority Weapons Group:

Mr. E. F. Newley, C.B.E.

MR. E. F. NEWLEY has been appointed director of the U.K. Atomic Energy Authority Weapons Group. Mr. Newley gained the degrees of B.Sc. (Mech. Eng.) and M.Sc. at the University of London, and spent the early part of his career at the Post Office Research Station, Dollis Hill. In 1949 he joined the Admiralty as a senior principal scientific officer, and left in 1955 to take up an appointment as deputy chief engineer at the Atomic Weapons Research Establishment, Aldermaston. Later he became chief of warhead development at Aldermaston and in 1959 was appointed deputy director, which position he has held until now.

### Chemistry in Brunel College of Advanced Technology:

Prof. K. S. W. Sing

DR. K. S. W. SING, head of the Department of Chemistry and Biology at Liverpool College of Technology, has been appointed to the chair of chemistry at Brunel College of Advanced Technology. Dr. Sing was educated at Teignmouth Grammar School and the University College of the South West of England (now the University of Exeter). In 1945 he gained a B.Sc. of the University of London in special chemistry with first-class honours, and remained at Exeter for a further three years as a research student studying the thermal decomposition of aluminium hydroxide, under the direction of Dr. S. J. Gregg. Dr. Sing was awarded a Ph.D. by the University of London in 1949 for his thesis dealing with adsorption of porous solids with particular reference to the adsorbents produced by heating gibbsite. After leaving Exeter in 1948, Dr. Sing spent a year in the research department of Imperial Chemical Industries, Ltd., Billingham. In 1949 he joined the staff of the Royal Technical College, Salford, where he was successively lecturer and senior lecturer. In 1956 he moved to Liverpool College of Technology, and under his headship the Department of Chemistry and Biology—already in a thriving condition—increased greatly in size, both in terms of accommodation and staff and student numbers, to the point where it is now one of the major departments of its type in a Regional College. Possibly the most striking development has been the increase in the amount of research carried out in the Department, much of it

sponsored by local industry. Dr. Sing's own research has included perhaps the first systematic attempts to investigate the conditions of formation of silica gel in relation to its surface properties, and investigations of the ageing of precipitated alumina at room temperature. His present interests include gas adsorption and the surface and structural properties of alumina and various mixed oxide catalysts.

### Brewing and Applied Biochemistry in the Heriot-Watt College, Edinburgh:

Prof. D. J. Manners

DR. D. J. MANNERS has been appointed to the chair of brewing and applied biochemistry at the Heriot-Watt College, Edinburgh, in succession to the late Prof. I. A. Preece (204, 1033; 1964). Dr. Manners was educated at Fitzwilliam House, Cambridge, where he obtained honours in the Natural Sciences Tripos (Biochemistry) in 1949 and a Ph.D. degree in 1952 for his research on carbohydrate biochemistry. He was appointed to a lectureship in chemistry at the University of Edinburgh in 1952 and to a readership in 1964. Dr. Manners's research interests have included investigations of the molecular structure of glycogen and polysaccharides from protozoa, algae and yeast, on glycogen storage diseases, on starch-degrading enzymes and on enzyme systems in marine algae. On the basis of this work he was awarded the Meldola Medal in 1957, and the D.Sc.(Edin.) degree in 1960. He was elected a Fellow of the Royal Institute of Chemistry in 1958, and has recently been appointed to the Editorial Board of the *Biochemical Journal*. His present research projects include several which are of direct interest to the brewing and related industries, so that the close links already established by Prof. Preece between the Heriot-Watt College and those industries will be maintained. It is expected that the College will attain university status at an early date and that, under the direction of Dr. Manners, the Brewing and Applied Biochemistry Department will take a full part in these developments.

### Sociology in the University of Bristol:

Prof. M. P. Banton

THE University of Bristol, the most recent of the established English universities to introduce courses in sociology, has appointed Dr. M. P. Banton to the newly

established chair of sociology. Dr. Banton was educated at King Edward's School, Birmingham, the University of Glasgow, and the London School of Economics. Appointed Noel Buxton research student in the Department of Social Anthropology, University of Edinburgh, in 1950, he undertook a study of African and West Indian immigration to Britain for which he was awarded a Ph.D. As Nuffield Research Fellow he then conducted an investigation of rural-urban migration and tribal life in Freetown, Sierra Leone. Dr. Banton was appointed assistant in social anthropology at Edinburgh in 1953, advancing to lecturer in 1955, to reader in 1962, and graduating D.Sc. in 1964. In 1959 he was secretary to the organizing committee for the Darwin Centennial Conference held in Edinburgh, and edited the Conference *Proceedings*. From February 1962 until January 1963 he was visiting professor of political science in the Massachusetts Institute of Technology where he was concerned with courses dealing with political and social development in Africa. In the 1964 Malinowski Memorial Lecture, delivered at the London School of Economics, Dr. Banton discussed the inter-relations of social anthropology and sociology, maintaining that in many situations it was more confusing than helpful to refer to them as separate subjects. In recent years he has undertaken research into British behaviour towards coloured people, and into the social characteristics of police work in Scotland and the United States. His concern to study interpersonal relations in sociological terms while drawing equally on the conclusions of social psychology and anthropology is most apparent in his recent work. Sociology teaching will start at Bristol in October 1966 and it is hoped to promote research on a broad front.

#### U.S.S.R.—United States Joint Desalination Scheme

A SIGNIFICANT step was taken on January 26 towards closer collaboration between the U.S.S.R., the United States and the International Atomic Energy Agency, when it was officially announced that the two countries had agreed to co-operate in the field of desalination. The agreement, which had been concluded between the two countries on November 18, 1964, and signed by Mr. A. I. Alexandrov, representing the U.S.S.R., and Mr. F. K. Hefner, representing the United States, provides for wide scientific and technical co-operation and for an exchange of scientific reports and other documents, periodic scientific meetings and visits of experts on a reciprocal basis. In order that the International Atomic Energy Agency and its members should receive benefits in full measure from this operation, it is stated in the agreement that the parties will give the Agency copies of accounts, reports and other documents which they exchange and also, in appropriate cases, invite observers to symposia and scientific meetings held by the parties.

#### Industrial Needs for Scientific Manpower

FIRST returns are now coming in from 11,000 employers in all sections of British industry who have been asked to participate in a survey aimed at providing adequate knowledge of the future needs of industry for scientific and technological manpower as a pre-requisite to meeting those needs. It is being carried out by the Ministry of Labour for the Committee on Manpower Resources for Science and Technology. This Committee, under the chairmanship of Sir Willis Jackson, replaces the former Committee on Scientific Manpower of the Advisory Council on Scientific Policy for whom similar surveys were carried out by the Ministry of Labour in 1956, 1959 and 1962. The latest enquiry is addressed to all establishments employing 500 workers or more and to a sample of those employing less than 500. To obtain more accurate information on the nation's technical manpower resources the range of this enquiry has been extended to cover "technicians and other technical supporting staff", as well as fully qualified scientists and technologists. The importance of this

supporting staff is being increasingly recognized and information about its supply and demand, complementary to that for professionally qualified staff, is essential. About 6,000 of the employers who have been approached are in the manufacturing industries, but service industries, including local and national Government Services, are also covered. The British Employers' Confederation, the Federation of British Industries and the National Association of British Manufacturers are giving full co-operation. Reports of the earlier surveys brought together, on a statistical basis, data on the output from the educational system, and of the utilization in employment of scientifically and technologically qualified manpower, together with forecasts three years ahead of the needs for this category of manpower. The results of the enquiry will be made available to the Committee on Manpower Resources for Science and Technology and published later this year.

#### Medical Research Council:

##### Brain Metabolism Research Unit

THE Medical Research Council has set up a Brain Metabolism Research Unit in the Department of Pharmacology, University of Edinburgh Medical School. The Unit, which will be under the honorary direction of Prof. W. L. M. Perry, aims to undertake experimental and clinical studies of the metabolic pathways of certain amino-acids and other substances in the brain and tissue fluids. The action of psychotropic drugs on these pathways will be used as a means of trying to determine whether there are metabolic defects in the various psychoses and ultimately whether such defects can be corrected.

##### Unit for Research on the Chemical Pathology of Mental Disorders

DR. F. A. JENNER has been appointed physician-in-charge of the Council's Unit for Research on the Chemical Pathology of Mental Disorders in the Department of Physiology, University of Birmingham Medical School, and at the Hollymoor Hospital, Birmingham, following Prof. I. E. Bush's resignation. The Unit will continue to carry out research into the development of new biochemical and other experimental methods for detecting chemical abnormalities in patients, but Dr. Jenner's appointment will lead to an increased emphasis on clinical research and a more concentrated study of physical problems associated with periodically recurring mental disorders.

#### Investment in Scientific and Technological Research in India

IN their paper, *Investment in Scientific and Technological Research during the Fourth Five Year Plan*, Dr. S. Husain Zaheer, A. Rahman and N. Sen discuss the quantum of investment in science and technology, particularly in the light of two investigations made by the Survey and Planning of Scientific Research Unit of the Council of Scientific and Industrial Research (Pp. 31. New Delhi: Council of Scientific and Industrial Research, 1964). The first examination indicated that support by State Governments had been limited to research on agriculture and animal husbandry, with a surprising neglect of research on industrial, technological and natural resources. It also showed that the support of the Central Government in several sectors was also unbalanced and the rates of growth of various sectors differed widely. The second investigation revealed an increase of 15–20 per cent per annum in scientific and technical personnel in the developed laboratories, and a more rapid increase in the younger laboratories. The cost of administration varied between 8 and 12 per cent per annum in these national laboratories, and recurrent expenditure was increasing by 12–20 per cent per annum, while capital expenditure, though varying from laboratory to laboratory, could be

estimated fairly accurately over a term of years. The average cost per scientist was also fairly steady, and a rate of growth of 10 per cent in investment was indicated. From these investigations, assumptions are made which lead to projections of expenditure on scientific and technological research, by the Central Government, and by the State Governments; of expenditure by the Central and State Governments on research in universities; and of expenditure by organized industry on research. For university research, figures of Rs. 46.0 million and Rs. 116.8 million, respectively, are suggested for the first and final years of the Plan, or Rs. 388 million in the university sector (4.4 per cent of the total investment in scientific research) during the quinquennium. Expenditure by organized industry is put at Rs. 193.4 million in 1966-67, rising to Rs. 343.1 million in 1970-71, or an investment of Rs. 1,317 million during the period, 14.8 per cent of the total investment on research.

### Art and Design

THERE is a widespread and growing interest in design among manufacturers and the general public, and in recent years the majority of students who have successfully completed a period of training at one of the recognized art schools have been able to find employment in some sphere of design. There is also a steady demand for art teachers. In most branches of art other than design, however, the openings occurring each year are to be reckoned in tens, not hundreds, and it takes time for even the most talented to establish themselves as free-lance artists. This information is given in a revised illustrated edition of *Art and Design* (Central Youth Employment Executive, *Choice of Careers*, No. 103. Pp. 55. London: H.M. Stationery Office, 1964. 2s. net). The booklet groups occupations in design under the following headings: the graphic arts; fashion design; package design; product design; design for woven textiles and printed materials; design for entertainment (set design, animated films and television). Particulars of educational qualifications for designers, professional training, examinations, cost of training, openings, and some indication of salaries, are also given.

### The National Museums of Southern Rhodesia

THE major task during 1963 for the National Museums of Southern Rhodesia was the installation of interior fittings and displays in the three new Museums (Report of the Trustees and Directors of the National Museums of Southern Rhodesia for the year ended December 31, 1963. Pp. 32. Salisbury: National Museums of Southern Rhodesia, 1964). In the initial planning of this work adequate display teams were envisaged. As so often happens in the museum field, in the event this was not forthcoming, though at the end of the year it was arranged that the opening of the three Museums would be in 1964. The Councils of the City of Bulawayo, the City of Salisbury and the Town Council of Umtali all made provision for the sites of the new buildings and in many ways have played a major part in making these developments possible. The three Councils continue to provide maintenance grants. In spite of their preoccupation with the moves to the new buildings the staff have been able to continue research work, as is testified by a series of publications. It is also pleasing to note that Captain E. F. Boulton continues as honorary keeper of arms and antiquities at the Umtali Museum.

### Natural History in the U.S.S.R.

THE weekly journal of the Russian book trade, *Novye Knigi* (No. 51; 1964), announces the forthcoming publication of a series of pocket-sized popular handbooks on the mammals, birds, reptiles, fishes, insects, trees and shrubs, grasses and flowering plants, and rocks and minerals of the U.S.S.R. The works, in Russian, are intended for the use of older school-children, amateur

naturalists and tourists, and will be extensively illustrated in colour. The edition is 50,000 copies of each, length 20 printers' sheets, price 1r. 75k. (about £1). The first book of the series, on mammals, will be published in 1965 and the others over the next three years.

### International Subject Bibliographies

*Maxwell's International Subject Bibliographies* No. 1 deals with electronics, including control engineering, covering the period 1960-63, but excluding medical and biological electronics and books on computers published in 1963-64, for which a special bibliography (No. 29) is available. *Special Subject Bibliography* No. 6 covers orthopaedics, including rehabilitation of the physically handicapped, but excluding myology, physical education in general and rehabilitation of the blind and deaf. This second edition covers the period 1961-63. *Special Subject Bibliography* No. 10 covers electrochemistry, including polarography, 1953-May 1964; No. 19 covers reinforced plastics, including polyester resins, plasticizers and glass film products, January 1960-June 1964; and No. 28 deals with machine tools and machine tool design, excluding drilling, polishing, cutting, grinding and pressing tools, which are covered by No. 9. This second edition refers to the period 1961-May 1964 (Oxford: Robert Maxwell and Co., Ltd.; Long Island City, N.Y.: Maxwell Scientific International, Inc., 1964).

### Wheat Research in Australia

THE annual report of the Wheat Research Unit of the Commonwealth Scientific and Industrial Research Organization for 1963-64 is a summary of work in progress, illustrating very clearly the possibilities of applying modern techniques to the investigation of the complex mixture of protein and carbohydrate that is the basis of bread dough (Pp. 15. Ryde, N.S.W.: Commonwealth Scientific and Industrial Research Organization, 1964). In fact, almost the entire effort of the laboratory is being devoted to the separation of homogeneous fractions of the complex in amounts sufficient for baking tests. The main research effort is concerned with the fractionation of the proteins comprising the gluten complex. A many-sided attack on this difficult problem is being made, using the techniques of column chromatography, starch-gel electrophoresis and gel-filtration. A particularly interesting aspect is the examination of the effect of ionic strength on the behaviour of gluten proteins during gel filtration on 'Sephadex'. This should ultimately provide a better understanding of the role of gluten proteins in relation to the physical properties of dough. Preparations of flour gum have been shown to be effective in increasing the volume of starch-gluten loaves, but pentosans from other sources can produce similar effects, the most effective being those with the main chain carrying large substituent groups. However, one surprising fact to be reported is the inhibition of yeast fermentation by the presence of 3 p.p.m. of chitosan (partially deacetylated chitin). Pentosan degradation investigations using "snail juice enzymes" have shown that, while loss of baking quality can result from the action of pentosanases, the neutralizing or improving effect of oxidizing agents such as potassium bromate cannot be explained by their ability to promote gelling of pentosans.

### Soils of the Middle Teign Valley District of Devon

THE Soil Survey of Great Britain normally prepares memoirs to accompany soil maps; it has now started to publish Bulletins dealing with investigations of special interest. The first to be issued is concerned with a district of eight parishes around Dunsford, of total area 40 square miles, of which the southern half comprises the Teign Valley between Great Haldon and Dartmoor (Bulletin No. 1: *Soils of the Middle Teign Valley District of Devon*. By B. Clayden. Pp. vii+111+map. Harpenden: Rothamsted Experimental Station, 1964. 25s. net).



Small mixed farms engaged in milk production predominate and the soil is an important factor in the economy, so that a soil survey was undertaken to assist the Nuffield Farm Project sponsored by the Nuffield Foundation and the Ministry of Agriculture, Fisheries and Food. The Bulletin gives a detailed account of the physical features, climate, geology and soil parent materials. Although the district as a whole is characterized by high precipitation and mild winters, local conditions give rise to wide variability from the average rainfall and temperatures. The methods of mapping and soil classification are described and coupled with numerous analytical data from representative profiles on Culm shales and granite, from brown earths and from gley soils. There are practically no calcareous soils, and soils of high base status are confined to those developed on the diabase, on red loamy head and on base-rich alluvium. The non-calcareous Culm rocks give markedly acid soils. A chapter on the history of agriculture in the area leads on to present-day farm practices, with statistics of farm sizes and enterprises and some discussion of the relationships between productivity and soil properties. There is a bibliography of scientific papers and an index.

### Spring-tails of the World

EVER since Sir John Lubbock, in his Ray Society Monograph of 1873, recognized that the Collembola (for which he proposed this name) should be regarded as a separate order of apterygote insects, the group has attracted enthusiastic devotees. They form a compact yet diverse group of insects, comprising three sub-orders, some sixteen families, and about 1,500 described species. Comparatively little is known about their ecology; but the vast numbers present in the soil suggest that they must be of the utmost importance in soil microbiology. Even less is known of their physiology. Dr. J. T. Salmon of the Victoria University of Wellington, New Zealand, a recognized authority on the group, has published a two-volume *Index to the Collembola* (Royal Society of New Zealand. Bulletin No. 7: Salmon. Vol. 1: Pp. 1-144; Vol. 2: Pp. 145-644. Wellington: Royal Society of New Zealand, 1964). This impressive work records the known genera and species of the Collembola of the world, their synonyms, type repositories, geographical distribution, taxonomic descriptions, morphology, embryology and ecology up to the end of 1962, and includes a key to genera throughout the world. The second volume consists of classified references to each species. There are something like 36,000 such references, each with an indication of the nature of the information it contains. This should prove to be the standard reference work to the 'spring-tails' for many years to come.

### Enteric Infections in Children

EVERYONE concerned with child welfare will be interested in the report recently issued by the World Health Organization Expert Committee, entitled *Enteric Infections* (World Health Organization Technical Report Series, No. 288, obtainable in the United Kingdom from H.M.S.O., 5s.). It is also published in French and Spanish. In many developing countries enteric infections are the most important causes of illness and death among children, and the report gives statistics of the illness and mortality due to them. In countries in which they are endemic they cause an infant mortality of more than 100 per 1,000 live births. A cardinal symptom of these diseases is diarrhoea, and the term enteric infections includes a variety of clinical syndromes of various aetiology. The most important causes are *Escherichia coli* and species of *Salmonella* and *Shigella*. Valuable sections of the report discuss the factors which contribute to the occurrence of these infections and their control. Many deaths due to them could be avoided by the application of knowledge already available, but the report discusses the diagnosis of the infections, the

further research needed and the requirements for control, including health education, environmental sanitation, control of flies, water supplies, child care—especially of ill-nourished children—and the expansion of health centres.

### Postgraduate Agricultural Studentships and Awards

A LIMITED number of postgraduate studentships are to be awarded by the Ministry of Agriculture, Fisheries and Food, and the Department of Agriculture and Fisheries for Scotland, for the academic year beginning October 1. These will cover the various branches of husbandry (including horticulture), farm management, agricultural or horticultural economics, agricultural or horticultural statistics, marketing, agricultural and dairy engineering (including farm mechanization), rural estate management and agricultural science. Applicants normally resident in England, Wales and Northern Ireland can obtain further information and forms of application from the Ministry of Agriculture, Fisheries and Food, Room 231A, Great Westminster House, Horseferry Road, London, S.W.1. Applicants normally resident in Scotland should apply to the Department of Agriculture and Fisheries for Scotland, Room 53, St. Andrew's House, Edinburgh, 1. The closing date for receipt of completed application forms is February 28.

Postgraduate awards are also offered by the Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1, for training in research. These are open to honours graduates in science or those holding a veterinary qualification, and to honours graduates in agriculture or horticulture who have shown a special interest in one of the basic sciences. Applications for awards in veterinary science should also be made to the Agricultural Research Council.

Awards are also available to postgraduate and post-doctoral students of agricultural science under the NATO Science Studentship and Fellowship Programme, which is intended to encourage the exchange of students between member countries in NATO. The scheme is administered for United Kingdom students by the Department of Scientific and Industrial Research, State House, High Holborn, London, W.C.1, to whom application should be made by March 1.

### Lady Tata International Grants, Scholarships and Fellowships for Research on Leukaemia and Allied Conditions

THE Trustees of the Lady Tata Memorial Trust invite applications for fellowships, scholarships and grants for research on leukaemia, in the academic year beginning October 1. In view of the affinity between leukaemia and other forms of neoplastic disease, candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukaemia will be eligible for consideration, and especially those studying leukaemogenic viruses in mammals, the epidemiology and natural history of leukaemia, and the immunogenetic aspects. The awards offered by the Trust are open to suitably qualified investigators of any nationality, working either in their own institutions or in other centres abroad. Applications must be submitted before March 31, and awards will be announced by the Trustees in June. Further information and forms of application can be obtained by writing to the secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London, S.W.3.

### The Leopoldina German Academy of Sciences: Elections

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Mathematics*, Prof. H. Heinrich

(Dresden); *Physics*, Prof. S. Tomonaga (Tokyo); *Geophysics*, Prof. G. Fanslau (Potsdam) and Prof. C. Junge (Mainz); *Chemistry*, Prof. R. Huisgen (Munich), Prof. T. Wieland (Frankfurt/M) and Prof. H. M. Pamir (Ankara); *Physical Chemistry*, Dr. M. Eigen (Göttingen); *Mineralogy*, Prof. F. Machatschki (Vienna); *General Biology*, Prof. H. Bauer (Tübingen), Prof. A. Gierer (Tübingen) and Dr. H. G. Wittmann (Tübingen); *Botany*, Prof. A. Lang (East Lansing, Michigan); *Anatomy*, Prof. J. Szentágothai (Budapest); *Physiological Chemistry*, Dr. A. Kornberg (Palo Alto, California) and Prof. J. Monod (Paris); *Internal Medicine*, Prof. H. Schulten (Cologne) and Prof. J. G. Waldenström (Malmö); *Dermatology*, Prof. S. Jabłońska (Warsaw), Prof. A. Wiedmann (Vienna) and Prof. W. Kalkoff (Freiburg/Br.), *History of Natural Science and Medicine*, Prof. G. R. A. Uschmann (Jena).

#### University News:

#### Churchill College

DR. J. R. KILLIAN, jun., chairman of the board of the Massachusetts Institute of Technology, has been elected into an overseas fellowship (Title F) at Churchill College.

#### London

PROF. R. H. S. THOMPSON, professor of chemical pathology at Guy's Hospital Medical School, has been appointed to the Courtauld chair of biochemistry tenable at Middlesex Hospital Medical School. Dr. D. Rogers, reader in chemical crystallography at the Imperial College of Science and Technology, has been appointed to the chair of chemical crystallography tenable at that College. Dr. T. S. West, reader in analytical chemistry at the Imperial College of Science and Technology, has been appointed to the chair of analytical chemistry tenable at that College. Mr. J. H. Kirk, under-secretary, Ministry of Agriculture, Fisheries and Food, has been appointed to the chair of marketing with special reference to horticulture tenable at Wye College.

#### Southampton

BUILDING work costing £2,250,000 is at present in progress. New buildings in course of erection include an eleven-storey Mathematics Building, a four-storey Students' Union Building, a single four-storey building to accommodate the Departments of Botany and Geography, a five-storey Physics Building and a smaller building to accommodate the new Department of Oceanography. Additional residential accommodation for 350 students is also being built. A Department of Oceanography has been established as a separate department in the Faculty of Science. The first head of the Department will be Prof. J. E. G. Rayment, who has transferred from the chair of zoology in the University. Apart from biological and chemical oceanography, which is being undertaken by the new Department, there will be an early development of physical oceanography. At present the new Department is entirely postgraduate and trains students for an M.Sc. course in oceanography as well as accepting students for research. Dr. L. Brent, of the Medical Research Institute, has been appointed to succeed Prof. Rayment as professor of zoology and head of the Department of Zoology.

#### Announcements

SIR GEOFFREY TAYLOR was awarded the James Watt International Gold Medal of the Institution of Mechanical Engineers on January 27, in recognition of his long and distinguished career and his many contributions to mechanical engineering.

PROF. R. G. W. NORRISH, of the University of Cambridge, delivered the twenty-fourth Faraday Memorial Lecture of the Chemical Society at the Royal Institution on January 14, and was afterwards presented with the Faraday Medal of the Society.

THE James Clayton Prize of the Institution of Mechanical Engineers, worth £1,700, has been awarded equally to Mr. F. H. Towler (Towler Brothers (Patents), Ltd.) for his work on the design, development and manufacture of heavy hydraulic equipment and related controls, and to Dr. A. E. W. Austen (C.A.V., Ltd.) for his research on the development of diesel engines and related equipment.

Dr. E. Ambler, Dr. R. Hayward, Dr. D. Hoppes and Dr. R. Hudson are to receive the 1964 Samuel Wesley Stratton Award of the U.S. National Bureau of Standards, for their work in demonstrating that the quantum mechanical law of parity conservation does not hold in weak interactions.

THE seventeenth technical exhibition of the Oil and Colour Chemists' Association will be held in London during March 22-26. Further information can be obtained from the General Secretary, Oil and Colour Chemists' Association, Wax Chandlers' Hall, Gresham Street, London, E.C.2.

A MEETING of the Oxford local section of the Institute of Metals will be held in Oxford on March 16. At the meeting, a lecture on "How and Why of Strong Magnetic Fields" will be delivered by Dr. N. Kurti. Further information can be obtained from the Institute of Metals, 17 Belgrave Square, London, S.W.1.

THE sixteenth Pittsburgh conference on "Analytical Chemistry and Applied Spectroscopy" and Exposition of Modern Laboratory Equipment will be held in Pittsburgh during February 28-March 5. Further information can be obtained from the programme chairman, W. G. Fateley, Mellon Institute, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213.

THE second symposium on "Progress in Biochemistry and Therapeutics" of the Carl Neuberg Society for International Scientific Relations will be held in New York City during March 23-24. The main topics of the symposium will be drugs, enzymes and diseases. Further information can be obtained from Mr. I. Greenberg, Carl Neuberg Society, 600 Lafayette Avenue, Brooklyn, N.Y. 11216.

A SERIES of lectures on "Railways Research", arranged through the courtesy of British Railways, will be held at the Imperial College of Science and Technology during the first half of 1965. The programme will include the following lectures: the application of automation to railways (March 1); riding of railway vehicles (March 22); communication with moving trains (May 10); fatigue problems in railway engineering (May 31); fuel cells for traction (June 14). Further information can be obtained from the Registrar, Imperial College of Science and Technology, London, S.W.7.

A CONGRESS on the "Protection of Tropical Crops", sponsored by the regional Chamber of Commerce and Industries, will be held in Marseilles during March 23-26. The programme will include sessions on: organization and methods of plant protection in the tropics; pesticides (mode of action, conditions of application, methods for testing in the field, equipment developed for their application); protection of stored products; protection of timber; control of birds, rats, locusts, root diseases, virus diseases; biological control. Further information can be obtained from Dr. G. Bouriquet, 45 bis avenue de la Belle Gabrielle, Nogent sur Marne, Seine.

CORRIGENDUM. The Editor has been informed by Dr. R. Kluthe *et al.* that in the communication entitled "Quantitative Estimation of Human Serum Haptoglobins by an Immunological Method" (*Nature*, 205, 93; 1965) the block for Fig. 1 and its key (but not legend) belong to Fig. 2, while the block for Fig. 2 and its key belong to Fig. 1.

## THE NATURE CONSERVANCY

ABOUT one-third of the fifteenth annual report of the Nature Conservancy\*, covering the year ended September 30, 1964, is again occupied by brief accounts of scientific research in progress, but some further scientific work is described in the account of the Scientific Advisory Services, for example, the work on grey seals and fisheries (on which the Consultative Committee on Grey Seals and Fisheries arranged a scientific symposium on September 9, 1964). Reference is also made in that section of the report to the establishment of a Biological Records Centre at Monks Wood Experimental Station, using as a nucleus the Botanical Society's records and data-processing machinery. (The work of the Scientific Advisory Services and the conservation work are considered on p. 631 of this issue of *Nature*.)

During the year, £104,297 was spent on research, studentships, training and special surveys, and £81,073 of this went to grants for research, studentships and training. In all, £63,027 was spent on research grants, including 12 new grants totalling £27,282, one of which (£9,500) went to the Wildfowl Trust towards the building and equipping of one floor of the Trust's new research centre at Slimbridge. Renewed grants included an interim £9,500 to the British Trust for Ornithology for investigations on bird populations and migration.

The Merlewood Research Station and the Moor House Field Station are now closely integrated and there is increasing collaboration with universities. The woodland botanists, who are mainly concerned with investigations on low-level oak woodlands, are now examining the nutrition of trees at 1,800 ft. at Moor House, and the investigations will provide important information on the possibilities of growing trees, not necessarily for the production of economic timber, but as ecological tools for the proper utilization of hill-land. Much attention is being directed to the circulation of mineral nutrients within the woodland and moorland ecosystem, including an examination of the rate at which nitrogen-fixing organisms can supplement the input by rain and so restore the major depletion of nitrogen—some 70 per cent when moorland vegetation is destroyed by burning.

Research at Furzebrook is concentrated on populations and physiography, including hydrology. The distribution survey of the Dorset heath (*Erica ciliaris*) has been completed and further sites selected for investigation. A stratigraphical and ecological examination of the valley bogs in Dorset has been started, including a detailed survey at Morden Bog National Nature Reserve, where pitfall trapping of spiders continued and was started at Studland Heath National Nature Reserve. A detailed historical investigation of the changing *Spartina* marsh pattern since the first appearance of the grass in Poole Harbour in 1900 has been completed. A detailed recent survey puts the area in Poole Harbour at about 1,700 acres, and it appears possible that the growth limits of marsh plants may be sensitive indicators of small-scale recent changes in sea-level. Investigations have also been made on the physiography, tidal submergence and chlorinity of the salt marshes in Poole Harbour, and coastal research has been continued at Bridgwater Bay, Orfordness, Braunton Burrows and Dawlish Warren, including examination of the chronology of their evolution. A survey of water balance programmes was concentrated on the peat uplands of the west and north and,

to a lesser extent, on the Woodwalton Fen area of Huntingdonshire.

At Monks Wood Experimental Station, work on moles (*Talpa europaea*) commenced, particularly in Monks Wood and Woodwalton Fen. Detailed measurements of seedling growth were made in the experimental blocks at Yarnar Wood, Monks Wood, Wychwood, Holme Fen and Castor Hanglands National Nature Reserves and the effects of various treatments are being assessed. Investigations by live trapping of populations of the bank vole (*Clethrionomys glareolus*) and wood mouse (*Apodemus sylvaticus*) continued, and the programme of sampling the invertebrate fauna of two contrasting grass-heaths in the East Anglian Breckland has been completed. The examination of the succession of aquatic invertebrates in a dyke at Woodwalton Fen was continued, as well as those of the changes in vegetation of a south-facing slope at Old Winchester Hill as a result of sheep-grazing, and of the effect of sheep-grazing on the floristic composition of chalk grassland on the Barton Hills, Bedfordshire.

New work in the Toxic Chemicals and Wild Life Section includes examinations of the arthropod fauna of hawthorn hedges in relation to the presence of associated herb species; of the plants and insects of roadside verges, with the view of providing a scientific basis for advice on the conservation aspect of roadside maintenance; of the invasion of fields by selected weed species from hedges; and of the rate of destruction of hedges. Investigations on the distribution and size of organochlorine insecticide residues in mammals, birds, birds' eggs and invertebrates have been greatly helped by a gas chromatographic apparatus.

In May 1964 the research programme on red deer on Rhum was extended to cover the feeding ecology of the animal and thus establish a link with the investigation of vegetation production. The survey for the vegetation map of Rhum has been completed and it will now be possible to estimate the approximate acreages of the plant communities grazed by the red deer. Censuses were again made throughout Orkney by the Grey Seals Research Officer, and examinations were made at breeding colonies in Colonsay, on islands in the Sound of Harris and in Orkney and Shetland. The substantial winter mortality of adult Soay sheep on Hirta and of the subsequent lambing was investigated in March–May 1964. An investigation of the ecology of birch in the Highlands has been started, while further improvements have been made in the techniques of collecting and extracting the seeds of trees and shrubs for use on reserves. Investigations of red grouse (*Lagopus lagopus scoticus*) at Blackhall and Kerloch, Kincardineshire, have concentrated on behaviour and nutrition in relation to a long-term population examination. At the Speyside Research Station much of the research of the Climatological Section concerns the climatological aspects of productivity investigations, particularly the water balance and changes in air humidity. Research at the Bangor Research Station continues to be concerned with investigations in Snowdonia on the influence of variations in local climate and geology on soils, vegetation (particularly grasslands and related arctic-alpine communities) and biological productivity. An X-ray fluorescence spectrographic method has been developed for the rapid analysis of the major elements in powdered rocks. Earlier studies of sheep population densities and their growth have been extended to an investigation of the increment of plant 'dry matter' and energy by different plant communities in summer.

\* Report of the Nature Conservancy for the year ended September 30, 1964. Pp. vii + 173 + 10 plates. (London: H.M.S.O., 1964.) 13s. net.



## THE NATIONAL PARKS COMMISSION

THE fifteenth annual report of the National Parks Commission\* covers the year ending September 30, 1964, and, like the present report of the Nature Conservancy, it makes special reference to the study conference, "The Countryside in 1970", held in November 1963. It also comments on the acute problem presented by coastal preservation and development and, like the Conservancy, welcomes the action of the National Trust in initiating 'Enterprise Neptune', the object of which is the conservation of selected stretches of coast line in co-operation with local planning authorities. On water supply problems the Commission regards with grave misgiving any scheme which would begin to abstract water from Ullswater, and adds that any proposals to flood the Winster Valley would be resisted most strongly. During the year under review new orders designating the forest of Bowland and Chichester harbour as areas of outstanding natural beauty were confirmed, but decisions are awaited regarding the Chilterns and the Solway coast areas. The Pennine Way long-distance footpath has now been completed, and provides a strenuous high-level walk from Edale, in the Peak District National Park, to the Cheviots and the Scottish border, passing through two other National Parks, the Yorkshire Dales and Northumberland. Special efforts have been made to assist local authorities in resolving difficulties, and in negotiating outstanding footpath agreements, in their areas regarding the Offa's Dyke path, but coastal erosion still presents difficulties in maintaining the south-west peninsular coast footpath. The definitive maps for the south Devon coastal area are now complete and should simplify consideration of outstanding public rights of way on the Devon south coast path, while some progress is reported with regard to the South Downs way.

The report includes brief accounts of progress during the year with each of the 10 national parks, but development questions continue to present the Commission with its main problems, and some 410 proposals for development affecting landscape beauty in national parks were referred to the Commission during the year. The Commission notes that the South Wales Electricity Board

proposed to carry out, during the year 1964-65, the first stages of an operation involving the expenditure of £50,000, primarily to improve visual amenities in the electricity distribution network area of South Wales. Strenuous efforts have been made by the two Yorkshire National Parks planning authorities, with the support of the Commission, to effect a change in the policy of the North East Electricity Board with regard to overhead supply lines. With regard to the 4,000-kV line in the southern counties, for which the Commission suggested alternative routes, the Commission has asked that, should the Minister of Power decide that the case for consent to the Electricity Board's proposed route is overwhelming, a definite time limit should be set for the removal of the lines, the towers and the bases, and that the period should be as short as possible. The Commission has also discussed with the Central Electricity Generating Board the project for a pump storage scheme in North Devon, with regard to the possible effects on the coast, including the approaches from land and sea and the recreational potential which the upper reservoir or reservoirs may have. The Minister of Power has again been approached with regard to the placing underground of the electricity lines in Martindale in the Lake District National Park.

Representations were made to the Minister of Transport regarding the proposal to withdraw passenger services from the Taunton to Barnstaple line, but without effect. The Minister of Transport, however, has decided to continue passenger services on the line from Llandudno junction to Blaenau Ffestiniog line, closure of which was also opposed by the Board. The Commission particularly welcomes the Minister of Transport's statement to the Ramblers Association regarding traffic restrictions in the remote countryside, particularly his view that he would expect the Highway Authorities to take into account the views of the National Park Planning Authorities in submitting improvement schemes to him for grant purposes. The Minister also said that he was ready to consider orders made on grounds of amenity so long as provision was made for carrying on the normal life of the district without imposing unreasonable burdens. Further reference to this question and also to the Commission's present views regarding recreational facilities in National Parks is made on p. 632 of this issue of *Nature*.

\* National Parks and Access to the Countryside Act, 1949. Fifteenth Report of the National Parks Commission for the year ended September 30, 1964. Pp. vii + 89 + 8 plates. (London: H.M.S.O., 1964.) 8s. net.

NUFFIELD FOUNDATION SCIENCE TEACHING PROJECT:  
PROGRESS REPORT

THE progress report of the Nuffield Foundation Science Teaching Project\* appeared towards the end of 1964. The work is now being extended to cover the teaching of science to children of all ages in most types of schools. Work began in 1961 on the design of courses leading to O-level examinations in physics, chemistry and biology. Programmes for the improvement of science and mathematics teaching have already been announced. The progress report now published describes some of the plans being made to design science courses for sixth forms and for 'Newsom' children (the less able half of the school population) in the 13-16 year range.

Since the preparation of the report, the Nuffield Trustees have allocated a further £115,000 to science teaching, chiefly to support A-level biology. So far

£550,000 has been set aside for science teaching, but this figure does not cover work in the physical sciences in the sixth form and the proposed programme for the 'Newsom' children. During the next year a sum of a quarter of a million pounds will have to be spent on the first printing of the teachers' guides and pupils' books due to be published in April 1966.

A hundred and fifty British secondary schools and a total of three thousand children in more than two hundred classrooms are being used in the controlled testing of the O-level courses. This is most necessary: far too many text-books are the results of their authors' attempts to dilute or re-present material from the standard works. Large groups of children will have to be the yard-sticks by which teaching material is validated. This absorbs time and money and may prove to be inhibiting to the over-facile writer of school text-books, but it is the only permissible way for the future.

\* The Nuffield Foundation Science Teaching Project. Progress Report, October, 1964. Pp. 81. (London: Longmans, Green and Co., Ltd., and Penguin Books. Published for the Nuffield Foundation.)

Another guiding principle is that science should be made intelligible and enjoyable, even in the work of the public examinations. Experimental work is a conspicuous part of the Nuffield courses. Many local authorities have helped to give the courses a fair trial by spending several hundreds of pounds a school on new scientific equipment.

Alternative O-level examinations for children using Nuffield courses in physics, chemistry and biology are to be provided by the public examinations in the years ahead, so that children engaged in the testing of new courses will not be hampered. A hundred titles in the first printing of about three million books will appear in spring

1966 and, in addition, there are films, film loops and other special items of equipment developed during the programme, and other visual aids.

The future of the O-level courses is said in the report to depend on the extent to which practising teachers will be helped to make the best use of the new methods. No charitable organization can be responsible for the work in the future, and the Department of Education and the local education authorities will have to give major support. The report outlines future developments, including a project to teach general science in the 11-13 age range.

W. L. SUMNER

## ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

VARIOUS aspects of antimicrobial agents, infectious diseases, microbiology, and chemotherapy were discussed in the 162 papers presented at the fourth Interscience Conference on Antimicrobial Agents and Chemotherapy. This meeting was held in New York during October 26-28, 1964, under the sponsorship of the American Society for Microbiology and organized with the co-operation of the Infectious Diseases Society of America. Dr. N. Bohonos (Lederle Laboratories) was chairman of the conference. 1,045 scientists attended this meeting, including representatives from eighteen countries in all.

The three-day conference opened with an address by Dr. M. Finland (retiring president of the Infectious Diseases Society) on problems in the clinical investigation of new drugs. The role of the U.S. Food and Drug Administration and other regulatory agencies was reviewed, and the necessity of allowing the investigator to exercise some initiative and responsibility was mentioned. The John Scott Award for meritorious scientific achievement was presented at this session by the City of Philadelphia (Pennsylvania) to Prof. J. C. Sheehan (Massachusetts Institute of Technology) for his research leading to the preparation of the 'semi-synthetic penicillins'.

Among the features of the 1964 meeting were four symposia. Prof. M. Hamburger chaired the first, which dealt with "Penicillins and Cephalosporins" and included reviews of the clinical status of cephalothin (by W. M. Kirby), ampicillin (by S. Ross), oxacillin and methicillin (by H. Simon), and nafcillin (by C. M. Martin). P. B. Bunn described his experience with penicillin allergy and emphasized the apparent lack of strong sensitivity to ampicillin of patients allergic to benzylpenicillin (penicillin G). It was obvious that some of these newer semi-synthetic penicillins were as clinically useful in treatment of certain Gram-negative infections as some of the other antibiotics, for example, chloramphenicol, polymyxin, and colistin. However, as Prof. Simon pointed out, we do not as yet have a 'panaceamycin' for all infections. M. R. Pollock, (National Institute for Medical Research, London) summarized his investigations of the  $\beta$ -lactamases of bacteria. These enzymes, which inactivate both penicillins and cephalosporins under laboratory conditions, may not be entirely responsible for the penicillin resistance noted in certain bacterial cultures.

Dr. H. C. Reilly (Sloan Kettering Institute) presided over a symposium on "Antineoplastic Antibiotics". S. A. Schepartz reviewed the programme of the Cancer Chemotherapy National Screening Center, in which searches are being made for anti-neoplastic antibiotics from fermentation processes. Thirty materials (isolated from more than 115,000 'crude' broths screened) have been considered for clinical evaluation, but none has shown 'great promise'. An investigation of the incidence of anti-neoplastic activity among antibiotics that act as inducers of lyso-genic bacteria was discussed by K. E. Price (Bristol Laboratories), who found a high correlation between anti-neoplastic activity and induction of lambda phage

in *E. coli* W1709. J. J. Fox (Sloan Kettering Institute) reported that gougerotin contains sarcosyl-D-serine in acyl-amino linkage to 4-amino-4-deoxy-hexuronic acid amide, and concluded that the structure proposed earlier for this antibiotic was in error. He pointed out that all known pyrimidine nucleoside antibiotics (elaborated by *Streptomyces* species) contain cytosine and 4-amino-hexose moieties. A. Bloch and C. A. Nichol (Roswell Park Memorial Institute) commented on the nucleoside antibiotics related to adenosine with special reference to their effects on adenosine phosphorylase, deaminase, and kinase. R. D. Sullivan (Lahey Clinic Foundation, Boston) pointed out that doses 2-2.5 times as great as those tolerated by single daily injection were well tolerated by prolonged intravenous infusion of actinomycin D, streptonigrin, and mitomycin C, and went on to review recent progress in the use of these antibiotics in cancer chemotherapy.

"The Future of Antiviral Drugs" was the subject of a session convened by E. C. Herrmann, jun. (The Mayo Clinic). F. M. Schabel (Southern Research Institute) and I. S. Johnson (Eli Lilly and Co.) discussed the theoretical basis for examining antiviral chemotherapy and problems in the detection of antiviral antibiotics. The successes in viral treatment following the use of 5-iodo-2'-deoxyuridine, cytosine, and 5-azaorotic acid were reviewed by P. Calabresi (Yale University); and R. L. Thompson (National Institutes of Health, Bethesda) summarized the tests with *N*-methylisatin- $\beta$ -thiosemicarbazone and other thiosemicarbazones. The promising laboratory and clinical results with 1-adamantanamine hydrochloride were reviewed by C. E. Hoffman (E. I. DuPont de Nemours and Co.).

"The Usefulness of Synthetic Antimicrobials" was the subject of a symposium chaired by Dr. D. Lehr (New York Medical College). Dr. J. Alban (Stanford University), E. H. Kass (Harvard University), J. P. Colmore (Oklahoma Medical Center), and D. F. McDonald (University of Rochester) gave information on the present status of the sulphonamides, nitrofurantoin, and nalidixic acid.

Among the new antibiotics mentioned at the Conference were:

(1) The *S*-ethyl homologue of lincomycin and *N*-demethyl analogue of lincomycin formed by adding DL-ethionine and  $\alpha$ -methylthiolincosamide, respectively, to lincomycin-producing fermentations according to Argoudelis *et al.* (The Upjohn Co.). (2) Everninomicin, a new antibiotic complex derived from *Micromonospora carbonacea* which inhibits Gram-positive bacteria and PPLO, as shown by Weinstein *et al.* (Schering Corporation). (3) Almarcetin, a new polypeptide antibiotic which may be useful against phytopathogens, according to Bachler *et al.* (Northern Regional Research Laboratory, U.S. Department of Agriculture). (4) Monicamycin, an acidic heptaene antifungal agent isolated by Gupta (Regional Research Laboratory, Jammu-Tawi, India). (5) Rubiflavin, a toxic anti-tumour antibiotic described by Aszalos *et al.* (Squibb Institute for Medical Research). (6) U-13714, a

toxic antiviral agent isolated by Vavra *et al.* (The Upjohn Co.) from *Streptomyces canarius*. (7) LL-AP191, a xanthomycin-type antibiotic inhibiting both Gram-positive and Gram-negative bacteria according to H. A. Whaley *et al.* (Lederle Laboratories). (8) LL-A1471E, a macrolide antibiotic inhibiting Gram-positive bacteria, reported by M. P. Kunstmann *et al.* (Lederle Laboratories). (9) U-11092, an antibiotic inhibiting Gram-positive bacteria *in vivo* when given either *per os* or subcutaneously, according to B. K. Bhuyan *et al.* (The Upjohn Co.). (10) Enteromycin (seligocidin) from a streptozotocin-producing fermentation, as reported by R. R. Herr *et al.* (The Upjohn Co.). (11) Enteromycin carboxamide isolated by S. DeVoe *et al.* (Lederle Laboratories) from a streptomycete fermentation.

A wide variety of topics were discussed in the sessions concerned with clinical problems and treatment in humans: (1) Animal infections examinations which were pertinent to understanding host resistance and drug action, which included: pneumococcal infections in splenectomized monkeys; prophylaxis of aerogenic Rocky Mountain spotted fever in monkeys; experimental histoplasmosis capsulitis endocarditis; coxsackie A-9 infection in adult mice treated with steroids and in mice with forced exercise; penicillin toxicity in guinea-pigs as related to changes in microbial flora; and renal infection with enterococcal protoplasts (in rats). (2) Clinical investigations concerned several chronic conditions, which included: fungal diseases in reticuloendothelial malignancies; infection in volunteers with penicillin-resistant gonococci; staphylococcal carriers; chronic bronchitis and pulmonary disease; retreatment of tuberculosis, *Salmonella* carriers, urinary tract infections and pathogenic studies in Gram-negative rod infections. (3) Studies of antibody responses, which included gonococcal and meningococcal infections, herpes simplex disease and staphylococcal states.

A series of papers on experimental infections covered: (1) *Mycobacterium fortuitum* in mice as a screen for tuberculosis; (2) hamycin in experimental mycoses in mice; (3) a standardized *Leptospira pomona* infection in hamsters; (4) experimental localized *Pseudomonas* infection (keratitis) in rabbits; (5) septicæmic anthrax in rhesus monkeys; (6) the use of sulphonamides in murine leprosy.

The modes of action of a number of antibiotics were mentioned, including D-cycloserine, vernamycin A, vancomycin, mitomycin C, porfomycin and streptonigrin. Among the synthetic antimicrobial agents reported for the first time were an antifungal agent, tolnaftate (O-2-naphthyl-m,N-dimethylthiocarbamate), 1-(5-nitrofurfurylideneamino)-2-imidazolidinone, and 4(5-nitro-2-furyl)-2-(3-pyridyl)thiazole.

Most of the papers presented at the Conference will appear in *Antimicrobial Agents and Chemotherapy, 1964*, which will be published by the American Society for Microbiology (Ann Arbor, Michigan) in April 1965. The *Proceedings* will be distributed to all registrants at the meeting, and will be available from the American Society for Microbiology or from Messrs. H. K. Lewis, London.

Plans for the 1965 conference are already under way. This meeting will be combined with the fourth International Congress of Chemotherapy and will be jointly sponsored by the American Society for Microbiology and the International Society for Chemotherapy. Programmes on infectious diseases will be organized with the co-operation of the Infectious Diseases Society of America. The sessions will be held in the Shoreham Hotel, Washington, during October 17–21. Dr. G. M. Savage (The Upjohn Co.) is general chairman, and enquiries concerning arrangements and participation should be sent to Mr. R. W. Sarber, Executive Secretary, American Society for Microbiology, 115 Huron View Boulevard, Ann Arbor, Michigan. D. PERLMAN

## METEOROLOGY AND LOCUST MIGRATIONS

A TECHNICAL Note recently published by the World Meteorological Organization covers a great deal of ground in the relationship between meteorology and the movement of locust populations\*. Work on locust problems started in 1929 under Prof. B. P. Uvarov at what has now become the Anti-Locust Research Centre, and Dr. R. C. Rainey, who was a glider before the Second World War, a wartime meteorologist, and again an entomologist working with the Centre after the War, is uniquely qualified to write on meteorological aspects of locust populations. The note is very well produced, as are most of the World Meteorological Office Technical Note series, with 24 figures and three instructive photographs of locusts in flight, which hint at the problem—the immensity of which is realized by figures such as a swarm density per square kilometre of about  $5 \times 10^7$  locusts, weighing  $10^5$  kilograms and eating their own weight of vegetation each day. Swarms can cover hundreds or even thousands of square kilometres. As an example of financial loss, Morocco's loss in the year 1954–55 was assessed at about 13 million dollars; and Morocco was only one of 23 countries which suffered losses in that year.

The main emphasis is on the detailed survey for the year May 1954–May 1955, during which the Anti-Locust Research Centre collaborated closely with the World Meteorological Organization mission in East Africa headed

by C. I. H. Aspliden, part author of Chapter 3. Considerable flying, both spotter and spraying, was undertaken, and swarm movements could be followed with far greater certainty than in any earlier work. Identifiable swarms flew for very variable distances up to 3,500 km, with a maximum followed flight time of around two months. After a short introduction outlining the life cycle of the locust, meso-scale consideration is given to the individual swarm behaviour, and the general movement downwind is brought out clearly. Earlier investigations sometimes found variation between winds and swarm movements; this was presumably largely a question of difficulties of reliable assessment by ground observers, who could be unduly influenced by individual locust movements. Even in light winds, swarms nearly always moved more slowly than the surrounding air, although individual locusts may fly more quickly. This is caused by a sort of rolling effect, where the swarm 'front' rolls forward, with individual locusts spending some time on the ground if the swarm is low; flight level varied widely, however, with swarms at many thousands of feet on occasions. This agreement between wind and swarm movement is very valuable, as, for example, arrival of swarms in a new area can be foreseen.

The special survey year is documented with excellent monthly charts showing the locust distribution over the whole area from India westwards to Morocco, and including Central Africa; the development stage of the locust (whether hoppers, etc.) is also brought out. Each area is briefly discussed, with an analysis of locust behaviour during the year.

\* World Meteorological Organization and Anti-Locust Research Centre. *Meteorology and the Migration of Desert Locusts: Applications of Synoptic Meteorology in Locust Control*. By R. C. Rainey. Pp. 115. (W.M.O. Technical Note No. 54—No. 138. TP. 64. Anti-Locust Memoir No. 7.) (Geneva: World Meteorological Organization; London: Anti-Locust Research Centre, 1964.) 25 Sw. francs; 42s.



Rainey puts forward the view that, broadly speaking, the locust populations are at their greatest to the south side of the Intertropical Convergence Zone (ICZ) and that frequently swarms of locusts follow by a day or two the northward moving ICZ. This is logical, since semi-tropical locusts must eventually get carried by northerly moving airstreams towards a convergence zone.

Locusts are essentially hot-climate insects, and the mostly light wind régimes which obtain in the semi-tropics do not give much opportunity for their movement outside the tropics. However, it does happen from time to time that an active depression tracking south of the Mediterranean carries the locusts bodily in the warm sector to areas north of the African coast. The classic migration example is quoted (with a trajectory map) of the locusts which reached the Isles of Scilly and Southern Ireland in October 1954—the first live locusts known to reach the British Isles since 1869. This type of trajectory technique has been applied more recently to moth migrations and spore movement with very similar types of track.

The pages of recommendations in Chapter 4 underline the importance of the type of collaboration found in meteorological networks and the inestimable value of aircraft both for spotting and for spraying. The control of locust movement by low-level winds is brought out, and also the importance of the ICZ. About the only point with which one might quarrel is the suggestion of a relationship between sunspot activity and the fluctuation of locust infestations which, viewed from a meteorological point of view, seems doubtful. Some of the diagrams such as those showing the effects of South Mediterranean depressions are not too happy; they have been stylized and appear distinctly confusing to a meteorologist.

There is no doubt of the value of the Note to anyone interested in either locust control problems or any insect migrations in general, and it is good to see such happy collaboration between such bodies as the Anti-Locust Research Centre and the World Meteorological Organization.

G. W. HURST

## PROTECTION AGAINST IONIZING RADIATIONS

THE informative and well-written *Protection Against Ionizing Radiations*\*, issued by the World Health Organization, concerns legislation on protection against the use of ionizing radiations. Apart from an introductory chapter outlining the need for control and making a general survey of existing legislation, the book is principally a detailed account of the legislation in force in 26 different countries. On these grounds it cannot fail to be of interest and a constant source of reference to all those concerned with radiological health and safety.

The scope of legislation varies widely from country to country, revealing many interesting aspects of their developing use of ionizing radiations and national characteristics. In some countries, it is extremely detailed, covering almost every aspect of protection, while in others it is quite general in character and greater reliance is placed on the voluntary action of users to maintain the required standards of safety. The need for legislation, in order to reduce the large numbers of accidents which were then taking place as a result of the use of X-rays, was discussed in Germany as long ago as 1905. Although no specific law resulted, nor is there any to-day in Germany covering the medical use of X-rays, the first rules for protection were drawn up and adopted in 1913 by the German Radiological Society. One of the earliest pieces of legislation was the New York City Ordinance of 1922, controlling the use of X-ray equipment. In the same year a law was introduced in Italy concerning the prospecting for, and use of, radioactive substances. Other countries introducing early legislation were Denmark, 1930, on the use of X-rays, Netherlands, 1931, X-rays and radioactive substances, and Finland, 1932, X-rays. It is not surprising that there was a great increase in regulations following the development and use of nuclear energy, and 16 of the 26 countries considered introduced legislation after 1945. In the United Kingdom, the first general law for the protection of both workers and public came into being with the passing of the Radioactive Substances Act of 1948, and in the U.S.S.R. the first general regulations appeared in 1953.

One example, concerning the employment of women on radioactive work, will suffice to demonstrate the importance of national characteristics, namely, that few countries would find it easy to follow Spain in enforcing a law which excludes from work with ionizing radiations all spinsters who are to be married within 3 months.

There is, however, general agreement between countries on the maximum permissible doses of radiation, and this is essentially the result of the fact that most legislation is based on the recommendations of the International Commission on Radiological Protection.

It is right that there should be international agreement about hazards which sometimes arise because of the gratuitous irradiation of people who have had no concern with the production of the source of the ionizing radiations, for example, from the testing of nuclear weapons, or, more peacefully, from the transport of radioactive sources. Furthermore, in view of the public's apprehension about the effects of radiation at the present time, it is, presumably, easier for most countries to fall in line, rather than disagree, with a body so authoritative and internationally constituted as the International Commission on Radiological Protection. Nevertheless, it will be surprising if, eventually, there are not some differences in the standards used in different countries, in any event for sources which are both generated and applied within their own frontiers. Already, the concept of maximum permissible dose is under review by the International Commission itself and there is a growing awareness of the statistical nature of the risks involved. According to present belief, there is no absolute safe limit on dose, only a diminishing risk as the dose decreases. It is not easy to see, therefore, how there can be international agreement about limits on dose which are equally acceptable to all countries. A degree of risk which is totally unacceptable in a well-developed country might seem trivial by comparison with the other risks of life, for example, from famine and disease, in a country the development of which may well depend on the use of nuclear energy. Mankind has always taken risks in order to improve his ultimate well-being, and no doubt, if the need arises, he will do so again in making use of ionizing radiations. Already, there have been some rejections of the standards set by the International Commission on Radiological Protection when their realization became either too inconvenient or too expensive. For example, some countries find it impracticable in certain industries to reach the present International Commission on Radiological Protection standard relating to the concentration of radon in air, a fact which is acknowledged by the International Atomic Energy Agency in its *Safety Series* No. 9, 1962. However, other countries, as exemplified in the World Health Organization's survey, have adopted the higher standards and presumably intend to reach them by

\* World Health Organization. *Protection Against Ionizing Radiations: Survey of Existing Legislation*. (Offprint from Vol. 15, No. 2 of the *International Digest of Health Legislation*.) Pp. 170. (Geneva: World Health Organization; London: H.M.S.O., 1964.) 6 Sw. francs; 10s.; 2 dollars.

increased expenditure and effort on improved ventilation and containment of the source of radon.

As this survey shows, there is wide divergence of views on the legislation which is necessary to ensure the safe use of ionizing radiations. It will be surprising if a future

edition of *Protection Against Ionizing Radiations* does not also reveal a greater diversity of opinions on the acceptable limits of dose which reflect more closely the needs of individual countries.

J. VENNART

## MULTIPLE HIT FREQUENCIES IN FULLY CORRELATED FIRE

By E. R. TERRY

Technical Operations Incorporated, Burlington, Massachusetts

IN evaluating the effectiveness of high-firing-rate guns as air defence and air-to-ground weapons we are often interested in hit frequencies other than the 'no hits', or miss, frequency. Assuming the aimpoint does not move during the firing interval, we can readily determine other hit frequencies by an extension of the 'salvo' or 'shotgun' formula. More precisely we will develop a model for determining the probability of exactly  $m$  hits with  $n$  rounds when the distribution of shots is circular normal and centred at a point  $h$  units from the target origin.

Assume that the pattern of weapons is released at a circular target of radius  $R$  with a bivariate circular normal aiming error characterized by a standard deviation  $\sigma_A$ . Each weapon in the pattern is distributed about the mean point of impact with a bivariate circular normal ballistic dispersion and is characterized by a standard deviation  $\sigma_B$ . The ballistic dispersion for each of the weapons comprising the pattern and the aiming error are mutually independent.

The probability that the  $i$ th round strikes the target of area  $A$  is:

$$p_i = \frac{1}{2\pi\sigma_B^2} \iint_A \exp\left[-\frac{1}{2\sigma_B^2}((x-h)^2 + y^2)\right] dx dy \quad (1)$$

Transforming to polar co-ordinates we obtain:

$$p_i = \frac{1}{2\pi\sigma_B^2} \int_{\theta=0}^{2\pi} \int_{r=0}^R \exp\left[-\frac{1}{2\sigma_B^2}(r^2 + h^2 - 2rh \cos \theta)\right] r dr d\theta \quad (2)$$

Rewriting equation (2) as follows:

$$p_i = \left(\frac{1}{\sigma_B^2} \exp\left[-\frac{h^2}{2\sigma_B^2}\right] \int_{r=0}^R r \exp\left[-\frac{r^2}{2\sigma_B^2}\right] \left(\frac{1}{2\pi} \int_{\theta=0}^{2\pi} \exp\left[\frac{rh \cos \theta}{\sigma_B^2}\right] d\theta\right) dr \right) \quad (3)$$

we see:

$$\frac{1}{2\pi} \int_{\theta=0}^{2\pi} \exp\left[\frac{rh \cos \theta}{\sigma_B^2}\right] d\theta = J_0\left[\frac{ih}{\sigma_B^2}\right] = I_0\left[\frac{rh}{\sigma_B^2}\right] \quad (4)$$

which is the zero-ordered Bessel function of pure imaginary argument. Accordingly equation (3) becomes:

$$p_i = \left(\frac{1}{\sigma_B^2} \exp\left[-\frac{h^2}{2\sigma_B^2}\right] \int_{r=0}^R r \exp\left[-\frac{r^2}{2\sigma_B^2}\right] \left(I_0\left[\frac{rh}{\sigma_B^2}\right]\right) dr \right) \quad (5)$$

Since the ballistic dispersions of all the rounds comprising the burst are independent of one another, the conditional probability that exactly  $m$  weapons will hit the target if a pattern of  $n$  weapons is released is  $\binom{n}{m} p_i^m (1-p_i)^{n-m}$  if we assume the resulting hit frequencies are binomially distributed. To obtain the unconditional probability that the centre of the distribution of shots is a distance  $h$  units from the target centre and that exactly  $m$  weapons will hit the target, we must multiply the expression above by the probability of the centre of this distribution of shots being so located.

The probability density function for determining whether the centre of the distribution of shots is within an annulus of width  $dh$  at a distance  $h$  units in any direction from the centre of the target is:

$$dp_h = \frac{1}{\sigma_A^2} \exp\left[-\frac{h^2}{2\sigma_A^2}\right] h dh \quad (6)$$

Multiplying equation (6) by the conditional probability that if the centre of the distribution of shots is so located exactly  $m$  rounds will hit the target, we get the corresponding probability density function:

$$\begin{aligned} dp_{[n,m]} &= dp_h \binom{n}{m} p_i^m (1-p_i)^{n-m} \\ &= \frac{1}{\sigma_A^2} \exp\left[-\frac{h^2}{2\sigma_A^2}\right] \binom{n}{m} p_i^m (1-p_i)^{n-m} h dh \quad (7) \end{aligned}$$

where the  $p_i$  are a function of the  $h_i$ . Integrating equation (7), we obtain for the unconditional probability that exactly  $m$  weapons will hit the target when a burst of  $n$  rounds is fired:

$$P_{[n,m]} = \frac{1}{\sigma_A^2} \int_0^\infty \exp\left[-\frac{h_i^2}{2\sigma_A^2}\right] \binom{n}{m} p_i^m (1-p_i)^{n-m} h_i dh_i \quad (8)$$

Equation (8) can be evaluated numerically by employing the Laguerre-Gauss quadrature formula.

The model outlined here has been programmed in *Fortran* for the IBM 7090/7094. Tables for facilitating a quick solution of equation (8) for any  $m$  and  $n$  are at present being compiled and will be submitted for publication in the near future.

## OPTICAL PROPERTIES OF URANIUM OXIDES

By DR. J. M. JONES and DR. D. G. MURCHISON

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WE were recently asked to investigate the optical properties of uranium oxides in the composition range  $\text{UO}_{2.00}$ - $\text{UO}_{2.25}$  as part of a research programme being carried out by the Reactor Group at the U.K.

Atomic Energy Authority Establishment, Dounreay, Scotland. It was hoped to establish: (1) that a satisfactory correlation existed between the optical properties of the oxides and their oxygen/metal ratios; (2) if this were the

case, that the method employed could give a sensitivity of  $\pm 0.01$  on the oxygen/metal ratios, particularly in the examination of sintered pellets and powders which are finding increasing use and application in technology and industry. The results obtained so far are encouraging and suggest that both aims stated above will be fulfilled.

Pellets have been prepared at Dounreay by sintering uranium oxide powder at  $1,450^\circ\text{C}$  in an atmosphere of carbon dioxide. A number of pellets in the composition range  $\text{UO}_{2.00}\text{--}\text{UO}_{2.21}$  have been provided. Sintered pellets are not, however, the most satisfactory materials for the collection of the initial optical data on which to establish reliable relationships with oxygen/metal (O/M) ratios. Attempts are therefore being made in this department to produce single crystals of uranium oxides with varying O/M ratios to act as reference materials. Up to now only crystals prepared in the manner described by R. G. Robins<sup>1</sup> have been examined optically. Briefly, this method involves electrolysis of uranyl chloride in a fused melt of potassium and sodium chlorides mixed in equimolar proportions. After chlorinating the melt to remove hydroxide impurities, and later to dissolve sufficient powdered uranium dioxide to yield an optimum concentration of 2.5 g  $\text{UO}_2/\text{Cl}_2/100\text{ g}$  solvent, the electrolysis is carried out for 20 h at  $830^\circ\text{C}$  using an applied potential of 100–250 mV and a cathode current density of 4 m.amp/cm<sup>2</sup>. So far as possible the optimum conditions recommended by Robins have been maintained; but certain changes in cell size and melt volume have been necessary. Despite the modifications, satisfactory crystals of cubo-octahedral habit of up to 0.5 mm in diameter have been obtained. Analysis by thermobalance of crystals from two sections of the platinum cathode gave O/M values of 2.002 and 2.003. X-ray analysis of crystals from the same areas of the cathode gave lattice parameters good for stoichiometric  $\text{UO}_2$ .

The optical parameter of the oxides measured is their specular reflectivity. This is obtained by comparing the intensity of light from the oxide with that from a standard of known reflectivity. In this case diamond has been used because of its low dispersion and the proximity of its reflectivity to those of the oxides. Reflectivities in air and oil of known refractive index have been measured, thus allowing calculation by Beer's equation of the refractive and absorptive indices of the oxides.

Because of the tendency of the uranium oxides to take on oxygen at a rate which is probably sufficient to cause a significant alteration of their surface properties over relatively short periods of time, the oxides are kept in an inert atmosphere. The reflectivity measurements and the last stages of polishing of the samples are therefore made in a glove box kept at a low positive pressure with nitrogen. A Leitz 'Epilux' microscope has been sealed just above the level of its vertical illuminator in the box and adjusted in the manner prescribed by M. Berek<sup>2</sup> for reflectivity measurements. After light from the specimen has left the microscope eyepiece, it passes into equipment similar to part of that designed by F. Gabler, W. Gubisch, W. Lipp and O. Rüker<sup>3</sup> for reflectivity determinations. This part consists essentially of a reflex housing, fitted immediately above the eyepiece, followed in the optical train by a plano-convex lens and then one of a series of apertures. These are contained on a silvered surface which makes it possible to observe with the reflex housing the size and position of the aperture in use in relation to the specimen in the field view of the microscope. The actual field sizes on which reflectivities can be measured vary from 4 to  $100\mu$ .

Before the light finally reaches the cathode of an 11-stage photomultiplier, it passes through one of a number of Schott interference filters. Output from the photomultiplier is fed into a pen recorder instead of more conventionally in reflectivity systems into a high-sensitivity galvanometer. This allows long series of measurements to be made without the operator having to remove his

hands from the box, thus preventing errors of measurement due to loss of focus on the microscope which occurs when the internal pressure of the box is altered by movement of the operator's hands.

Difficulties may arise when measurements are made on sintered samples because of the pores, flaws and defects resulting from the sintering process. To measure reflectivities on the surfaces of such samples, as small a field of view as possible has to be used. With a small aperture and a high-power air objective (N.A. 0.85, mag.  $\times 45$ ), the diameter of the field can be reduced to  $4\mu$ . Repeatability of measurements on sinters lies within  $\pm 1$  per cent of the reflectivity of the oxide, but on single crystals that yield polished surfaces of very high quality, greater accuracy is possible.

Reflectivities in air of three sinters with different O/M ratios, and one set of pure single crystals, have been measured at 4030, 4480, 5020, 5460, 5900, 6520 and 7090 Å. These results are plotted in Fig. 1. It appears that for the uranium oxides the maximum change of reflectivity with O/M ratio occurs towards the extremes of the visible spectrum with much less differentiation around 5000 Å. This observation corresponds in general with the results of R. J. Ackermann, R. J. Thorn and G. H. Winslow<sup>4</sup>, who plotted optical densities of uranium dioxide films against wave-lengths, and with the trends of diffuse reflectance with wave-length on bulk uranium dioxide reported by Companion and Winslow<sup>5</sup>.

It is unfortunate in the present experiments that the convenient high-intensity tungsten filament lamp used as a light source for the microscope has a low intensity in the blue and that the photomultiplier now in operation has a much reduced sensitivity around 7000 Å. These factors at present reduce the accuracy of measurement in the two most desirable regions of the visible spectrum. Measurements are now primarily being made at 4030 Å, where the spectral response of the photomultiplier is strong. The results so far suggest that, even with the tungsten lamp and its low efficiency at this wave-length, the desired differentiation between the different sintered oxides will be achieved. It does seem, however, that the reflectivity values measured on the sinters will be much more liable to influence of polishing technique than other materials such as coal constituents and many ore minerals.

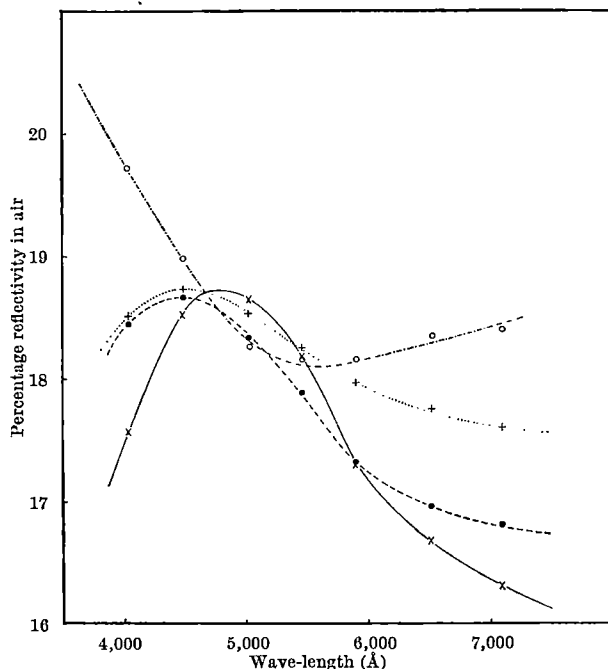


Fig. 1. Reflectivities of uranium oxides with different oxygen/metal ratios plotted against wave-length. x,  $\text{UO}_{2.002}$ ; ●,  $\text{UO}_{2.104}$ ; +,  $\text{UO}_{2.151}$ ; ○,  $\text{UO}_{2.203}$ .



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## MOLECULAR HYPOTHESIS OF FLAGELLAR ACTIVITY

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**ANALYSES** of the effect of temperature on flagellar movement involve a consideration of chemical kinetics and, following a recent investigation of this topic<sup>1</sup>, we have been tempted to speculate at a molecular level on the basis of flagellar contraction.

Several stroboscopic and high-speed cine-photographic investigations<sup>2-5</sup> have revealed that cilia generally execute an oar-like beat followed by a recovery stroke offering little resistance to movement, while many flagella maintain two- or three-dimensional waves along their length. Hydrodynamic equations derived by several authors<sup>6-13</sup> relate the propulsive velocity of a flagellated organism to the wave-length, amplitude and frequency of beat. Equations of a similar nature<sup>13,14</sup> enable one to evaluate the energy expended externally by an undulating flagellum, while the presence of a sustained amplitude along its length is held to show<sup>7,15</sup> that chemical energy is used over the whole extent of an organelle.

The fine structure of cilia and flagella has been well established by electron microscopy<sup>16-18</sup> although the functions of the several components are the subject of discussion. Within the flagellar membrane is the well-known 9 + 2 array of fibrils extending the length of an organelle. Each of the nine peripheral fibrils is in fact a doublet and possesses pairs of arms pointing towards an adjacent fibril. Between the central pair and the outer nine is a set of nine secondary fibrils or radial spokes joining the peripheral to the central fibrils.

The reactivation of glycerol-extracted cilia and flagella by ATP<sup>20-23</sup> has led to the suggestion that the dislocation of the terminal phosphate bond in ATP may be the source of energy for their movement. The way in which the chemical energy is released in a controlled manner is uncertain, but it is accepted that a motile flagellum must possess elements for contraction and compression and, further, a means for propagating waves along its length.

From the foregoing summary it is evident that there are many diverse approaches to the problem of flagellar motility, but in general the discussions in the literature have tended to be confined within the limits of a particular discipline. Now that more structural and chemical knowledge is becoming available and the interpretation of electron micrographs is concerned with structural sub-units of molecular dimensions, it will be useful to have some working hypothesis formulated in molecular terms which will provide a basis for general discussion. In the model that we propose below we have not attempted to satisfy or explain the multitude of observations in the literature, but have tried rather to devise a plausible system which will suggest further lines of thought.

**Résumé of relevant data.** The main items that should be incorporated in a flagellar model would seem to be: (i) A cyclic system of local contractions. (ii) The outer nine fibrils of the flagellum. (iii) The composition of the fibrils and probable existence of sub-units. (iv) The theory of mechanical propagation of contractile stimuli put forward by Machin<sup>15,24</sup>. (v) The ATPase activity of flagella and the dependence of their wave-form and frequency on ATP concentration. (vi) The relation

between the flagellar system and the actinomyosin system, especially in the light of a recent paper on the behaviour of F-actin<sup>25</sup>.

Because a sliding-filament mechanism operates in striated muscle it has been considered a possibility in the movement of flagella. Afzelius<sup>16</sup>, Gibbons and Grimstone<sup>18</sup> and Satir<sup>26</sup> discuss such a mechanism, but it is discarded by Gibbons and Grimstone on the grounds that to sustain a wave on a flagellum would require simultaneous extensions and contractions at different points on the same fibril. A more attractive system is one which assumes<sup>19,24</sup> active longitudinal contraction of regions of the flagellar structure. The nine peripheral fibrils are generally credited with the contractile function<sup>27</sup>, and we shall follow this assumption, in which each fibril is supposed to be made up of a series of contractile units<sup>15,7</sup>. The structure of these double fibrils, with their typical 'figure-of-eight' appearance in cross-section and their rows of projections or 'arms', has been described by several authors<sup>16,18,19</sup>, and more recently evidence of finer structure has been adduced. André and Thiéry<sup>28</sup> have observed the fibrils to be composed of 35-Å diameter protofibrils with a longitudinal periodicity of about 80 Å, and Pease<sup>29</sup> has found substantially the same arrangement.

A means by which local contractions may be propagated along a fibril has been suggested by Machin<sup>15</sup>. In this mechanical process a contractile unit is activated on deformation by a passively propagated wave from a neighbouring region of the flagellum. If the relation between tension and length of a contractile unit is non-linear, and there is a delay between the change in length and the development of tension, Machin<sup>24</sup> has shown that waves will arise spontaneously on a flagellum. In such a system the frequency of propagation can be controlled by altering the mechanical impedance at the proximal end of the flagellum. Holwill<sup>30</sup> considers that, although a mechanical system could be responsible for wave propagation, a control system is necessary for the initiation of a wave.

Chemical investigations of cilia<sup>27,31</sup> are not yet at the stage of characterizing the protein of the fibrils, but Gibbons<sup>27</sup> has reported the existence of a 25 S particle in solutions of ciliary protein. The particle has ATPase activity, is possibly associated with the 'arms', and is suggested by Gibbons to be a naturally occurring sub-unit of the cilium. The ATPase activity of flagella has also been demonstrated in the outer fibrils by Nelson<sup>32</sup> using cytochemical techniques, and the dephosphorylation of ATP is generally agreed<sup>21</sup> to be the source of energy for flagellar movement.

**Stimulation of the contractile unit.** The observations summarized here suggest that chemical energy supplied to the flagellum is used in the change of shape of contractile units composing the fibrous structure, and the theory of Machin<sup>15</sup> suggests how the contraction of individual units may be stimulated. In this theory the stimulus to contraction is conveyed mechanically, by the passive propagation of the flexional wave along the flagellum. The physical implications of this process appear to be satisfied

in practice<sup>30</sup> so that so far as we know this is a feasible system. We may imagine that the contractile unit in the fibril, before the peak of the contraction wave reaches it, is in some physico-chemical metastable state, and that the passive deformation conveyed by the wave momentarily alters the steric and electrostatic environment of the unit so that it is able to contract into an energetically more favourable conformation. The flagellum thus undergoes a kind of forced simple harmonic motion. The velocity of the passive wave along the flagellum is inherently dependent on the mechanical properties of the organelle and of its environment, but at the same time determines the rate of transmission of the forcing vibration. The stimulus to contraction could take effect in at least three ways, two of which we hope to eliminate at a later stage:

(a) The stimulus makes it possible for the contractile unit to interact with ATP molecules in its local environment, and use the dephosphorylation energy in changing its shape. (b) The unit is already 'primed' with ATP and the stimulus merely initiates the dephosphorylation and consequent contraction. (c) The stimulus raises the unit over a potential energy barrier so that it is able to contract to a state which is physically at a lower energy level; ATP is only used afterwards in the expansion of the unit to its previous metastable condition.

*Relation between chemical and structural sub-units.* A question which arises at this point is the relation between the contractile units we envisage and the observed fine structure of the outer fibrils. The experiments of Brokaw<sup>21</sup> with glycerinated models of *Polytoma* flagella may give a clue to the answer, for from his published measurements one can estimate (very approximately) the number of contractile units per flagellum. From his graphical presentation of the dephosphorylation of ATP by a suspension of flagella, we find the rate,  $V$ , of this process was in one instance 0.02 g of P per ml. per min. The concentration of flagella was  $1.5 \times 10^8$  in 20 ml. and that of the ATP was  $5 \times 10^{-5}$  M. At this concentration of ATP the rate  $V$  was almost independent of the substrate (ATP) concentration.

At high substrate concentrations the Michaelis-Menten law of enzyme kinetics (which is obeyed by the present reaction) reduces to the statement:

$$V = k[E]$$

where  $k$  is the limiting rate constant and  $[E]$  is the total concentration of enzyme<sup>33</sup>. If each contractile unit in the flagellum is activated  $f$  times per second, where  $f$  is the flagellar beat frequency, then  $f$  is also the limiting rate constant of the several chemical reactions which must occur in each contraction-relaxation cycle<sup>1</sup>. Let us define the size of the contractile unit to be that which is associated with one ATP molecule. If there are  $x$  units per flagellum and there are  $F$  flagella per litre, we can replace the molar concentration  $[E]$  by  $Fx/N_A$ , where  $N_A$  is Avogadro's Number. Thus we obtain:

$$V = \frac{fFx}{N_A}$$

Brokaw's values give  $V = 1.1 \times 10^{-8}$  M sec<sup>-1</sup> and  $F = 7.5 \times 10^9$  l.<sup>-1</sup>, so that  $fx = 9 \times 10^5$  units per flagellum per sec. The frequency of beat at an ATP concentration of  $5 \times 10^{-5}$  M is not given specifically by Brokaw, but is probably somewhere between 1 and 5 per sec. By making a guess at  $f = 3$  per sec, one finds a value for  $x$  of  $3 \times 10^5$  contractile units per flagellum.

On our hypothesis the contractile units are located in the outer fibrils so that  $3 \times 10^5$  units have to be fitted into 9 structures with an average length of about  $15\mu$  ( $15 \times 10^3$  Å). A short calculation shows that the effective length containing a contractile unit is equal to the number of units occurring in the cross-section of a fibril, multiplied by 4.5 Å. If, for example, one takes 18, the number of

35-Å 'protofibrils' suggested to be in the fibril by André and Thiéry<sup>28</sup>, one obtains an effective length of about 80 Å. The agreement of this length with the observed periodicity is coincidental, but these calculations do show that it is possible for the molecular units responsible for ATPase activity to be identical (in position or distribution) with the sub-units of which a fibril is composed.

*Role of ATP in the contraction phase.* The energy supplied by the measured rates of dephosphorylation of ATP has been noted by several authors<sup>21,34</sup> to be much in excess of the energy calculated to be necessary to propel the organism. The remainder of the energy is generally supposed to be dissipated in irreversible processes such as the non-elastic deformation of the flagellar structure, but another possibility is that there may be continuous ATPase activity in that part of the fibril which is under tension, in a way analogous to that suggested by Asakura, Taniguchi and Oosawa<sup>25</sup> for the isometric contraction of *F*-actin. However the energy is used, we know that something like  $10^5$ - $10^6$  contractile units per sec are activated in a flagellum, and that, if they are packed in the outer fibrils, they are probably separated longitudinally by a distance of the order of 100 Å. Since the wave velocity along flagella has been measured (about 250  $\mu$ /sec in *S. oncopelti*) (ref. 30) one can calculate the rate at which successive units are stimulated mechanically by the passage of the wave—for the values mentioned, the rate of progress of the stimulus along the fibril would be  $2.5 \times 10^4$  contractile units/sec. We can use this rate in order to decide whether type (a) of our proposed effects of stimulation is possible, that is, whether in fact it is possible for ATP to be exchanged in the contraction process within a reasonable time of stimulation.

The collision theory of reaction rates enables one to estimate the number of times per sec an ATP molecule in the local environment of the fibrils will make an effective collision with a sub-unit, and we can compare this rate with the rate of propagation of the stimulus. The number of effective collisions of unlike molecules  $A$  and  $B$  per c.c. per sec is given by the expression:

$$N_A N_B \sigma_{AB} \left\{ 8\pi RT \left( \frac{1}{M_A} + \frac{1}{M_B} \right) \right\}^{\frac{1}{2}} e^{-E/RT}$$

in which  $N$  is the concentration in molecules/c.c. and  $M$  the molecular weight of one reactant, and  $\sigma_{AB}$  is the mean diameter of the two molecules<sup>35</sup>. We put  $N_B = 1$  for the sub-unit (s.u.) in order to find the number of collisions/sec/s.u. and make the unit effectively stationary by letting  $M_B \rightarrow \infty$ . If we insert a physiological concentration of ATP ( $10^{-4}$  M) and a mean diameter of 30 Å, and use values for the molecular weight and activation energy of ATP of  $M_A = 500$ ,  $E = 10$  kcal/mole, we obtain a frequency of effective collisions of just less than 10 per sec at  $T = 300^\circ$  K. Since our calculated rate of stimulation is  $2.5 \times 10^4$  s.u. per sec it seems that collision processes are not able to supply ATP quickly enough for it to be exchanged in the contraction phase.

An alternative to exchange is that before the stimulus arrives, the ATP is already in position in the form of an ATP-sub-unit complex, the two components being presumably bound electrostatically in some way. Brokaw's finding<sup>21</sup>, that the dephosphorylation rate with glycerinated flagella became independent of ATP concentration above a certain level, is consistent with this idea, as this behaviour is characteristic of enzyme reactions in which an enzyme-substrate complex is formed<sup>33</sup>. The binding of ATP by molecules of a fibrous protein is not unlikely, as, for example, actin is known to form complexes with nucleoside phosphates and act as a dephosphorylating enzyme in certain circumstances<sup>25</sup>. The suggestion is that the ATP-sub-unit complex is the contractile unit of the flagellum, and that dephosphorylation occurs as a fairly fast process in the contraction phase. Since the total process is a cyclic one, this implies that the ADP

produced by contraction must be replaced by ATP in the relaxation phase, before the sub-unit is able to contract again.

*The relaxation phase.* On the foregoing hypothesis, it is in the relaxation phase and in the regeneration or replacement of ATP that the rate-limiting step occurs which must control the frequency of beat. In the living organism this step may be in some subsidiary metabolic process concerned with the re-phosphorylation of ADP (which could be still bound to the contractile unit, or free in 'solution' in the flagellum). It is to this limiting reaction that the values of activation enthalpy and entropy would apply which are derived, for example, from measurements of the thermal dependence of flagellar activity<sup>1</sup>.

In glycerinated models<sup>20,21,23</sup> the frequency of beat increases with increasing concentration of ATP in the medium, so that it seems in this case the ADP may be replaced directly by ATP by a diffusion-collision process. The rate of such a process would be directly dependent on the ATP concentration, and would control the rate of 'relaxation' of the contractile units and hence the frequency. It is interesting that the effective collision frequency which we calculated for an ATP concentration of  $10^{-4}$  M is about 10 per sec, while Brokaw<sup>21</sup> found that *P. uvella* models at this concentration had frequencies of beat "probably greater than 5 beats/sec". A collision process is thus able to supply ATP at rates of the right order of magnitude to sustain the flagellar motion.

The commonly noted phenomenon that excess ATP has a 'softening' or 'plasticizing' effect on flagella<sup>20,32,34</sup> may arise because the replacement of ADP by ATP on the sub-unit is necessarily concomitant with the release of tension. Nelson<sup>32</sup> states that flagella frequently appear rigid when ATP is washed out, which seems to agree with this hypothesis. We have suggested elsewhere<sup>1</sup> that the effect of ionic strength on the flagellar frequency may arise because the limiting reaction involves ions of like charge. If, as we propose, the limiting step is the replacement of ATP in the relaxation phase, the 'softening' effect of an increase in ionic strength<sup>20</sup> may be due merely to the speeding-up of the rate at which this replacement occurs.

The action of ATP as a 'relaxing factor' is also indicated by the effect of ATP concentration on the flagellar amplitude. If the bending of a flagellum is due to the contraction of a number of sub-units, each by a fixed amount, then the amplitude of bending will be related to the number of sub-units which are in the contracted state at a given time. The shorter the time after stimulation for which the contractile unit maintains tension, the smaller will be the number of units under tension at a given time (see Fig. 1). Since the tension 'decay time' will depend on the rate of interaction of sub-units with the relaxing factor one would expect the amplitude to decrease as the concentration of this factor increases. This, in fact, is the behaviour described by Brokaw<sup>21</sup>, who found that as

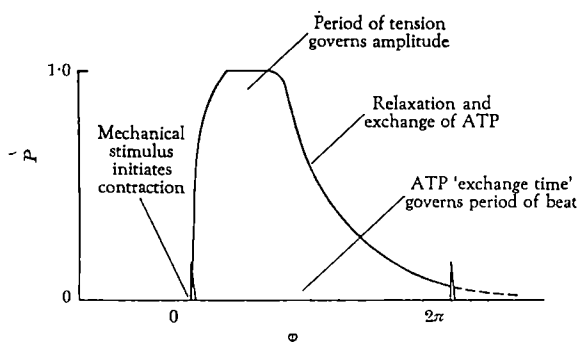


Fig. 1. Proposed events in the contraction-relaxation cycle of a hypothetical flagellar model.  $P$  is the probability that a given contractile unit will be in the contracted state. The phase,  $\phi$ , in the cycle can be interpreted either as a measure of time at a fixed point in the flagellum or as a measure of distance along the flagellum at a given instant.

the ATP concentration was raised towards  $10^{-3}$  M frequency increased but amplitude decreased—thus ATP is both regenerating the system (controlling frequency) and relaxing tension (controlling amplitude).

*The occasion of ATPase activity.* Although in the preceding discussion we have implicitly taken alternative (b) of the effect of stimulation (that is, that the contractile unit dephosphorylates bound ATP in the contraction phase) the merits of (c) have yet to be considered. In (c) the contraction is purely a physical process in which the unit contracts to a lower energy state, and in contrast to (b) the dephosphorylation occurs in the relaxation phase on restoring the unit to its expanded state. In both (b) and (c) the interaction with free ATP occurs on relaxation, so that most of the previous argument on the effect of ATP on frequency and amplitude applies to either alternative.

One way to distinguish between the two cases would be to find whether dephosphorylation accompanies the contraction or the expansion of the unit. Because the flagellar action is a cyclic one there is little hope of determining this unless the flagellar proteins can be isolated, when one might hope to follow conformational changes in the ATPase component by the methods of physical chemistry. Such an approach, if successful, could overcome another disadvantage of the cyclic system, namely, that observations on the motion of whole flagella can only yield activation energies. If the contractile protein itself were available there would be a possibility of determining equilibrium constants in the contraction process and hence of finding the free-energy change associated with dephosphorylation.

Another point which distinguishes (b) from (c) is that we conceive the contractile unit in (b) to be bound to a nucleoside phosphate molecule for most of the period of the contraction-relaxation cycle, whereas this is not a necessity for the case in which the contraction is purely a physical process. One can only judge between the two systems on the basis of probabilities, but we have an interesting comparison in the behaviour of *F*-actin under sonic vibration. Asakura, Taniguchi and Oosawa<sup>25</sup> found that the *F*-actin could split ATP when in a sonic field, although normally this protein is associated with bound, non-reactive ADP<sup>27</sup>. They explain the details of this behaviour briefly as follows. The partial disruption of the fibrous structure which is produced by mechanical means allows bound ADP to be replaced by ATP from the medium, which is then split as ruptured intermolecular bonds are re-formed. The chemical energy provided by the ATP-splitting accelerates the restoration of the filament to its uninterrupted state. Continuous sonic bombardment thus results in a cyclic process in which regions of the filament are continually being interrupted and re-formed, with the consequent splitting of ATP. The parallels are obvious between this system and the proposed mechanical stimulation of flagellar contraction, and favour that alternative in which the contractile unit remains bound to nucleoside phosphate and splits ATP in the contraction phase. (If the outer fibrils of the flagellum do, indeed, behave in this actin-like way, it would explain many of the muscular properties which are exhibited by flagella.)

*The proposed model.* It remains now to summarize the hypothetical system at which we have arrived in this discussion. To begin with the structure, we suppose that the outer nine fibrils of the flagellum are capable of longitudinal contraction at localized regions. The contractile capacity is a property of units in the fibril which are identical in distribution (and probably *in toto*) with the structural sub-units of which a fibril is composed. The contractile units are associated with a bound adenosine phosphate molecule for most of the period of the flagellar beat.

The contraction cycle begins (Fig. 2) when a mechanical deformation, conveyed passively along the beating flagel-



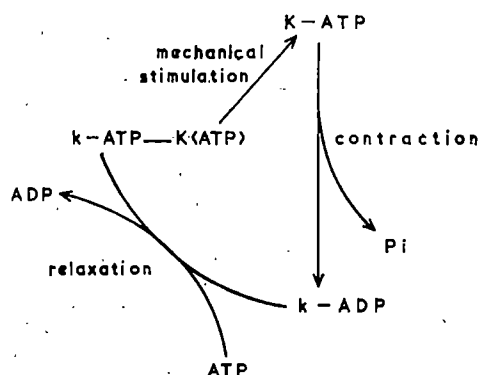


Fig. 2. Proposed chemical changes in the contraction-relaxation cycle of a hypothetical flagellar model. *K* denotes the 'normal', and *k* the contracted, state of a fibrillar sub-unit. The ATP shown in parenthesis is presumed to be masked and inactive. The processes, *k*→*K* and the exchange of ATP, could be simultaneous or in the reverse order.

lum, stimulates a particular sub-unit to split the ATP with which it is bound. Energy from the dephosphorylation enables the unit to change its conformation and, in so doing, perform external work on the flagellar system. The contraction follows quickly after stimulation, so that the contraction phase probably lasts for less than a tenth of the flagellar period. After contraction, the unit maintains tension until 'relaxed' by some factor which may be ATP itself. The bound ADP which remains after dephosphorylation is then exchanged with free ATP in the local environment. The rate of the relaxation process governs the amplitude of beat. When the unit again has a bound ATP molecule, it is ready to take part anew in the contraction cycle. The rate of replacement of ATP controls the frequency of beat.

In considering this model we have only discussed the possible role of the outer fibrils, from a rather mechanical point of view. The functions of the rest of the intricate flagellar structure, and the chemical aspects of ATPase activity and relaxation, promise levels of complexity which at present are beyond speculation. The few quantitative results on which we could draw, even for the

present discussion, show how much remains to be done in this field; however, the model may serve as a framework for further experiment, to be discarded when a better hypothesis can be devised.

We thank Prof. R. E. Burge and Mr. M. R. Watson for helpful discussions.

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## INCREASED SENSITIVITY OF MICROSOMES FROM PHENOBARBITAL-TREATED RATS TO SYNTHETIC MESSENGER RNA (POLYURIDYLIC ACID): LACK OF EFFECT ON RIBOSOMES

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THE *in vivo* administration of phenobarbital increases the activity of several rat liver microsomal enzyme systems<sup>1</sup>. Present evidence suggests that the phenobarbital-induced increases in enzyme activity are due to enzyme synthesis. Thus, the stimulatory effect of phenobarbital on one of these enzymes, amino-azo dye demethylase, is prevented by the simultaneous administration of puromycin<sup>2</sup>, an inhibitor of protein synthesis at the microsomal level. Further, microsomes isolated from phenobarbital-treated rats have a greater activity both *in vivo*<sup>3</sup> and *in vitro*<sup>4</sup> in the incorporation of DL-leucine-<sup>14</sup>C into protein than do microsomes isolated from control animals. As a part of an investigation on the mechanism of the increased incorporation, we examined the effect of

phenobarbital pretreatment on the ability of isolated microsomes and ribosomes to incorporate phenylalanine into protein and the sensitivity of these preparations to the addition of synthetic messenger RNA, that is, polyuridylic acid.

Groups of four Sprague-Dawley female rats, weighing 160–170 g, were injected intraperitoneally with 80 mg/kg of sodium phenobarbital in 0.9 per cent sodium chloride at 42 and 18 h before decapitation. Controls were given saline only. After the 40-h period, the livers were removed, homogenized in 5 volumes of 0.25 M sucrose, and the pooled homogenates were centrifuged for 10 min at 12,000g to sediment the nuclear and mitochondrial fractions. The microsomal fraction was separated by centrifugation at 100,000g for 2 h and then re-suspended in 10<sup>-3</sup> M tris-HCl pH 7.5, 1.5 × 10<sup>-3</sup> M MgCl<sub>2</sub> and 5 × 10<sup>-3</sup> M KCl at a protein concentration of 17.5 mg/ml.

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Liver ribosomes were isolated by treating an aliquot of the 12,500g supernatant with sodium deoxycholate according to the method of Korner<sup>5</sup>.

Livers from phenobarbital-treated rats weighed about 20 per cent more than livers from control animals; moreover, microsomal protein recovered per g of wet liver was increased about 24 per cent and microsomal RNA was increased generally by less than 10 per cent. Thus, the RNA/protein ratio was slightly decreased in phenobarbital microsomes. However, the ribosomal protein and RNA recovered/g of liver was about 25 per cent and 10 per cent respectively less in phenobarbital-treated animals.

Table 1. PHENYLALANINE INCORPORATION BY LIVER MICROSOMES AND RIBOSOMES FROM CONTROL AND PHENOBARBITAL-TREATED RATS

Contents	Pre-incubation	Additions	Phenylalanine-(U)- <sup>14</sup> C incorporation (c.p.m./mg protein)		
			Control	Pheno-barbital	Diff. (%)
Microsomes	None	None	87	186	+114
Microsomes	None	Polyuridylic acid (300 µg)	205	333	+62
Microsomes	Pre-incubated	None	3	5	—
Microsomes	Pre-incubated	Polyuridylic acid (300 µg)	215	430	+100
Ribosomes I	None	None	150	205	+37
Ribosomes I	None	Polyuridylic acid (300 µg)	274	283	+3
Ribosomes I	Pre-incubated	None	6	7	—
Ribosomes I	Pre-incubated	Polyuridylic acid (300 µg)	337	367	+11
Ribosomes II	None	None	170	200	+14

The reaction mixture contained in a final volume of 1.7 ml. the following: 20 µmoles potassium phosphate buffer pH 7.4, 2.5 µmoles ATP, 0.5 µmoles GTP, 87.5 µmoles sucrose, 100 µmoles reduced glutathione, 10 µmoles MgCl<sub>2</sub>, 40 µmoles phosphocreatine, 250 µg creatine phosphokinase, 0.2 ml. of 100,000g supernatant from control preparations containing 2.0 mg of protein and 0.4 ml. of the microsomal preparation (7.0 mg of protein) or 0.4 ml. of the ribosomal preparation (ribosomes I, 3.5 mg protein, ribosomes II, 3.0 mg RNA) and 0.085 µmoles of L-phenylalanine-(U)-<sup>14</sup>C (spec. act. 9.13 mc./mmole). Incubation was at 37° C for 15 min. The reaction was stopped with 1.7 ml. of 10 per cent trichloroacetic acid containing 0.1 per cent non-radioactive L-phenylalanine. The proteins were washed and counted as previously described<sup>6</sup>. The results given are the average of duplicate determinations. Pre-incubation\* was at 37° C for 12 min in the presence of all the co-factors but in absence of radioactive phenylalanine. After pre-incubation, the flasks were placed in ice and additional creatine phosphate (40 µmoles) of creatine phosphokinase (250 µg) and phenylalanine <sup>14</sup>C were added. Polyuridylic acid was added where indicated and the flasks were incubated as previously described.

Table 1 shows that microsomes isolated from phenobarbital-treated rats have 114 per cent greater L-phenylalanine-(U)-<sup>14</sup>C incorporating activity than do microsomes from control animals. In the presence of 300 µg of polyuridylic acid, the phenobarbital microsomes incorporate 62 per cent more L-phenylalanine-(U)-<sup>14</sup>C. Table 1 also shows the effect of phenobarbital pretreatment on the amino-acid incorporating activity of rat liver ribosomes. In contrast to the large differences observed in the microsomes, phenobarbital treatment caused only a 37 per cent increase in the incorporating activity of ribosomes. In the presence of 300 µg polyuridylic acid there was essentially no difference in the phenylalanine incorporating activity of control and phenobarbital ribosomes.

The addition of polyuridylic acid to different preparations of microsomes or ribosomes stimulates phenylalanine incorporation in varying amounts. Under the usual conditions in which polyuridylic acid is added to non-pre-incubated preparations, it is difficult to determine the sensitivity of the preparation to the added messenger RNA since one cannot differentiate between the amount of incorporation directed by the endogenous RNA compared with that directed by added messenger RNA (poly-U). In order to determine the sensitivity of the microsomes or ribosomes to added messenger RNA (poly-U), we removed endogenous RNA by a pre-incubation procedure<sup>6</sup> which makes phenylalanine incorporation totally dependent on added poly-U.

The phenobarbital microsomes are considerably more sensitive to added poly-U than are normal microsomes. Thus, after pre-incubation (Table 1) the addition of 300 µg of polyuridylic acid caused a 100 per cent greater phenylalanine incorporation in the phenobarbital micro-

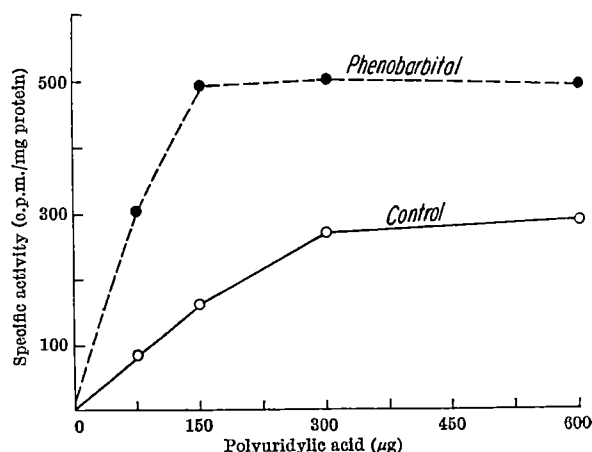


Fig. 1. Effect of polyuridylic acid on phenylalanine incorporation

somes than in the normal control microsomes. Fig. 1 shows the effect of varying amounts of polyuridylic acid on phenylalanine incorporation in pre-incubated phenobarbital and control microsomes. Over a greater than four-fold range of added polyuridylic acid there is greater phenylalanine incorporation in the phenobarbital microsomes. At saturating levels of polyuridylic acid, the phenobarbital microsomes are 90 per cent more active than are control microsomes. This indicates that one of the effects of phenobarbital is to increase the sensitivity of the microsomes to the addition of messenger RNA. Our results are not due to a possible phenobarbital-induced decrease in microsomal nuclease activity and hence a decreased destruction of added messenger RNA, since the addition of more than twice the saturating level of polyuridylic acid does not increase incorporation in either preparation. One possible mechanism for the increased sensitivity of phenobarbital microsomes would be an increase in the number of microsomal sites capable of binding to messenger RNA.

Although phenobarbital microsomes are more sensitive to added poly-U, the sensitivity of ribosomes from both control and phenobarbital-treated rats was essentially the same. Poly-U addition stimulated phenylalanine incorporation in normal and phenobarbital pre-incubated ribosomes to the same extent. Thus, the increased sensitivity of phenobarbital microsomes seems related to factors which are removed by deoxycholate treatment. The major known component solubilized by deoxycholate treatment is the endoplasmic reticulum. It thus appears that phenobarbital alters the endoplasmic reticulum in a way that enables the ribosome-membrane complex (microsomes) to have a greater sensitivity for messenger RNA.

Our results are consistent with the suggestion<sup>8</sup> that the endoplasmic reticulum is an important component of mammalian protein synthesizing systems. Remmer and Merker have shown by electron microscopy that phenobarbital increases the amount of smooth membranes of the hepatic endoplasmic reticulum<sup>7</sup>. The results reported here are consistent with these findings and suggest the possibility that newly synthesized membrane components have increased activity in supporting amino-acid incorporation.

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# RIBOSOMAL AGGREGATES IN GAMMA-GLOBULIN SYNTHESIS IN THE RAT

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IT has been established in a number of instances that proteins are synthesized on polyribosomes (clusters of ribosomes held together by messenger-RNA). This mechanism was demonstrated to apply, among others, to the synthesis of haemoglobin,  $\beta$ -galactosidase and collagen<sup>1-3</sup>. While some of these proteins are made on polysomes of a size which is fairly uniform and roughly proportional to the size of the protein formed, collagen appears to be assembled on large polysomes of heterogeneous size distribution. Stenzel *et al.*<sup>4</sup> found that antibody synthesis in the rabbit spleen occurs on single ribosomes and concluded that antibodies may be composed of short protein or polypeptide sub-units much smaller than the light and heavy chains of 20,000 and 60,000 molecular weight units, respectively, that have been reported to constitute the gamma-globulin molecule, and that these short sub-units would then, by combination in different patterns, form antibodies of different specificities. Other recent work indicates, however, that rat liver proteins of average size can be formed on single ribosomes<sup>5</sup>.

We have investigated gamma-globulin synthesis in rat lymph nodes stimulated by *Salmonella* antigens and have found evidence indicating that, in this immune system, protein synthesis appears to take place both on single ribosomes and on ribosomal aggregates exhibiting unusual susceptibility to proteolytic enzymes and resistance to ribonuclease.

Rats were injected in the footpad with 0.1–0.4 ml. of suspensions of *Salmonella* antigens (obtained from Difco, Inc.). Some of the animals were killed during the primary responses after five days, others were given a second antigenic stimulus after a few weeks and killed four days later. The popliteal lymph nodes were excised and incubated for 10 to 30 min in Eagle's medium MEM (ref. 6) in which <sup>14</sup>C-labelled algal protein hydrolysates replaced the usual amino-acids. After the incubation the tissue was chilled, homogenized and subjected to density gradient centrifugation as previously described for a different system<sup>3</sup>. Fractions of approximately 1 ml. were collected and the absorbancy at 260 m $\mu$  and the trichloroacetic acid-precipitable radioactivity, indicating respectively the amount of ribosomal material and the protein-synthesizing activity, were determined in the individual fractions.

In Fig. 1 the distribution over the gradient of absorbancy and radioactivity of the ribosomal material derived from non-immunized rats is compared with that of material from immunized rats. In the homogenate from non-immunized lymph nodes the absorbancy at 260 m $\mu$  shows a maximum in the region of the single ribosomes. A small amount of polysomal material is broadly distributed over the gradient. There is very little amino-acid-incorporating activity, mainly located in the single ribosome region. In lymph node homogenates from immunized animals, on the other hand, a second, broader peak of absorbancy at 260 m $\mu$  and of radioactivity was consistently found in the heavier regions of the density gradient. No difference was noted between the distribution patterns of lymph node material derived from rats during the primary and the secondary response.

Paper electrophoresis of the medium after incubation indicated that radioactive gamma-globulin was being formed by the immunized tissue but not by the non-immunized tissue.

In many protein-synthesizing systems not treated with detergents such as sodium deoxycholate before gradient

centrifugation, a pellet of lipid-containing material forms on centrifugation. This pellet is presumably derived from the endoplasmic reticulum to which the ribosomal material is believed to be bound in such systems. Deoxycholate

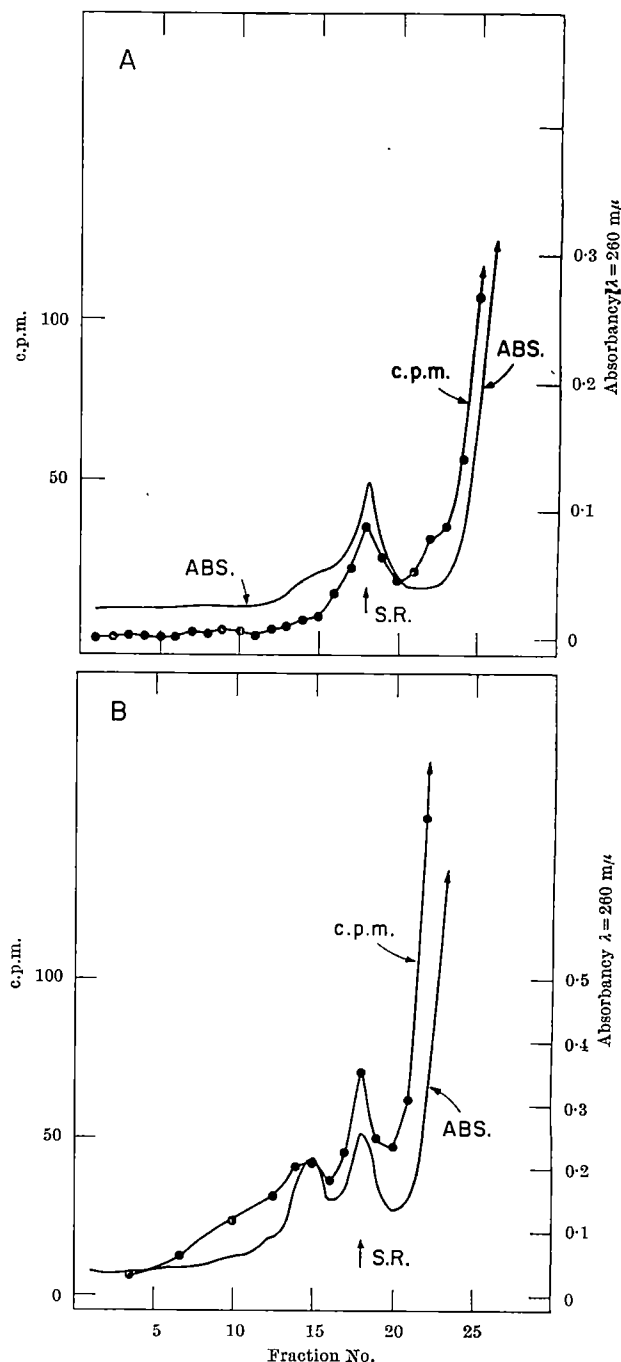


Fig. 1. Distribution over a sucrose density gradient of ribosomal material and of amino-acid-incorporating activity in unimmunized (A) and immunized (B) rat popliteal lymph nodes. Lymph nodes were incubated in Eagle's medium MEM supplemented with <sup>14</sup>C-amino-acids for 20 min, cooled to 0° and homogenized. The homogenate was freed of heavy cell components by low-speed centrifugation, layered over a sucrose density gradient 15–30 per cent w/w containing 0.01 M KCl, 0.0015 M MgCl<sub>2</sub> and 0.01 M *tris* HCl, pH 7.4, and centrifuged for 120 min at 25,000 r.p.m. in *STV* 25 rotor of Spinco ultracentrifuge. S.R., single ribosomes



dissolves the membrane material and liberates the ribosomes or polysomes linked to it. In the lymph node system used by us no pellet was formed and deoxycholate was without effect on the distribution of the ribosomal material. This is surprising since plasma cells, which are generally accepted as the major source of immune proteins, are presumably rich in endoplasmic reticulum. The present observations suggest the possibility that in this system antibody formation occurs on ribosomes very loosely associated with the endoplasmic reticulum, or perhaps that some early transitory cell relatively poor in endoplasmic reticulum may in fact be involved in gamma-globulin formation.

Electron-microscopic examination of the material in the heavier peak showed a heterogeneous population of ribosomal aggregates ranging from closely-spaced dimers and trimers to less closely-spaced aggregates of up to six ribosomes. The wider spacing of these larger aggregates presumably accounts for the fact that they sediment more slowly than the closely-spaced polysomes found, for example, in the reticulocyte system<sup>1</sup>.

Characteristically, polysomes have been found to be disaggregated by mild ribonuclease treatment to form single ribosomes. This has been taken as supporting evidence for messenger-RNA being the agent which links the ribosomes together into polysomes. Unexpectedly, the ribosomal aggregates from immunized rat lymph nodes were found to be uncommonly resistant to ribonuclease action even at concentrations considerably higher (up to 10 times) than those sufficient to disaggregate polysomes in other systems. Fig. 2 shows that after ribonuclease treatment of lymph node homogenates from immunized rats the characteristic 'immune' peak of absorbancy and radioactivity is still present, while the small amounts of polysomes in the other regions of the gradient have been disaggregated in the usual manner. This resistivity to ribonuclease attack was also found when Taka-ribonuclease was used instead of pancreatic ribonuclease. Deoxyribonuclease was also without effect on the systems.

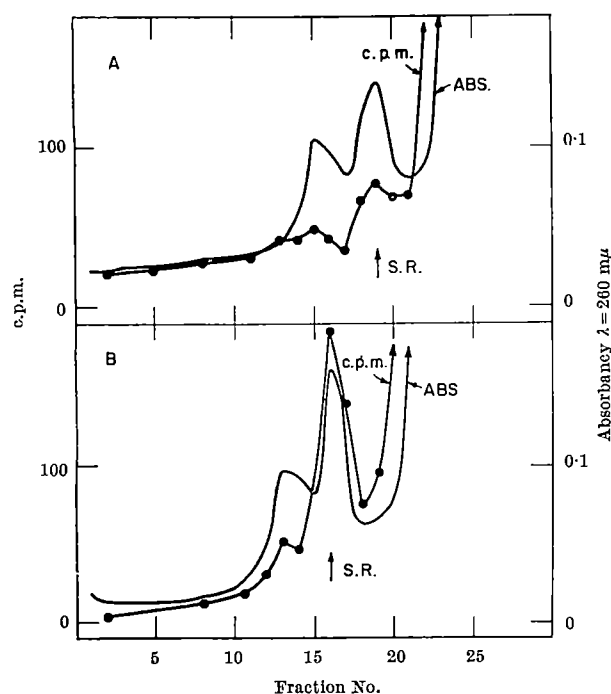


Fig. 2. Effect of ribonuclease on ribosomal aggregates from immunized rat lymph node homogenates. Conditions of incubation and centrifugation as in Fig. 1. *A*, No ribonuclease treatment. *B*, Homogenate treated with 6  $\mu$ g/ml. pancreatic ribonuclease at 0° for 10 min before gradient centrifugation

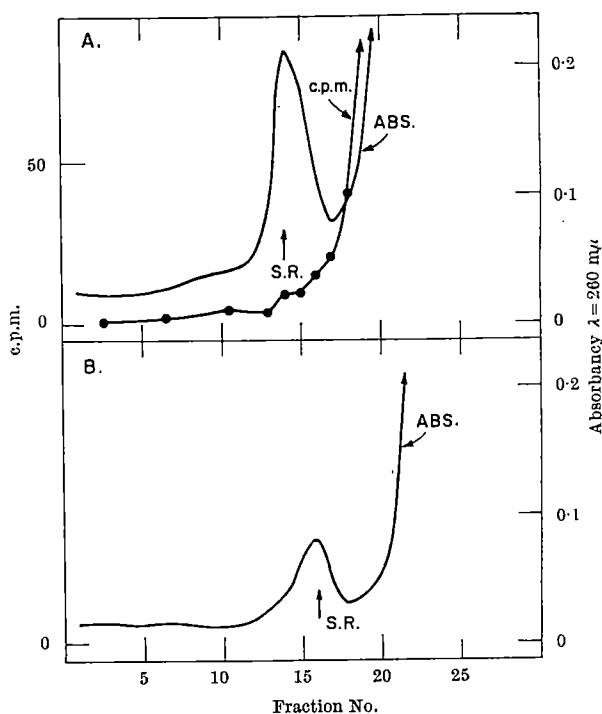


Fig. 3. Effect of trypsin and chymotrypsin on ribosomal aggregates from immunized rat lymph node homogenates. Conditions of incubation and centrifugation as in Fig. 1. *A*, Homogenate treated with 50  $\mu$ g/ml. trypsin at 20° for 5 min before gradient centrifugation. *B*, Homogenate treated with 50  $\mu$ g/ml. chymotrypsin at 20° for 5 min before gradient centrifugation

E. Bell has found (personal communication) that under mild conditions proteolytic enzymes can disaggregate polysomes to ribosomes rather than to ribosomal sub-units. In our investigation, preliminary experiments showed that mild trypsin treatment of chick embryo homogenates results in limited disaggregation of heavy polysomes and a corresponding increase in the amount of smaller and lighter polysomes. On the other hand, trypsin treatment of 'immune' lymph nodes under identical conditions results in almost complete disaggregation of the ribosomal aggregates and a corresponding increase in the single ribosomes (Fig. 3). Most of the radioactivity is lost from the particulate fraction by this treatment as the trypsin degrades the nascent polypeptide chains attached to ribosomal material into slowly sedimenting fragments. Identical results were obtained when mild chymotrypsin rather than trypsin treatment was applied. Control experiments with mixtures of chick embryo and lymph node homogenates revealed extremely low levels of endogenous nucleases in the lymph node homogenates and thus ruled out the possibility that the proteolytic enzymes remove a protective protein from the messenger-RNA and so allow subsequent attack by endogenous ribonucleases. Rather, it is concluded that the substance linking together the individual ribosomes of the 'immune' aggregates is indeed a polypeptide or protein. It is not clear whether such a protein serves specifically to link the ribosomes or whether the linkage is, in fact, through interaction of the nascent gamma-globulin chains.

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# NEOPLASMS IN THYMECTOMIZED MICE FOLLOWING ROOM INFECTION WITH POLYOMA VIRUS

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SOME strains of mice, particularly *C57BL*, are highly resistant to the oncogenic effects of polyoma virus even when virus is injected in the immediately postnatal period<sup>1,2</sup>. Highly susceptible mice of the strains that have been examined also usually do not develop neoplasms if they are 2 weeks old or older at the time of virus injection<sup>1</sup>.

In the case of *C57BL* mice, salivary gland rudiments in organ culture show the same proliferative response and transformation to the neoplastic state<sup>3</sup> as the gland rudiments of susceptible *C3H* mice to polyoma virus infection<sup>4</sup>. The process of control of the neoplastic conversion, therefore, appears to rest within the intact animal.

The obvious explanation for the susceptibility of most neonatal mice to polyoma virus is the inadequacy of the immune response and the readiness with which tolerance to foreign antigens can be established at this time. *C57BL* mice become immunologically competent soon after birth and are indeed relatively resistant to the induction of tolerance<sup>5,6</sup>. This explanation becomes more plausible when it is recognized that neoplasms induced with polyoma virus possess virus-specific new cellular antigens<sup>7,8</sup>. Both Habel<sup>7</sup> and Sjogren *et al.*<sup>8</sup> have interpreted their findings of resistance to challenge with isogenic transplantable polyoma tumours using the concept that polyoma virus transforms normal cells to neoplastic cells in the new-born and adult; the transformed cells containing the new cellular antigen provoke an immune response in adult but not in new-born mice. Neoplastic cells therefore arise after virus infection in the early postnatal period; they are tolerated and grow progressively. Adult animals are capable of rejecting in a homograft type reaction these tumour cells containing 'foreign' antigen.

That the low susceptibility of *C57BL* mice to polyoma virus oncogenesis has, in all probability, an immunological basis was shown in investigations using the early thymectomized animal<sup>9,10</sup>. Thymectomy during the immediately postnatal period is known to impair the maturation of immunological faculty<sup>11</sup>. More recently, it was shown that *C57BL* mice thymectomized at birth, and reconstituted with adult syngeneic spleen cells had their capacity to resist polyoma virus oncogenesis restored<sup>2</sup>.

The investigation recorded here was initiated to examine the effect of early thymectomy on the development of spontaneous mammary cancer in the females of a high mammary tumour strain and at the same time to investigate the effect of thymic removal on methylcholanthrene-induced fibrosarcomas in their male litter-mates.

*C3H/HeN* strain mice from the NIH colony were used. These were thymectomized at 3 days of age. Completeness of thymectomy was checked by serial sections of tissue obtained at necropsy from the upper mediastinal area. At least two members of each litter were left as intact controls. Litters were raised by their own mothers and at 4-5 weeks were separated according to sex, but with thymectomized and intact mice remaining together, 6-8 mice per cage. These mice were housed in an animal room containing other experimental mice, some of which had received polyoma virus parenterally. Females received no further treatment; males received 0.1 mg of 3-methylcholanthrene (MCA) dissolved in 0.2 ml. tri-

caprylin, subcutaneously, at 8 weeks of age. The mice were examined daily for neoplastic growths. Unexpectedly an untreated female developed bilateral parotid gland tumours when 4½ months old. At this time polyoma HI antibodies<sup>12</sup> were determined in the sera of both males and females. The members of 6 out of 15 litters of the untreated females showed HI antibodies ranging from 1:400-1:12,800. An HI antibody survey taken when the mice were 2 months of age showed all to have titres of < 1:100 (HI negative). Later, 9 additional females presented polyoma-type neoplasms but only those among the 23 thymectomized mice comprising the 6 HI-positive litters. All intact mice, showing nevertheless polyoma virus infection, remained tumour-free, as did all members of the 9 HI-negative litters (see Table 1).

Table 1. POLYOMA-TYPE NEOPLASMS IN *C3H/HeN* FEMALE MICE: 3-DAY THYMECTOMY AND ROOM INFECTION

Group	Polyoma HI antibodies (according to litter)			No. neoplastic No. mice	Latent period (months)
	+	-	Titre		
Thymect.	6	—	1:4,500*	10/23 (44%)	5.8 (4.5-6.5)
Intact	6	—	< 1:100	0/84	—
		9	1:600	0/15	—
		9	< 1:100	0/18	—

\* Seven mice that either had parotid gland tumours, or later became tumorous, had HI antibody titres of 1:6,400 or higher; otherwise the mean titres of polyoma-infected mice were nearly identical in thymectomized and intact mice.

The neoplasms, grossly, were confined to one or both parotid glands. Histological examination, however, also revealed numerous neoplastic areas in other salivary glands. The histological appearance of these neoplasms was similar to that described earlier<sup>13</sup>. There were poorly-defined tubular structures with a tendency toward loss of epithelial character and gain of spindle-cell features.

No differences were observed in the frequency or latent period of MCA-induced fibrosarcomas between 3-day thymectomized males and their intact litter-mates. Eighty-one per cent (47/58) of thymectomized mice, and 79 per cent of the controls (23/29), had neoplasms; the average latent periods (after carcinogen introduction) were 76 and 74 days, respectively. Among the 3-day thymectomized *C3H/HeN* males, only 23 were at risk to polyoma virus infection from 4½ to 6½ months, during the period when parotid gland tumours appeared in their female litter-mates; yet only one male mouse developed parotid gland neoplasms, and this was at 6 months of age although more than half had evidence of polyoma infection.

Mammary tumours are now appearing in the remaining *C3H* females, beyond 7½ months of age, and these have the typical morphology of such neoplasms arising in mice with the Bittner milk agent, reproducing mammary acini in many areas and for the most part classified as adenocarcinomas. The frequency of these neoplasms, however, is so far nearly the same in thymectomized and intact females (see ref. 14).

The pattern of spontaneous polyoma virus infection in animal colonies housing experimentally inoculated mice is in harmony with the observations reported earlier; *C3H* mice in our colony showed no polyoma HI antibodies at 2 months of age. At from 3 to 8 months approximately 30 per cent were infected and the infection rate increased to approximately 70 per cent at 12 months<sup>15</sup>, yet polyoma-type tumours were not observed in our *C3H* colony. Probably the only way in which the mouse, naturally

infected as an adult, can develop a polyoma-induced neoplasm is by the chance occurrence of an event which reduces its immunological competence. Thymectomy at 3 days appears to be more effective than X-irradiation in this respect. C3H mice, in the same colony, which received 300 r. or 150 r.  $\times$  4 whole-body X-irradiation, and of which 80 per cent had high levels of polyoma HI antibodies, failed to develop polyoma-type neoplasms.

The present findings are in accord with the concept that the basis of resistance to polyoma virus oncogenesis is a cellular immunity of the homograft type directed against virus-specific new antigen contained in the neoplastic cells. In the immunologically disturbed animal these antigens are not capable of provoking an immunological attack on the cells transformed to neoplasia by polyoma virus. These clones of cells therefore grow progressively into frank neoplasms.

The new cellular antigen must indeed be qualitatively similar to a minor histocompatibility antigen since the 3-day thymectomized C3H mice in this investigation showed normal bodily growth, were not deficient in lymphocytes (peripheral blood or lymphoid organs), were

capable of forming haemolysins in normal fashion to sheep red blood cells and rejected allogeneic skin grafts (AKR) at the same time as intact litter-mates.

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## EFFECTS OF ADRENERGIC NEURONE-BLOCKING AGENTS ON THE SUBMAXILLARY GLAND OF THE CAT

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EMMELIN and Engström<sup>1</sup> reported that bretylium caused salivation in the submaxillary gland of the cat as a result of its muscarinic properties. They found that bretylium was without effect on the secretion of saliva and the vasodilatation evoked by electrical stimulation of the chorda tympani nerve. In contrast to the results of Emmelin and Engström, we have found that in addition to abolishing the vasoconstrictor and secretory effects of sympathetic stimulation, and possessing muscarine-like activity in this organ, bretylium reduces the secretory and vasodilator effects of chorda stimulation. This was found to be a general property of adrenergic neurone blocking agents. Fig. 1 shows that guanoxan, guanethidine, and bretylium antagonize not only the vascular and secretory effects of sympathetic stimulation but also those of parasympathetic stimulation.

According to Hilton and Lewis<sup>2-4</sup>, there is no vasodilator innervation in the cat submaxillary gland; and both the atropine-resistant vasodilatation evoked by chorda stimulation and the after-vasodilatation which follows sympathetic stimulation are caused by a plasma kinin which is indistinguishable from bradykinin. These conclusions have been challenged by Schachter and his colleagues<sup>5,6</sup>, who consider that the chorda tympani nerve contains vasodilator fibres; this view is consistent with that originally held by Dale and Gaddum<sup>7</sup>. The antagonism of the vasodilator effects of chorda stimulation by adrenergic neurone-blocking agents suggested to us the possibility that an adrenergic mechanism may be involved in the vasodilatation. For example, acetylcholine released from the nerve endings of the chorda tympani may excite the nerve endings of sympathetic postganglionic adrenergic C fibres and release noradrenaline/adrenaline in close proximity to vascular  $\beta$ -receptors; or the chorda tympani nerve may innervate chromaffin tissue within the submaxillary gland.

It could be argued that the antagonistic effects of adrenergic neurone-blocking agents on the atropine-resistant hyperaemia evoked by chorda stimulation resulted from a general change in the state of the systemic circulation. However, it can be seen, in Fig. 2, that close arterial injections of small amounts of guanethidine directly into the blood supply to the submaxillary gland abolished the vasodilatation evoked by chorda stimulation at a time when vasoconstriction still accompanied sympathetic stimulation. The secretory effects of sympathetic stimulation and the after-vasodilatation which follows sympathetic stimulation were abolished by even smaller amounts of guanethidine injected close-arterially; these amounts did not affect the secretory and vasomotor effects of chorda stimulation (Fig. 2).

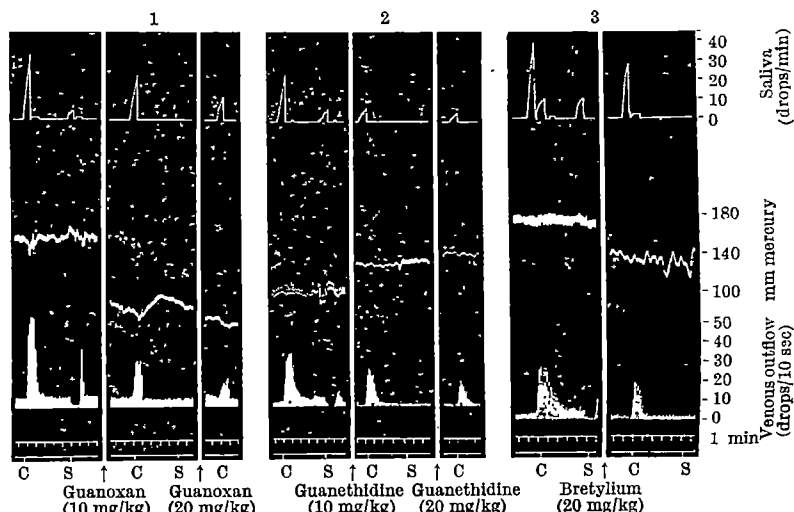


Fig. 1. Effects of intravenous injections of guanoxan (1), guanethidine (2) and bretylium (3) on the cat's submaxillary gland. Chloralose anaesthesia. Top tracing: secretion of saliva. Middle tracing: mean arterial pressure. Bottom tracing: venous outflow. Stimulation of the chorda tympani (C) and cervical sympathetic (S) with supramaximal pulses (20 V; 0.5 msec; 10/sec; 60 sec). Note: guanoxan, guanethidine and bretylium in addition to antagonizing the effects of sympathetic stimulation also antagonized the effects of parasympathetic stimulation.



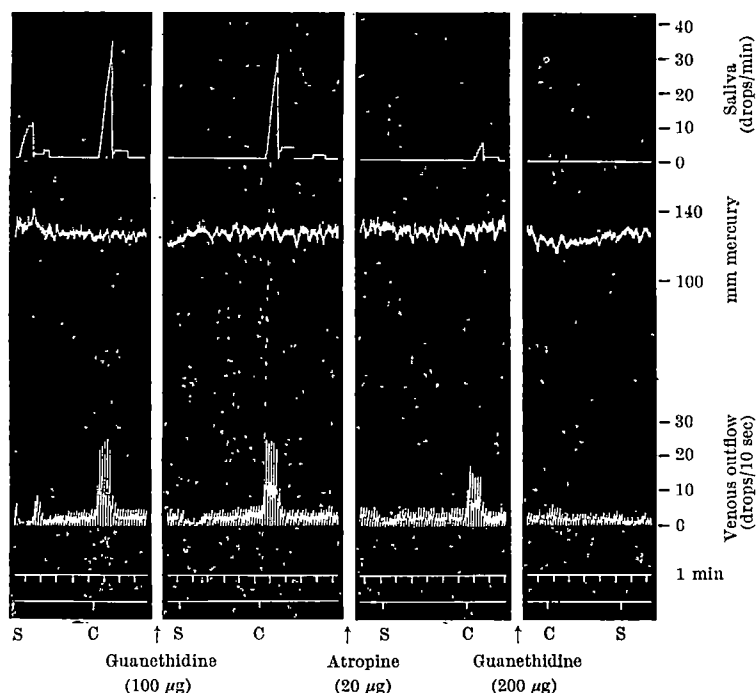


Fig. 2. Cat, 2.5 kg, ♀. Chloralose anaesthesia. Record of salivary secretion from the submaxillary gland (top), mean arterial pressure (middle) and venous outflow (bottom). Stimulation of the chorda tympani (C) and the cervical sympathetic (S) with supramaximal pulses (20 V; 0.5 msec; 10/sec; 60 sec). Guanethidine and atropine given by close-arterial injection. Note: guanethidine abolished the after-vasodilatation which followed sympathetic stimulation and the atropine-resistant hyperaemia evoked by chorda stimulation

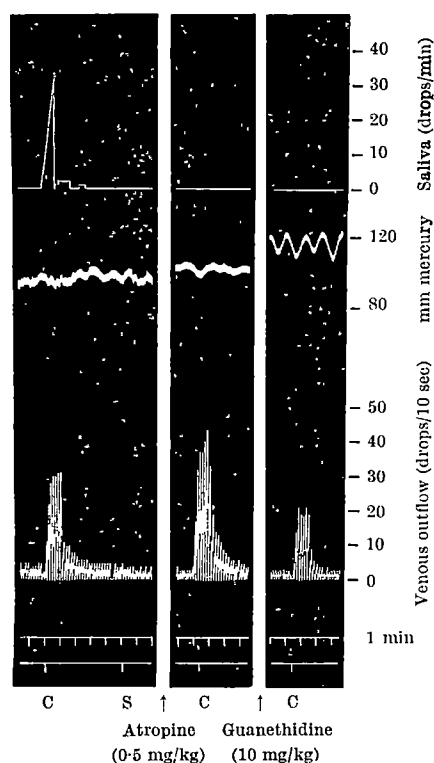


Fig. 3. Cat, 3.0 kg, ♂. Chloralose anaesthesia. Reserpine (1.0 mg/kg) injected subcutaneously 24 h before experiment. Record of salivary secretion from the submaxillary gland (top), mean arterial pressure (middle) and venous outflow (bottom). Stimulation of the chorda tympani (C) and the cervical sympathetic (S) with supramaximal pulses (20 V; 0.5 msec; 10/sec; 60 sec). At first arrow atropine (0.5 mg/kg i.v.), at second arrow guanethidine (10 mg/kg i.v.). Note: the initial potentiation of the vasodilatation by atropine and its reduction by guanethidine; prior treatment with reserpine does not alter the secretory and vasodilator effects of chorda stimulation

In cats treated with reserpine (1 mg/kg) 24 h previously an essentially normal vasodilatation and salivary response were evoked by stimulation of the chorda tympani nerve despite the fact that the effects of sympathetic stimulation were abolished (Fig. 3). Similarly a normal vasodilatation and increase in the rate of secretion of saliva accompanied chorda stimulation in the submaxillary glands of cats from which the ipsilateral superior cervical ganglion had been removed 14–21 days before the acute experiment.

It would therefore seem that an adrenergic mechanism is not involved in the atropine-resistant vasodilatation in the submaxillary gland of the cat. The effects of adrenergic neurone-blocking agents on the responses to chorda stimulation are probably related to their antagonistic effects on cholinergic ganglionic transmission<sup>8</sup>. However, the abolition by adrenergic neurone-blocking agents of the after-vasodilatation which follows sympathetic stimulation would appear to be a result of their effects on adrenergic transmission, since this after-vasodilatation is also abolished by the  $\beta$ -adrenergic receptor blocking agent pronethalol (Fig. 4). These observations suggest that the vasodilatation which follows sympathetic stimulation is caused by the sympathetic transmitter stimulating vascular  $\beta$ -receptors and is not due to the formation of bradykinin as suggested by Hilton and Lewis.

Small specimens of cat submaxillary salivary gland and the chorda tympani nerve were freeze-dried, treated with formaldehyde gas, embedded in paraffin wax, sectioned (8–10  $\mu$ ), and mounted for fluorescence microscopy<sup>9</sup>. In addition, portions of

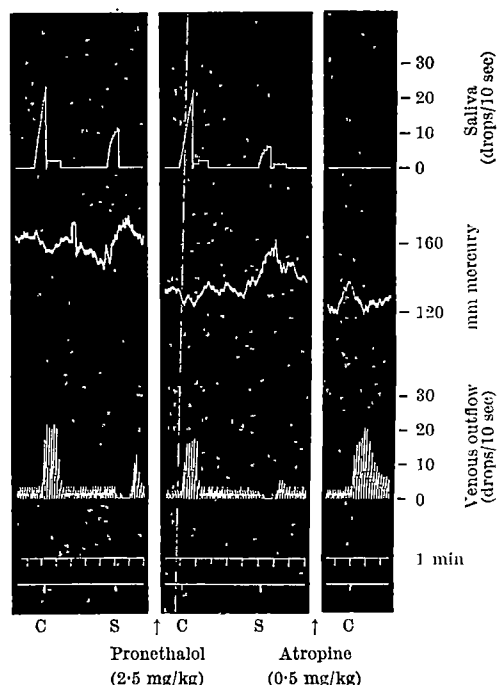


Fig. 4. Cat, 3.5 kg, ♂. Chloralose anaesthesia. Record of salivary secretion from the submaxillary gland (top), mean arterial pressure (middle) and venous outflow (bottom). Stimulation of the chorda tympani (C) and the cervical sympathetic (S) with supramaximal pulses (20 V; 0.5 msec; 10/sec; 60 sec). At first arrow pronethalol (2.5 mg/kg i.v.), at second arrow atropine (0.5 mg/kg i.v.). Note: Pronethalol antagonizes the after-vasodilatation which followed sympathetic stimulation



Fig. 5. Fluorescent adrenergic nerve fibres in cat submaxillary salivary gland. ( $\times 250$ )

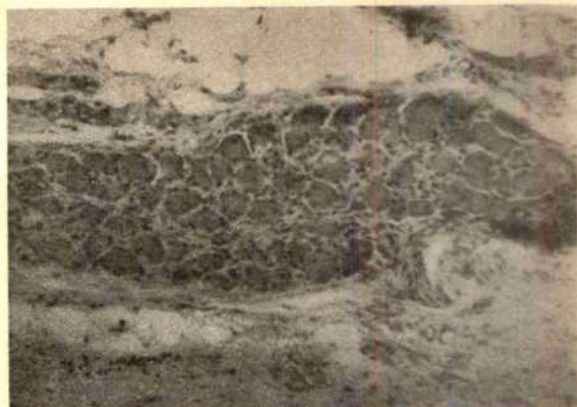


Fig. 6. Collections of ganglion cells in the core of the cat chorda tympani nerve (haematoxylin and eosin;  $\times 250$ )

salivary gland and nerve were fixed in Orth's fluid and unstained sections were examined for chromaffin tissue.

No chromaffin tissue was found either in the chorda tympani or in the salivary gland. In the salivary gland a network of fine fibres which had an intense yellow-green fluorescence was found, surrounding the mucous alveoli (Fig. 5). No fluorescent nerve cells were seen although groups of non-fluorescent ganglion cells were seen within connective tissue bands.

No fluorescent adrenergic fibres or ganglion cells were found in serial sections of the chorda tympani. Typical adrenergic fluorescent fibres were seen surrounding blood vessels in the epineurium.

After examination in the fluorescence microscope paraffin was removed with xylene and the sections were stained with haematoxylin and eosin. Large collections of ganglion cells were found in the core of the chorda tympani nerve (Fig. 6).

In conclusion it may be said that although the vasodilatation evoked by chorda stimulation is abolished by adrenergic neurone-blocking agents, the underlying

physiological mechanism is not adrenergic. However, an adrenergic mechanism and not a plasma kinin would appear to be responsible for the vasodilatation which follows sympathetic stimulation. Finally, we should like to suggest that the pain in the region of the salivary glands experienced by patients treated with bretylium may well be ischaemic in origin, and not the result of an excessive hyperaemia as suggested by Emmelin and Engström. A reduction in the functional hyperaemia in association with a meal could result in a shortage of oxygen with ischaemic pain. The increase in gland cell activity induced by bretylium would further contribute to the oxygen deficiency.

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## ELECTRON MICROGRAPHY OF ANTIBODY-PRODUCING CELLS

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INVESTIGATIONS on the identification of antibody-producing cells have made use of immuno-fluorescent histological techniques<sup>1</sup> and of microdrop isolation techniques<sup>2,3</sup>. In the first method, identification usually relies on the antigenic properties of the antibody molecules produced by the cell and not on their antibody activity<sup>4,5</sup>. In the second method there is no ambiguity about the antibody-synthesizing capacity of the cells. Both methods, however, are based on optical microscopy.

Electron microscopy was recently applied to the investigation of immunocytes (antibody-producing cells) using the ferritin technique<sup>6,7</sup>. Identification of the immunocytes based on their capacity to fix ferritin-tagged antigen is not unequivocal because of a considerable amount of non-specific fixation of ferritin by reticulo-endothelial cells.

The introduction of the method of localized haemolysis in gel (LHG) by Ingraham and Bussard<sup>8,9</sup>, and by Jerne and Nordin<sup>10</sup> has provided a means by which the cell producing antibody can be identified without any doubt. The morphology of the antibody-producing cell can be examined by any kind of microscopic examination.

The production of anti-sheep erythrocyte (SE) haemolysis by lymph-node cells of immunized rabbits, in a physiological gel, has been investigated by the method described by Ingraham and Bussard. Minor modifications of the gel method have been introduced, enabling the antibody-producing cells to be examined with the electron microscope.

Popliteal lymph-node cells of adult rabbits in a stage of secondary reaction (4 days after booster injection of SE in the foot-pad) were prepared and counted for viability by the trypan blue exclusion method (see ref. 9).

Preparations for electron microscopy were made only when the population of the lymph-node cells had an activity greater than 200 active cells/million viable cells. Suspension of the active lymph-node cells ( $10^7$ /ml.) were added to carboxy-methoxy-cellulose gel, containing SE ( $5 \times 10^9$ /ml.) and guinea-pig complement as previously described<sup>9</sup>. When the activity of the lymph-node cell suspension was very high a concentration of  $10^6$  lymph-node cells/ml. was used. This measure increased the probability of finding only one lymph-node cell in each zone of haemolysis. A small amount of the gel containing



cells (0.1 ml.) was pipetted into the bottom of a glass tube (10 mm diameter) and incubated at 37° for 2 h. When an aliquot preparation, on a microscope slide, showed that the number of plaques of haemolysis had reached its maximum, Palade fixative was layered on the bottom of the tube on top of the gel. After 30 min of fixation, the osmic acid was removed and the pellet washed, dehydrated and finally embedded in 'Epon'.

The pellet of dehydrated gel was removed by breaking the tube and then fixed on a Porter-Blum microtome. The whole pellet was cut in serial sections—thick ones at first (1 $\mu$ ) for observation with the optical microscope, until the boundary of a zone of haemolysis was reached; then thin sections (200–300 Å units) were made for electron microscopy. After cutting through the lymph-node cells, thick sections were made again so long as no leucocyte was found. As soon as a thick cut showed the presence of a leucocyte as seen by phase-contrast microscopic examination, thin cuts were resumed through the cutting of the leucocyte. By such a technique a whole series of haemolysis zones were completely examined without losing a section of the preparation and we were able to observe and photograph all the cells present in each zone of haemolysis.

In our attempts to obtain serial sections through zones of haemolysis containing only one or two white cells, we were successful in twelve cases. Only those haemolytic zones in which all sections could be examined have been included in our results.

(a) The haemolytic zones in which all the red blood cells have lost their haemoglobin and have become ghosts are as easy to recognize with the phase-contrast, as with the electron, microscope. These zones have a characteristic appearance which is constant from one experiment to another. Although the size of the haemolytic zone may vary from one experiment to the next, in any one experiment their size is similar. In cross-section the zones are round with a diameter of 70–120 $\mu$  (Fig. 1). The volume of the zone varies with the activity of the cells and the duration of the incubation.

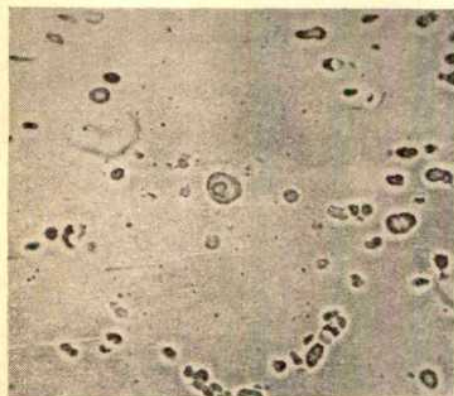


Fig. 1. Phase-contrast micrograph of a zone of haemolysis with the antibody-producing cell in the centre

All the dimensions and the shape of the zones can be determined from the known shape and thickness of the sections and the total number of sections in the zone. The haemolytic zones are not spherical but elliptical, with their long axes lying along the length of the tube. This flattening occurs not only in the zone as a whole but also in the individual cells in the zone. Thus, the plasma cells are 15–20 $\mu$  long and are not more than 3 $\mu$  thick.

(b) The type of white cells found in the haemolytic zone have been determined. In all cases plasma cells have been found. These are readily identified with the phase-contrast microscope (Fig. 1) and by their large content of ergastoplasm as seen in the electron microscope

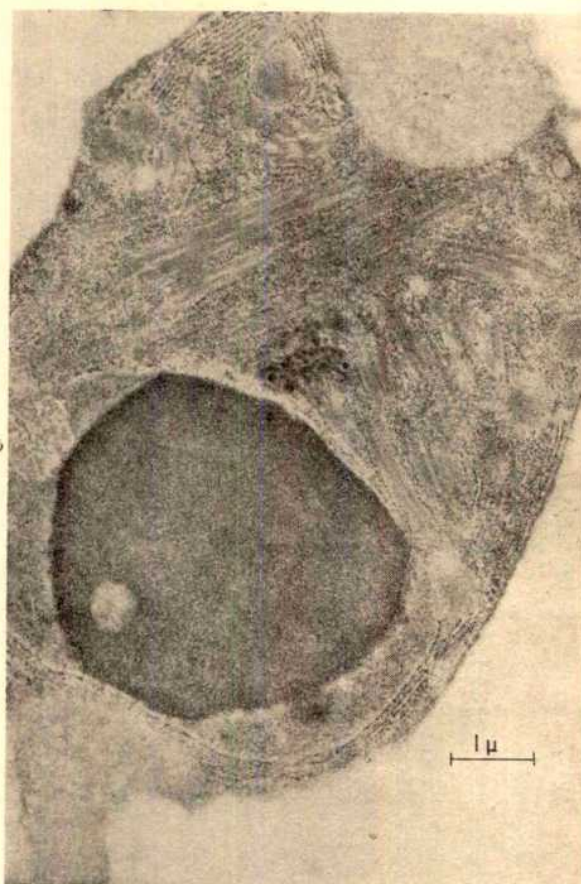
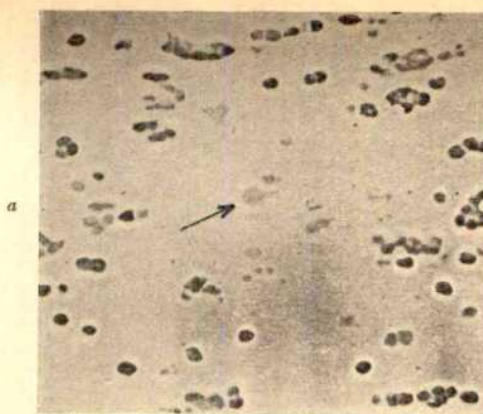


Fig. 2. a, Phase-contrast micrograph of a zone of haemolysis with a plasma cell in the centre; b, electron micrograph of the plasma cell from the centre of the zone shown in a

(Fig. 2b). In seven out of twelve cases plasma cells were isolated in the centre of the haemolytic zone (Fig. 2a); in a single case two plasma cells were found. In each of two other zones a lysed white cell was found which resembled plasma cells under phase contrast but which could not be identified in the electron microscope. Finally, in each of two other zones a lymphocyte (Fig. 3c) in addition to a plasma cell (Fig. 3b) was found. One of the lymphocytes had a very well-developed centriole.

(c) The appearance of the plasma cells in the electron microscope was not unusual. Inclusions could not be discerned in their nuclei. Many dilated ergatoplasmic sacs were seen in the cytoplasm (Fig. 2b). A dense electron-opaque material close to the Russell bodies could be seen between the ergatoplasmic sac surfaces. Frequently, haemolysed red blood cells were found adhering



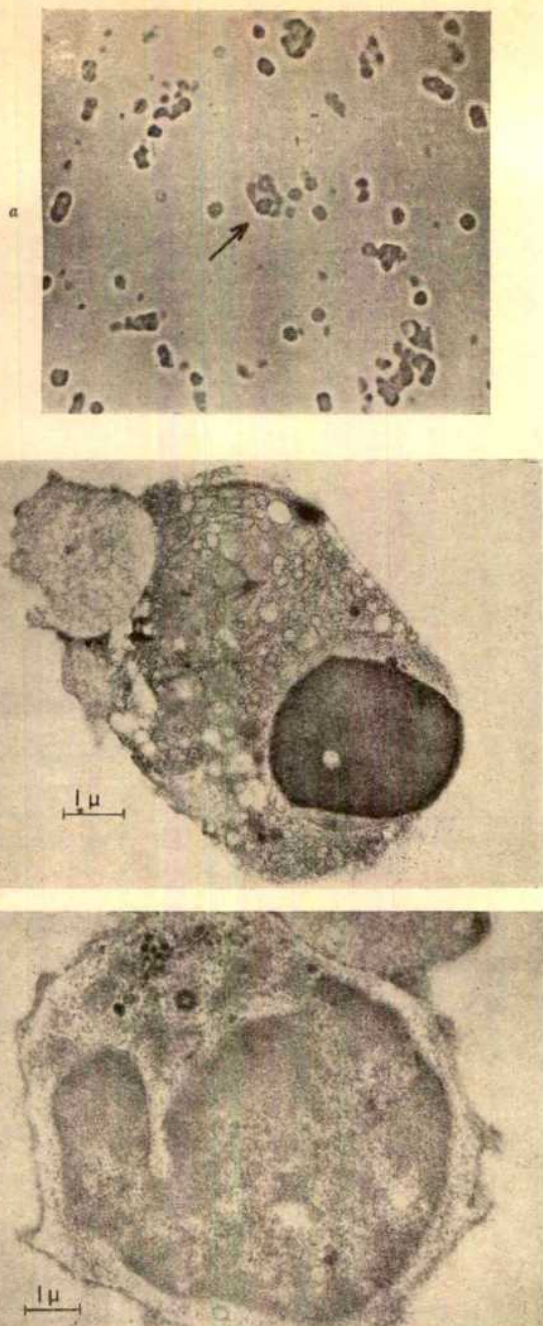


Fig. 3. *a*, Phase-contrast micrograph of a zone containing two lymph-node cells at different locations in the plaque; *b*, electron micrograph of the plasma cell from the centre of the zone; *c*, electron micrograph of the lymphocyte slightly peripheral in position

to the cytoplasmic membrane. Occasionally, openings in the cytoplasmic membrane were seen at the site of attachment of the red blood cells; it is, however, difficult to ascribe a function to the openings. Most of the haemolysed red blood cells were not in contact with the plasma cell and sometimes red blood cells which were not haemolysed were found adhering to the plasma cell.

(*d*) It was found by sucrose gradient ultracentrifugation<sup>14</sup> that most of the haemolytic antibody in rabbit serum was of the 19 *S* type. However, some samples of serum contained appreciable amounts of 7 *S* haemolysin (as much as 20 per cent). Thus, not all haemolysin is of the 19 *S* form, at least in the rabbit in a secondary phase of immune response.

Mercapto-ethanol (0.1 M-30 min at 37° C) completely inhibited the haemolytic activity of all samples of serum

including those containing 7 *S* antibody. Thus, mercapto-ethanol inhibition of an antibody activity cannot be used as a specific proof that the antibody involved is of the 19 *S* type.

Several points merit discussion: characteristics of true haemolytic zones as seen by the electron microscope; the differences between true haemolytic zones and artefacts; evidence that the lymph-node cell is responsible for the formation of the haemolytic zone and that the haemolysin is synthesized *de novo* in the gel; the nature of the responsible cell; the type of antibody synthesized and its relation with the cytological type of the active white cell.

(1) In the preparations devised for work with the electron microscope the shape of the lytic zone is different from that obtained under a cover-slip on a glass slide. The placing of a thin layer of gel near the bottom of a test-tube for electron microscopy results in a haemolytic zone of ellipsoidal shape the long axis of which is parallel to the long axis of the tube. The ellipsoid is flattened by the process of dehydration, necessary for viewing in the electron microscope. A lymph-node cell is always found at the centre of the zone of haemolysis. In a given plaque practically all the red blood cells are lysed, with the occasional exception of some red blood cells which are adherent to the lymph-node cell.

Haemolytic zones formed by a white cell are easy to distinguish from artefactual clear areas. In the latter there are no red blood cells or ghosts or there may be extensive agglutination with only slight partial haemolysis of red cells. Further, white cells are not found in the centres of these areas.

(2) Earlier work<sup>8,9</sup> supports the conclusion that the lymph-node cell is responsible for the localized haemolysis. Briefly the evidence is: only cells from immunized animals give rise to the zones—the number of haemolytic plaques is proportional to the number of lymph-node cells in the preparation—and at the centre of every plaque there is only one lymph-node cell. There is good evidence that haemolysis is due to antibody newly synthesized by the cells in the gel and not simply to the diffusion of pre-formed antibody since the formation of the haemolytic zone is markedly reduced by dinitrophenol, actinomycin and puromycin<sup>9</sup>. The cells in the haemolytic zones have a well-developed endoplasmic reticulum which is known to be associated with protein synthesis.

(3) Since, in almost all cases, a single plasma cell was found at the centre of the haemolytic plaque, one can conclude that it is at least one of the types of cell which produces haemolysin. In the plaque with one plasma cell and one lymphocyte it is not possible to say which cell was the haemolysin producer. Since we did not find plaques with only a lymphocyte present we cannot say whether they are capable of haemolysin synthesis.

(4) The haemolytic antibody found in the serum of the immunized rabbits from which the lymph-node cells were derived was of the 19 *S* type. This was shown by ultracentrifugation in sucrose gradient<sup>14</sup>. If we assume that in our case the essential producers of these 19 *S* antibodies were the lymph nodes, then we may conclude that the lymph-node cells in the plaques observed with the electron microscope were 19 *S* producers. In the work of de Petris, electron microscopy was applied to the examination of cells producing anti-ferritin in hyper-immune animals<sup>6</sup> and it is most probable that these cells were synthesizing 7 *S*-type antibodies in contrast to the cells in our investigations which formed mainly 19 *S* antibodies.

Synthesis of 19 *S*  $\gamma$ -globulin in pathological cases, such as the Waldenström disease in man, has been ascribed to the lymphocytic cell<sup>5,11,12</sup>. Identification of the antibody-producing cell has been made, in these macroglobulinaemia cases, by immuno-fluorescence histology<sup>13</sup>. These cells have been described as "large- and medium-sized lymphocytes and lymphoblasts". However, it is possible that with further characterization under the electron micro-



scope they might be considered plasma cell intermediates with well-developed endoplasmic reticulum.

We thank Dr. Bessis for his interest and help, and Mr. J. C. Mazie and Mlle. Vinzens for their assistance.

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## COMPARATIVE EFFECTIVENESS OF ADENOSINE ANALOGUES AS INHIBITORS OF BLOOD-PLATELET AGGREGATION AND AS VASODILATORS IN MAN

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THERE is increasing evidence that the aggregation of blood platelets brought about by adenosine diphosphate (ADP) is involved in haemostasis and in thrombogenesis<sup>1-6</sup>. This aggregation can be inhibited by substances related to ADP<sup>7</sup>, particularly by adenosine and by some of its analogues<sup>3,8</sup> not only *in vitro* but also *in vivo*<sup>9,10</sup>. One analogue already known to have inhibitory activity greater than that of adenosine itself is 2-chloro-adenosine<sup>11</sup>. We have now found other substances related to adenosine which have inhibitory activity and we have investigated further the molecular structure required for this inhibition. Adenosine and several of its derivatives are known to be vasodilators<sup>12,13</sup>. We show here also that the relative potency of some of these substances in inhibiting platelet aggregation corresponds with their relative potency as vasodilators.

Platelet aggregation and its inhibition were measured by a turbidimetric method already described<sup>14</sup>. Two different models of the apparatus were used<sup>6,7</sup> and the results agreed satisfactorily. Human platelet-rich plasma containing 0.1 volume of 3.8 per cent (w/v) trisodium citrate was incubated at 37° C and stirred at 1,000 r.p.m. The compound to be tested for inhibitory activity, dissolved in 0.154 M saline, was added after 3 min. Five minutes later a solution of ADP in saline was added to give a standard concentration, usually  $5 \times 10^{-6}$  M. Aggregation of platelets was measured by the 'rate' and 'extent' of the decrease in optical density of the platelet-rich plasma. Inhibitory activity was indicated by diminutions in these parameters of aggregation. With each compound, the inhibitory activities of different concentrations were compared with that of a standard concentration of adenosine, usually  $2 \times 10^{-5}$  M. The inhibitory activity of each compound was expressed as a percentage of that of adenosine, that is:

$$\frac{\text{concentration of adenosine selected for the comparison}}{\text{concentration of inhibitor required to produce the same inhibition as the chosen concentration of adenosine}} \times 100$$

As contamination of the compounds with adenosine could give a false indication of inhibitory activity, those with more than 10 per cent of the activity of adenosine were examined for such contamination by subjecting them to ascending chromatography on Whatman No. 4 paper using *n*-butanol : acetic acid : water (12 : 3 : 5 by volume)

as solvent. This system separated these compounds except 2-aza-adenosine, from adenosine.

The results are shown in Table 1. Only a few compounds had inhibitory activity comparable with that of adenosine. We confirmed that its activity was exceeded only by 2-chloro-adenosine<sup>11</sup>. Some other derivatives substituted in position 2 had activity approaching that of adenosine, notably 2-bromo- and 2-fluoro-adenosine. 2-Aza-adenosine and adenosine-1-*N*-oxide also inhibited quite strongly, showing that alterations of the molecule in this region are less critical than elsewhere. Thus, inhibitory activity was markedly diminished by almost any other modification (see also ref. 8), for example, substitution in or replacement of the 6-amino group; alterations in the purine ring except 2-aza-adenosine; substitution of the ribosyl moiety in other positions; and replacement of ribose by some other sugars. Certain alterations of the ribosyl moiety, notably removal of the oxygen in position 3', or substitution of phosphate in position 5' or at both 2' and 5', left up to 10 per cent of the activity, while others, such as substitution of sulphate at position 5', abolished activity altogether. It is interesting that two of the compounds which inhibited aggregation, 3'-deoxyadenosine and adenosine-1-*N*-oxide, cause platelet aggregation as the 5'-diphosphates<sup>15</sup>.

Thus inhibitory activity requires a highly specific molecular structure. The specificity is indicated also by the fact that the most potent compounds inhibited aggregation in concentrations of the same order as the concentration of ADP causing aggregation. In this respect, inhibition by adenosine analogues differs from inhibition caused by certain anti-histamines and local anaesthetics<sup>16</sup> and by esters of certain amino-acids<sup>17</sup>. The high specificity of the adenosine inhibitors implies a correspondingly specific receptor. The chemical nature of this receptor is still unknown.

Vasodilator activity was measured in the human forearm by venous occlusion plethysmography using mercury-in-rubber strain-gauges; the hand was excluded by a wrist cuff inflated to a pressure of 250 mm mercury. Sterile saline was infused continuously through a needle inserted into one brachial artery; this infusion caused no significant change in blood flow. Flow was also measured in the other forearm as control. Seven compounds with widely differing activities as inhibitors of platelet aggregation were chosen for comparison with adenosine as vasodilators.

Table 1. ACTIVITY OF COMPOUNDS RELATED TO ADENOSINE AS INHIBITORS OF AGGREGATION OF HUMAN PLATELETS BY ADP

	Activity relative to adenosine (per cent)	Chromatographic purity (per cent)	Source of compound
2-Substituted adenosines			
* 2-Chloro-adenosine	> 150 < 300	> 95	Dr. J. A. Montgomery, Southern Res. Inst., Birmingham, Alabama
2-Bromo-adenosine	> 50 < 100	> 95	
2-Fluoro-adenosine	> 30 < 60	> 95	Dr. G. B. Brown, Sloan-Kettering Inst. of Cancer Res., Rye, N.Y.
2-Methylthio-adenosine	> 10 < 25	> 95	Dr. E. Fitzgibbon, Southern Res. Inst., Birmingham, Alabama
2-Acetamido-adenosine	5	< 5	
* 2-Hydroxy-adenosine (isoguanosine; erotenoside)	< 1	—	
* 2-Amino-adenosine (2,6-diaminopurine riboside)	< 1	—	
6-Substituted purine-9- $\beta$ -D-ribofuranosides			
6-Dimethylaminopurine riboside (that is, N <sup>6</sup> ,N <sup>6</sup> -Dimethyladenosine)	> 5 < 10	—	Dr. K. Burton, M.R.C. Unit for Research into Cell Metabolism, Oxford
6-Furfurylamino-adenosine (that is, N <sup>6</sup> -Furfuryladenosine)	< 10	—	Zellstoffabrik Waldhof, Mannheim, Germany
6-Chloropurine riboside	< 10	—	
6-Methylthiopurine riboside	< 5	—	Dr. J. A. Montgomery (see above)
6-Hydrazinopurine riboside	< 5	—	Dr. A. D. Welch, Yale Univ. School of Med., Conn.
6-Hydroxylaminopurine riboside	< 5	—	
Substitution of and in the ribosyl moiety of adenosine			
Adenosine 2', (3'), 5'diphosphate	> 5 < 20	> 95	Dr. A. D. Welch (see above)
* Adenosine-5'-monophosphate	10	> 95	Böhringer und Sohne, G.m.b.H., Mannheim, Germany
Adenosine-3'-monophosphate	< 4	—	Prof. Kunio Yagi, Nagoya Univ., Japan
Adenosine-5'-monosulphate	< 5	—	I.C.I., Ltd., Pharmaceuticals Div., Alderley Park, Cheshire
3'-Deoxy-adenosine ('Cordycepin')	> 5 < 10	> 95	California Corporation for Biochemical Res., Los Angeles
* 2'-Deoxy-adenosine	< 1	—	Zellstoffabrik Waldhof (see above)
2',3'-O-isopropylidene adenosine	< 10	—	I.C.I., Ltd., Pharmaceuticals Div. (see above)
Adenosine-9- $\beta$ -D-mannopyranoside	< 4	—	Dr. E. J. Reist, Stanford Res. Inst., Menlo Park, California
Adenosine-9- $\beta$ -D-arabinofuranoside	< 2.5	—	Dr. J. A. Montgomery (see above)
<i>Cis</i> -3-(6-amino-9H-purin-9-yl) cyclopentanemethanol	< 6	—	
Other adenosine derivatives			
Adenosine-1-N-oxide	> 15 < 30	> 95	Dr. G. B. Brown (see above)
6,2',3',5'-Tetra-acetyladenosine	< 5	—	Zellstoffabrik Waldhof (see above)
Substitution of the ribosyl moiety of adenosine at other positions on the purine nucleus			
Adenosine-7- $\beta$ -D-ribofuranoside	< 5	—	Dr. J. A. Montgomery (see above)
Adenosine-3- $\beta$ -D-ribofuranoside	< 5	—	Dr. Hogenkamp, Dept. of Biochem., Iowa State Univ.
Modification of the purine ring in adenosine			
2-Aza-adenosine	> 40 < 100	—	Dr. G. B. Brown (see above)
4-Amino-7-D-ribofuranosyl-pyrrolo[2,3-d]pyrimidine (tubercidin)	< 5	—	Dr. A. D. Welch (see above)
4-Amino-1-D-ribofuranosyl-pyrazolo[3,4-d]pyrimidine	< 5	—	I.C.I., Ltd., Pharmaceuticals Div. (see above)

\* The effects of these compounds have been described in earlier publications<sup>8,11</sup>, but are included here to give a more complete picture.  
 < Indicates no detectable inhibition by the highest concentration tested.

The compounds were dissolved in saline and sterilized by autoclaving at 115° C for 30 min. Two ml. of solution was injected into the brachial artery in 6–7 sec. Since some compounds were available only in small quantities we were unable to compare the concentrations required to produce a given response, as was done when comparing their effects on platelet aggregation. Instead, the effect of a given dose of each compound was compared with that of the same dose of adenosine on the basis of the time integral of blood flow (see Fig. 1).

Table 2 shows that the relative activities of the compounds were the same in causing vasodilatation as in inhibiting platelet aggregation, with one exception. This was 2-methylthio-adenosine which, because of its low solubility, was injected in such low concentrations that

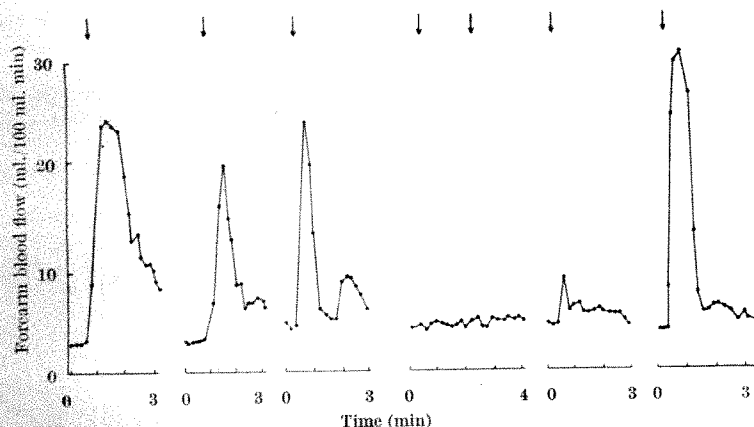


Fig. 1. Effect on forearm blood-flow of rapid injections of adenosine and analogues, each in 2 ml. saline. The sequence of injections was, from left to right: 2-chloro-adenosine, 2-bromo-adenosine, and 2-aza-adenosine each at  $2.5 \times 10^{-4}$  M; followed by 6-chloropurine riboside, adenine-9- $\beta$ -D-mannopyranoside 3'-deoxyadenosine and adenosine, each at  $8 \times 10^{-4}$  M.

Table 2

Compound	No. of experiments	Response to compound	Response to adenosine	Mean
2-Chloro-adenosine	3	105	180	270
2-Aza-adenosine	4	71	66	72
2-Bromo-adenosine	3	88	54	55
3'-Deoxy-adenosine	3	11	0	0
2-Methylthio-adenosine	3	4	0	0
6-Chloropurine riboside	3	0	0	0
Adenosine-9- $\beta$ -D-mannopyranoside	3	0	0	0

The response of forearm blood flow to injection of adenosine analogues is compared with the response to the same dose of adenosine (results from 9 subjects). The response is expressed as the area under the curve of blood flow against time.

reliable measurements of its effect were not possible. The two compounds which had no activity against platelet aggregation also had no detectable vasodilator activity even when injected at a concentration of  $3 \times 10^{-3}$  M. The large increases in forearm blood flow which were produced by the active compounds were not accompanied by flushing of the skin, indicating that the vasodilatation was in the muscles. The four most potent compounds, particularly 2-chloro-adenosine, caused pain in the forearm. When adenosine was infused an initial large increase in blood flow was followed by a decrease to a new rate which was still higher than the control (Fig. 2).

The results show that, among adenosine analogues, the structure/activity relationships for the inhibition of platelet aggregation and for vasodilatation are similar in man. Calculation shows that vasodilatation was produced by adenosine in blood concentrations as low as  $1-5 \times 10^{-4}$  M, which is similar to the concentration that inhibits platelet aggregation. These facts suggest



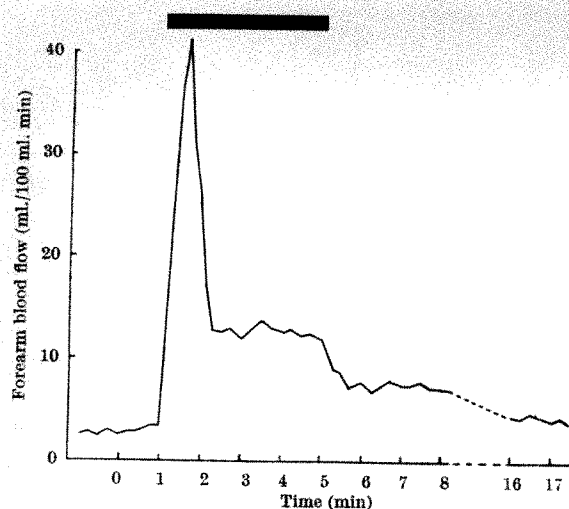


Fig. 2. Effect on forearm blood flow of an intra-arterial infusion of adenosine ( $5 \times 10^{-4}$  M) at a rate of 1.0 ml./min for 4 min (indicated by the black line)

that the effects of the adenosine analogues on platelet aggregation and on vascular smooth muscle are mediated by similar receptors. An implication of this is that other related compounds which may be found to inhibit platelet aggregation may also cause vasodilatation. In

the cat, adenosine and some of its analogues cause hypotension<sup>12</sup>. If effective inhibitors of platelet aggregation of the adenosine type also cause hypotension in man, this may complicate any attempt to use them therapeutically in thrombotic diseases.

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## A MODEL PREDICTING CHARACTERISTICS OF GENETIC MAPS IN *Neurospora crassa*

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**R**ESTRICTION of recombinant frequencies to an intermittent series was suggested by the unexpected recurrence of certain values observed among random ascospore isolates from crosses of linked markers<sup>1,2</sup>. The suggestion has been strengthened by recognition, in large samples of spore octets from individual asci, of a recurring ratio, approximately 9:1:8, of octets which are non-recombinant (parental ditype, PD), totally recombinant (non-parental ditype, NPD) or half recombinant (tetra-type, T) (ref. 3). This ratio may be used as a basis for calculating a series in which the degree of linkage rises by steps that reproduce recombinant frequencies indicated as recurrent ones by random spore data. To obtain the first member of the series, 9:1:8, a basic ratio, 1:1:4, indicating no linkage, is adjusted as follows: PD  $\times$  4.5; NPD  $\times$  0.5; T unchanged. For the next member, the correction of the basic ratio is PD  $\times$  9, NPD  $\times$  0.25, and so on.

In order to illustrate the applicability of this series to spore octet data, the distribution patterns of individual markers, in linear asci, must be taken into account. A marker showing regular 4m:4+ segregation may be distributed by fours in two ways (simple patterns) or by pairs in four ways (complex patterns). Equal frequencies of the six patterns will, of course, give an S:C ratio of 1:2 but this ratio is not uniformly observed. Often S exceeds C. To account for this, present theory assumes that the division products of the two centromeres of a chromosome pair are regularly distributed by fours, following meiosis in the ascus, and that crossing over between the centromere and a marker locus allows pair-wise distribution of the marker<sup>4</sup>. Thus the S:C ratio is taken as a measure of the degree of linkage, or the map distance, between the centromere and the marker locus.

On the basis of the definition of linkage used here, namely, a significant deviation from a 1:1 ratio of recombinant to non-recombinant classes for any given pair of markers, recent findings are in agreement with the idea that S:C ratios greater than 1:2 reflect linkage, since these ratios, like recombinant frequencies, appear to form a discontinuous series. Observed values suggest 1:1, 2:1, 4:1, 8:1, etc. A series of ratios, not easily distinguishable from these experimentally, may be obtained through application of the linkage correction factors given here, with the assumption that there are two basic ratios, N:0 and 1:2, and that intermediate values reflect different degrees of linkage either directly or indirectly. To illustrate, a cross involving markers *a*, S:C = N:0, and *b*, S:C = 1:2, gives, with no linkage, three of the seven phenotype distribution classes characteristic of two-marker crosses, PD1 and NPD1 (*a* and *b* simple) and T1 (*a* simple, *b* complex), in a ratio of 1:1:4. First-degree linkage between *a* and *b* will change the class ratio to 9:1:8 and the apparent S:C ratio of *b* to 10:8. Successively higher degrees of linkage will give apparent ratios of 37:16, 145:32, 577:64, etc. If *a* is assumed to represent markers which affect the phenotype inconspicuously, then intermittent S:C ratios will be expected from, apparently, single marker crosses. (It is not necessary to assume that all S:C = N:0 markers are inconspicuous.) Since the degree of linkage between *b* and a given *a* may differ from strain to strain and also since different strains may reasonably be assumed to sometimes carry different *a*-type markers, the model predicts the changes in S:C ratios often observed from single marker crosses when markers are re-isolated or crossed to different *m*<sup>+</sup> parents<sup>5</sup>. Observed ratios which do not coincide with any member of the above series may be attributed to indirect linkage, to be considered below, or to non-

uniformity of fruiting bodies within a cross<sup>3</sup>. Common occurrence of the latter situation would have escaped recognition because of experimental difficulties involved in the serial isolation of sufficiently large numbers of spore octets from individual fruiting bodies.

Introduction of additional conspicuous markers allows prediction of phenotype distribution class frequencies observed from more complex crosses, as shown in Table 1. For the first cross,  $c + cot \times + col +$ , the linkages assumed are as follows: fourth-degree linkage of  $c$ ,  $S : C = 1 : 2$ , to  $a-1$ , inconspicuous,  $S : C = N : 0$ , giving the expected  $S : C$  ratio for  $c$  as 577 : 64; 2nd degree linkage of  $col$ ,  $S : C = 1 : 2$ , to  $a-2$ , also  $N : 0$ , inconspicuous, hence  $col$  is given an expected  $S : C$  ratio of 37 : 16; no direct linkage of  $cot$ ,  $S : C = 1 : 2$ , to an  $N : 0$  factor but first-degree linkage between  $col$  and  $cot$ , thus the expected  $S : C$  ratio for  $cot$ , reflecting its indirect linkage to  $a-2$ , becomes, approximately, 1 : 1.2. For the  $c, col$  combination, expected relative frequencies of the phenotype distribution classes are calculated in the following way:

$$\begin{aligned} PD1 + NPD1 &= S c \times S col / S + C c \times S + C col \\ T1 &= S c \times C col / S + C c \times S + C col \\ T2 &= C c \times S col / S + C c \times S + C col \\ T3 + PD2 + NPD2 &= C c \times C col / S + C c \times S + C col \end{aligned}$$

Since  $c$  and  $col$  are unlinked, the PD1 and NPD1 classes are expected to be equally frequent. The last three classes are distributed on the basis of equality of PD and NPD plus the common-sense one that T3, which is half recombinant and half non-recombinant, will be twice as frequent as either PD2, totally non-recombinant, or NPD2,

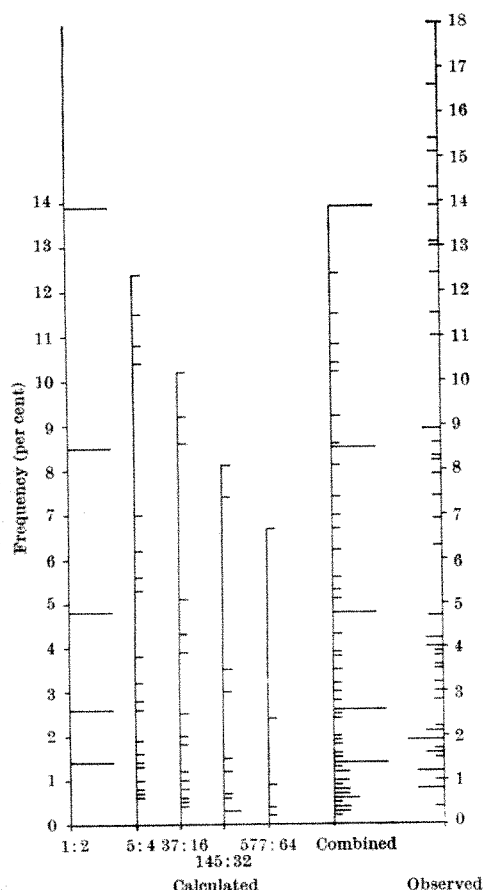


Fig. 1. Comparison of the distribution of observed recombinant frequencies, given in Table 2, with that of frequencies calculated for all combinations of the  $S : C$  ratios, 1 : 2, 5 : 4, 37 : 16, 145 : 32, 577 : 64, with five degrees of linkage for each combination. Lengths of the horizontal bars indicate the number of occurrences, 1-6, of each frequency.

Table 1. FREQUENCIES OF PAIR-WISE PHENOTYPE DISTRIBUTION CLASSES FROM THREE-MARKER CROSSES

Cross 1, $c + cot \times + col +$						
	$c, col$		$col, cot$		$c, cot$	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
PD1	110	111.2	119	123.5	75	72.5
NPD1	108	111.2	11	13.8	61	72.5
T1	96	96.2	112	109.7	178	173.7
T2	24	24.6	23	23.8	17	18.0
T3	6	5.3	29	23.8	10	9.6
PD2	5	2.7	55	53.5	8	4.8
NPD2	5	2.7	5	5.9	5	4.8

Cross 2, $col cot dn \times + + +$						
	$col, cot$		$cot, dn$		$col, dn$	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
PD1	22	25.8	15	16.8	13	15.6
NPD1	3	2.9	1	1.9	5	5.4
T1	26	23.0	14	14.9	33	30.6
T2	5	5.0	9	9.0	7	6.6
T3	5	5.0	8	9.0	5	6.6
PD2	12	11.2	26	29.2	10	9.8
NPD2	1	1.2	1	2.2	1	2.3

Expected frequencies are calculated on the basis of first-degree linkage between  $col, cot$  and  $cot, dn$  and with the following  $S : C$  ratios assumed:  $c$ , 577 : 64;  $col$ , 37 : 16;  $cot$  and  $dn$ , 1 : 2. Observed frequencies of the three-marker distribution classes are given elsewhere<sup>1,4</sup>.

totally recombinant. Expected relative frequencies for  $col, cot$  without linkage may be calculated similarly and the correction for first-degree linkage then applied as follows:  $PD \times 4.5$ ;  $NPD \times 0.5$ . Expected values for  $c, cot$  are obtained in a more complicated way, from expected frequencies of the 29 three-marker distribution classes, as described in detail elsewhere<sup>3</sup>. Briefly, expected values for the  $c, col$  pair-wise combinations are distributed according to expected relative frequencies, with first-degree linkage, of the corresponding  $col, cot$  combinations. In the few cases in which three-marker classes differ only with respect to  $c, cot$  combinations, distribution can be made on the basis of the general conclusions that in the absence of linkage  $PD = NPD$  and  $T3 = PD2 + NPD2$ .

The second cross shown in Table 1,  $col cot dn \times + + +$ , is of interest, even though the sample is small, because it formed the basis of a map of linkage group IV published some years ago<sup>6</sup>. Expected frequencies were calculated with the following assumptions: 2nd degree linkage of  $col$ ,  $S : C = 1 : 2$ , to  $a$ , inconspicuous,  $S : C = N : 0$ , but no direct linkage between  $a$  and  $cot$  or  $dn$ , both  $S : C = 1 : 2$ ; first-degree linkage for  $col, cot$  and  $cot, dn$  but no direct  $col, dn$  linkage. Frequencies of the 29 three-marker classes were obtained by first calculating values for the  $col, cot$  pair-wise combinations directly and then distributing these, as outlined here, according to expected relative frequencies of the corresponding  $cot, dn$  combinations, with the assumptions of first-degree  $cot, dn$  linkage and no direct  $col, dn$  linkage. Expected frequencies of  $cot, dn$  and  $col, dn$  pair-wise combinations were then obtained by adding the appropriate three-marker classes. Direct  $a, col$  linkage gives the expected  $S : C$  ratio of  $col$  as 37 : 16. Indirect linkage of  $cot$  and  $dn$  to  $a$  gives expected ratios of approximately 1 : 1.2 and 1 : 1.7 for these two markers. Indirect linkage between  $col$  and  $dn$  gives a recombinant frequency which is somewhat less than the sum of the  $col, cot$  and  $cot, dn$  frequencies. The expectations are 27.8 per cent for  $col, cot$  and  $cot, dn$  and 40.2 per cent for  $col, dn$ . Thus a satisfactory 'linear map' is obtained showing the order, 'centromere'— $col$ — $cot$ — $dn$ .

In order to make a more general comparison between calculated and observed recombinant frequencies, expected frequencies of one of the two recombinant classes among random spores were calculated for all pair-wise combinations of the  $S : C$  ratios, 1 : 2, 5 : 4, 37 : 16, 145 : 32, and 577 : 64, with five degrees of linkage for each combination. These are plotted in Fig. 1 with frequencies of the ++ recombinant class observed from the two-marker crosses

listed in Table 2. The observed values represent direct counts, not estimates, made under essentially uniform conditions by the same observer (myself). All such data at hand are included if linkage is indicated and if the count of the recombinant class = 25 or more. A rough

Table 2. OBSERVED FREQUENCIES OF THE ++ RECOMBINANT CLASS FROM CROSSES OF THE FORM  $m-l \times m-2^*$

Mutant <sup>a</sup>	Parent strains		Linkage group	Progeny count	
	Reisolate and mating type	Mutant Reisolate and mating type		Fraction ++	% ++
C84	260-3 A	33933 R7 a	V	304/1691	18.0 <sup>7</sup>
C84	260-3 A	37401 1197-5 a	V	133/4124	3.2 <sup>7</sup>
C84	260-3 A	44411 H3 a	V	72/806	8.9 <sup>1</sup>
C94	a	C115 A	I	247/1492	16.6 <sup>8</sup>
C94	3750-3 a	C136 R3 A	I	39/5033	0.8 <sup>1</sup>
C94	5275-1 A	C140 5498-3 a	I	143/8436	1.7
C94	5275-1 A	T1710 5493-1 a	I	25/1668	1.5
C94	a	35203 95-5 A	I	67/1712	3.9 <sup>7</sup>
C102	R2 A	C141 R a	IV	65/1866	3.5 <sup>8</sup>
C102	R6 A	C141 R1 a	IV	343/11558	3.0 <sup>8</sup>
C102	R1 a	28610 R4 A	IV	53/2542	2.1 <sup>1</sup>
C102	R5 a	34556 A	IV	180/15109	1.2 <sup>8</sup>
C102	8025-1 a	37301 8025-7 A	IV	71/1760	4.0
C102	8334-1	37301 8334-8	IV	50/965	5.2
C102	3704-1 A	37803 4937-4 a	IV	79/718	11.0
C102	R1 a	37815 369-5 A	IV	124/1391	8.9
C102	R5 a	37815 R1 A	IV	376/3027	12.4 <sup>8</sup>
C102	R1 a	38502p† 12953-7 A	IV	296/2621	11.3 <sup>8</sup>
C102	R6 A	38502p† R7 a	IV	825/7179	11.5 <sup>8</sup>
C102	8025-1 a	49001 R1 A	IV	34/490	6.9
C102	R5 a	70007 1315-4 A	IV	568/3771	15.1 <sup>8</sup>
C102	8025-1 a	70007 3264-1 A	IV	71/501	14.2
C115	A	47403 a	I	76/1605	4.7 <sup>8</sup>
C117	A	51602 a	VI	198/2405	8.2 <sup>8</sup>
C117	2159-4 A	66204 R1 a	VI	57/1500	3.8 <sup>1</sup>
C132	R7 a	C170 R2 A	I	51/1425	3.6
C132	8560-7 a	C170 8560-1 A	I	38/920	4.1
C136	5268-2 a	C170 R2 A	I	57/1349	4.2
C136	5268-2 a	T1710 R7 A	I	46/2129	2.2 <sup>1</sup>
C136	R3 A	15069 5634-4 a	I	45/1622	2.8 <sup>1</sup>
C136	R3 A	30300 1081-1 a	I	80/4902	1.6 <sup>1</sup>
C136	R3 A	30300 5697-4 a	I	92/2305	4.0 <sup>1</sup>
C141	R5 A	28610 (cot) R2 a	IV	773/8979	8.6
C141	(cot)† R1 A	28610 H5 a	IV	163/8478	1.9 <sup>6</sup>
C141	(cot)† R1 A	34556 R2 a	IV	105/13884	0.8 <sup>6</sup>
C141	R A	37301 798-1 a	IV	681/4891	13.9 <sup>7</sup>
C141	R1 a	37815 R1 A	IV	446/3116	14.3 <sup>8</sup>
C141	R A	38502p R1 a	IV	320/2448	13.1 <sup>7</sup>
C141	(cot) R1 A	70007 1247-4 a	IV	380/1950	19.5
C170	R2 A	39303 a	I	27/431	6.3
263	R3 A	70007 1315-4 A	IV	42/4180	1.0 <sup>1</sup>
B1312	A	51602 a	VI	69/869	7.9 <sup>1</sup>
T1710	R7 A	35203 R2 a	I	32/2694	1.2 <sup>1</sup>
3254	4021-5 a	51602 R2 A	VI	31/6783	0.4 <sup>1</sup>
3254	4021-5 a	70007 R2 A	VI, IV	158/1220	13.0
15069	R6 A	30300 1081-1 a	I	37/1950	1.9 <sup>1</sup>
16117	A	33933 R7 a	V	203/5736	3.5
28610	(cot) R2 a	34556 A	IV	101/8210	1.2 <sup>8</sup>
28815†	R1 a	29997 R1 A	I	38/1910	1.9 <sup>1</sup>
33442	R1 A	37301 798-1 a	IV	45/2836	1.6
37803	4937-4 a	70007 1315-4 A	IV	80/4152	1.9 <sup>8</sup>
37815	R4 a	70007 1315-4 A	IV	33/4113	0.8 <sup>8</sup>
38502d†	R1 A	38502p R7 a	IV	64/2752	2.3
38502d†	R3 a	38502p (cot) R1 A	IV	94/4827	1.9 <sup>8</sup>
38502d†	(cot) R2 A	38502p R7 a	IV	189/9106	2.1 <sup>8</sup>
38502d†	(cot) R3 a	38502p (cot) R1 A	IV	43/2314	1.9 <sup>8</sup>
38502p	R7 a	70007 1315-4 A	IV	516/2523	20.4 <sup>8</sup>
45502	1507-1 A	51602 a	IV, VI	178/1155	15.4 <sup>1</sup>
45502	1508-1 a	51602 R2 A	IV, VI	283/3381	8.3 <sup>1</sup>
45502	R9 A	70007 1247-4 a	IV	265/5571	4.7 <sup>1</sup>
51602	a	66204 R3 A	VI	313/7417	4.2 <sup>1</sup>
51602	a	75001 R1 A	VI	122/1654	7.4 <sup>1</sup>

\* If frequencies from two or more crosses involving different reisolates of the same mutants differ by less than 2 per cent, only the value from the largest sample is plotted in Fig. 1.

† Isolate 38502 is a double mutant the closely linked components of which are *pyr-2* and *dn*; 28815 is also a double mutant, *ad lys*, the *lys* component being the one used here.

‡ The symbol (cot) preceding a reisolate number indicates that the temperature-sensitive marker, *cot*, was present but unexpressed at the temperature of incubation used.

correspondence in overall distribution is apparent even though the calculated values are restricted to the results of direct linkage, which is not necessarily true of the observed values. It is also apparent that if a system such as that under consideration were operating, one might expect to become aware, even from determinations on the modest scale presented, of recurring frequencies without being able to recognize sharp peaks.

Consideration of relative frequencies of spore octet classes in the same series of hypothetical two-marker-cross progenies allows a general prediction concerning mapping with respect to 'centromeres'. Briefly, the prediction is that directly linked markers, tested by pairs, will map on the same side of the 'centromere', if they give an unambiguous result in this respect. This reflects the effect of linkage in increasing the frequency of parental ditypes relative to that of tetratypes since one of the former classes, PD2, switches from a single to a double cross-over class while one of the latter, T2, does the reverse, when two markers are assigned to positions on opposite sides of the 'centromere'. If, on the other hand, two markers are assumed to be indirectly linked through linkage of both to the same inconspicuous marker,  $S:C = N:0$ , the expected result might well be interpreted as locating the 'centromere' between the two conspicuous markers. Such a situation may be illustrated with a hypothetical case in which two conspicuous markers, *b* and *c*,  $S:C = 1:2$ , show third- and second-degree linkage to *a*, inconspicuous,  $S:C = N:0$ . The three possible *a, b* classes, PD1, NPD1 and T1, occur in the ratio of 144:1:32. These values are distributed among the 11 three-marker classes according to the expected PD1:NPD1:T1 ratio, 36:1:16, for *a, c*. Expected relative frequencies of the *b, c* classes, all seven of which are represented, are then obtained by adding values for the appropriate three-marker classes and are, approximately, as follows: PD1, 51.8; NPD1, 1.8; T1, 23.2; T2, 11.8; T3, 2.6; PD2, 1.3; NPD2, 1.3. Thus the double cross-over classes, T3, PD2 and NPD2, are less frequent than either of the single cross-over classes, T1 and 2, and the result is consistent with the mapping of the 'centromere' between *b* and *c*. Since the situation involving indirect linkage appears, superficially at least, to be more exacting, one might expect that it would be observed less often than the result of direct linkage. This expectation would be in accord with an observed situation, namely, that, up to 1959, essentially all markers assigned to five of the seven linkage groups were mapped on the same side of their respective 'centromeres', despite the fact that cytologists have described all 'centromeres' as non-terminal<sup>10</sup>.

In conclusion it may be said that the proposed model, which is mainly an extrapolation from observations and does not include the assumption of a direct relationship between recombination maps and the spatial organization of genetic material, predicts observed situations which are unpredicted or ambiguous in terms of conventional theory. This result is thought to offer hope that adoption of a broader point of view in evaluating genetic data may be helpful in approaching a more complete understanding of mechanisms involved in the distribution of phenotypes in ascii and in recombination of linked markers.

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## LETTERS TO THE EDITOR

## RADIO ASTRONOMY

## Occultation of Taurus-A by the Solar Corona at 430 Mc/s in June 1964

DURING June of every year, the radio source Taurus-A passes within a small angular distance of the Sun. This particular situation, commonly referred to as the occultation of Taurus-A by the Sun, permits an investigation of the Sun's outer corona as the radio source is viewed through it. The measurements made over the past several years have demonstrated that the radiation from the source is scattered at the electron density irregularities in the corona; as a result one observes an apparent broadening of the source accompanied by a decrease of its intensity. The effect becomes larger with decreasing angular separation of the source from the Sun. This phenomenon has been observed in the range of frequencies 26-178 Mc/s<sup>1-5</sup>. At wave-lengths of 6 and 18 cm the effect has been reported to be negative<sup>6,7</sup>, except that a positive result has been reported on 10- and 25-cm wave-lengths<sup>8</sup>. The results on all metre and decametre wave-lengths are consistent and are in accordance with the theory of small-angle multiple scattering, in which the angular size of the scattered distribution is proportional to the square of the wave-length. However, the increase of intensity on 169 Mc/s at  $7 R_0$  ( $R_0$  is the photospheric radius) from the centre of the Sun is not consistent with the theoretical prediction based on the lower frequency data<sup>5</sup>. In order to investigate if the coronal scattering is still effective at frequencies higher than 169 Mc/s, and in particular to see if the increase of intensity is also observed on 430 Mc/s, we undertook to observe at 430 Mc/s the occultation of Taurus-A by the solar corona in June 1964, using the 1,000-ft. dish of the Arecibo Ionospheric Observatory.

The 1,000-ft. dish has a half-power pencil beam of 9.5 arc at 430 Mc/s, and because of its large collecting area it is particularly suitable for studying the variation

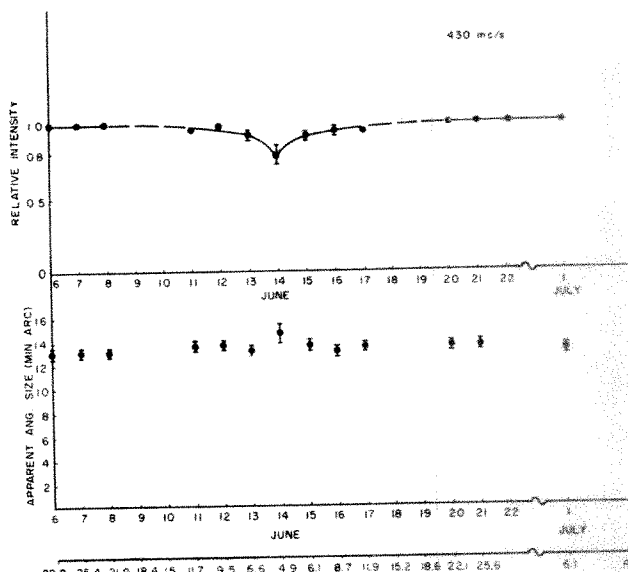


Fig. 2. Relative intensity and apparent angular size of Taurus-A as a function of its angular separation from the Sun in solar radii. The short vertical lines indicate probable errors, including uncertainties in subtracting the solar contribution due to side lobes of the antenna.

of intensity of Taurus-A as its radiation passes through different regions of the corona. However, the beam has undesirable side lobes and special measurements had to be made to eliminate their effects.

The experimental procedure adopted was to have 5-6 drift curves, including the meridian drift of Taurus-A and 2-3 declination scans on each day observed. The receiver was calibrated for high-level operation, and a 1,000° K calibration signal was used. In addition, the radio source 3C123 was observed.

The Sun appeared to be relatively quiet, and fairly good data were obtained during the entire period of observation, including June 14 when the radiation from Taurus-A passed through the corona as close as  $5R_0$  from the Sun's centre. This was fortunate since there are not many data available at this distance.

The results are presented in Figs. 1 and 2. As can be seen from these results, no increase of intensity of Taurus-A was ever observed, in contrast to the result on 169 Mc/s<sup>4</sup>. On the contrary, a significant decrease of intensity was observed on June 13, 14, 15 and 16, the maximum decrease of about 20 per cent being observed on June 14 when the angular separation of Taurus-A relative to the Sun was about  $5R_0$ . On June 13, 15 and 16, the decrease was roughly 8 per cent. The declination scans show approximately the same intensities as the drift curves; the difference in intensity, if any, is no higher than the experimental uncertainties of removing the solar side lobe contribution. Because of the presence of side lobes in the records of Taurus-A, it is not easy to determine the change in its angular size. However, we seem to have observed a very small increase in size of about 1' arc on June 14. This small increase of angular size is in accord with the theoretical prediction if one extrapolates from lower frequency data on the assumption that the  $\lambda^2$  dependence of the angular extent of the scattered distribution holds down to 430 Mc/s. No change in position of the source was observed, indicating that the effect of refraction, if present, is small.

No difficulty was experienced in determining the base line of Taurus-A drift curves (or of declination scans) on

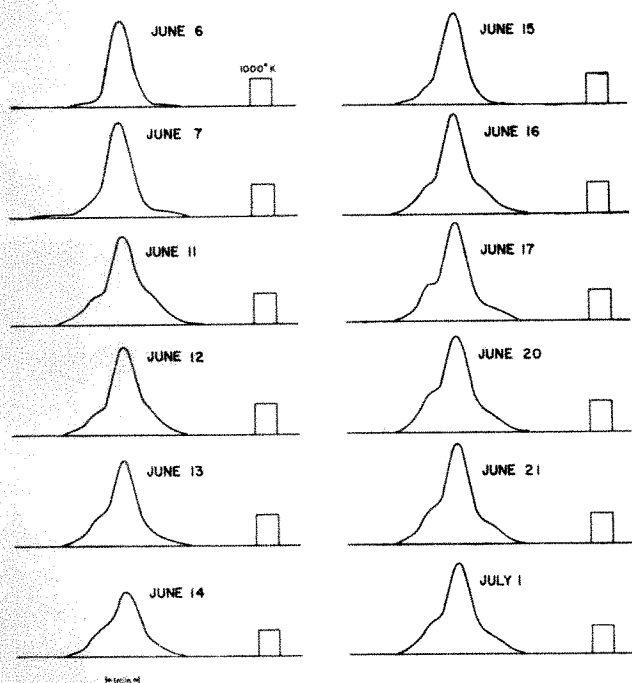


Fig. 1. Drift curves of Taurus-A near meridian transit, reconstructed after eliminating the solar contribution. The rectangular boxes on the right represent 1,000° K calibration signals.



any day except June 14, since the record of Taurus-A is situated on a rather flat portion of a side lobe due to the Sun. The situation is, however, different on June 14, when the right ascension of Taurus-A differs from that of the Sun by only 8 sec, with the result that the Taurus-A record is superimposed on a peak of a solar side lobe. In order to allow for the solar contribution on June 14 as well as on June 13 and 15, we determined the solar contribution by pointing the antenna two days later towards positions which the source had relative to the Sun on the occulting days of June 13, 14 and 15. This was done near the meridian transit as well as at other hour angles, in order to approximate the same antenna configurations as actually used. A good opportunity came on June 27, when the Sun had practically the same declination as on June 14, and the solar side lobe contribution was once again determined by simulating the occultation situation of June 14. Eliminating the solar contribution, we found, indeed, that the Taurus-A intensity significantly decreased on June 14.

It has been shown by several authors<sup>2,3,5,9</sup> that small-angle multiple scattering at electron density irregularities of the coronal streamers can account for the increase of angular size as well as the decrease of intensity of Taurus-A as its radiation passes within a small distance of the Sun. The observed decrease of intensity on 430 Mc/s and the small, but apparently significant, increase of angular size indicate that the same scattering mechanism may be applicable over the entire range of frequencies 26-430 Mc/s.

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## ASTROPHYSICS

### Soft X-ray Image of the Sun

ON August 11 a soft X-ray photograph of the Sun (Fig. 1) was obtained from a three-axis stabilized rocket vehicle. The head of a Skylark rocket launched from Woomera, Australia, was stabilized by a three-axis system developed by Elliott Bros., Space, and G.W. Division, under contract to the Royal Aircraft Establishment.

The firing was successful and during the exposure time of the pinhole camera the pointing noise was 3 arc.min peak to peak (p.t.p.), and 8 arc.min p.t.p., in the lateral planes and less than 5° p.t.p. in the roll axis. During the time of the exposure there was also a movement in the datum of one lateral axis of 6 arc.min.

The X-ray pinhole camera device consisted of nine pinholes, covering five wave-length ranges, mounted above an armoured film cassette. The cassette was provided with two spring-loaded shutters operated by solenoids to start and finish the X-ray exposure. The photograph reproduced was obtained from a camera of focal length 7.5 in. with a 0.005-in. pinhole. The filter used over the pinhole was of 1.5 $\mu$  plastic foil with a 1000-Å coating of aluminium. This filter passes soft X-rays in the region up to about 25 Å and also in the region 44-70 Å.

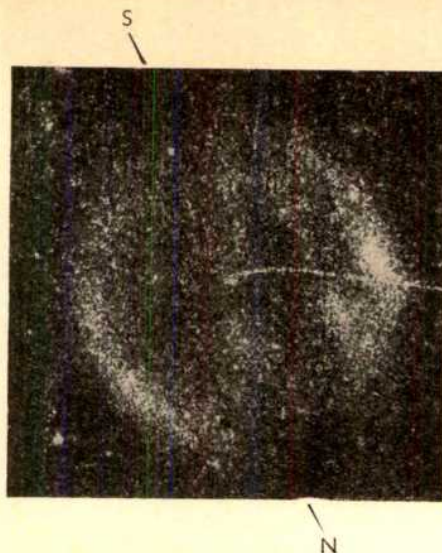


Fig. 1. Soft X-ray pinhole photograph of the Sun, 05:40 U.T., August 11, 1964. (The line running from the centre to the east limb is a scratch on the negative)

The photograph was obtained by exposure between 118 sec and 287 sec of the rocket flight, corresponding to 365,000 ft. on the upward journey to 362,000 ft. on the downward journey; peak altitude was 473,000 ft. The photographic medium was specially prepared by Mr. Hercock and his staff of the Products Research Laboratory, Ilford, Ltd., and had no supercoat covering the emulsion.

Comparison of this photograph with the Ca K plage photographs of Tokyo Observatory (Fig. 2) and the Fraunhofer Institut solar map (Fig. 3) shows a good correlation between the plage areas and the patches on the X-ray photograph. The X-ray emission defining the external edge of the disk of the photograph does not correlate with the plage areas and is thought to be due to coronal emission of soft X-rays and the limb brightening which would be expected due to the optically thin nature of the corona at these wave-lengths.

Comparison with the 9.1-cm spectroheliograph (Fig. 4) obtained by the Radio Astronomy Institute, Stanford, on August 10, 1964, 8 h before the X-ray photograph, shows generally good correlation of the bright patches and the limb areas.

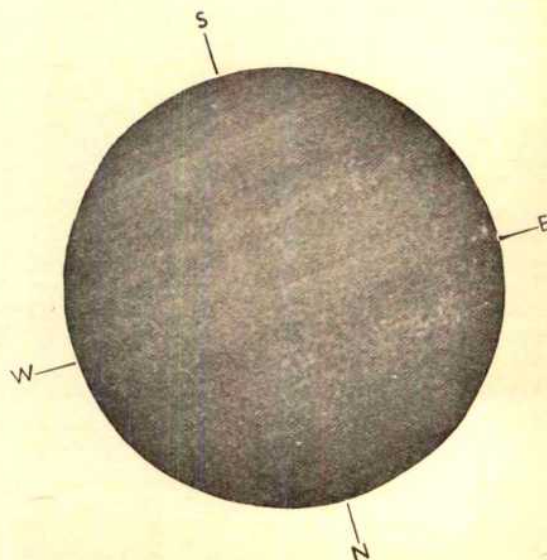


Fig. 2. Ca K photograph of the Sun, 04:16 U.T., August 11, 1964. (By courtesy of Tokyo Observatory)



## PHYSICS

## Improved Test Cell Material for the Tetrahedral Anvil Apparatus

Test cells for ultra-high-pressure apparatus of the 'belt' or 'anvil' types<sup>1</sup> are typically made from pyrophyllite, a naturally occurring aluminium silicate. It is unlikely, however, that this material possesses an ideal combination of mechanical properties for this purpose, a view which is supported by the shape of the load/pressure calibration curve. Fig. 1 (curve A) shows this experimental relation for a 0.75-in. edge National Bureau of Standards type tetrahedral anvil apparatus using pyrophyllite tetrahedra. The usual resistance transitions in bismuth at 25.4 kbars (ref. 2), thallium at 36.7 kbars (ref. 2), and barium at 59 kbars (ref. 3) were used, with the specimen geometry shown. It will be noted that although at 25.4 kbars loads close to the nominal pressure (that is, load/anvil area) line B are obtained, at higher pressure there is a more or less linear fall-off towards high loads, the efficiency of pressure generation falling away rapidly. No explanation of this has as yet been published, but two likely hypotheses may be put forward: either (a) it is due to the load on the compressible gaskets, or (b) it is due to stress difference in the pyrophyllite (which can be high at high pressures)<sup>1</sup>, since the maximum principal stress would be expected to be normal to the anvil face in the pyrophyllite just below the surface. Experiments have been made with specially profiled and lubricated anvils<sup>4</sup>: these indicate that gasket loads are probably not significant throughout the pressure range, so that (b) is the more likely hypothesis. Since this involves a fundamental mechanical property, an alternative test cell material was sought with a different relation between shear strength and compressibility.

Two-phase materials were thought promising, and curve C of Fig. 1 shows the load/pressure relation for tetrahedra made from an epoxide resin filled with magnesia: 50–60 per cent by volume of 200-grade MgO was added to the resin ('Araldite' type MY740), and a liquid anhydride hardener. This was then cast into tetrahedral moulds and cured for 16 h at 120°C, plus 4 h at 150°C and 2 h at 200°C. A minimum of machining is required to produce finished tetrahedra, and the process is thus much simpler and cheaper than that using pyrophyllite. Curve C shows that the new tetrahedra are much more efficient at high pressures; further, that the load/pressure relation is now fairly close to the nominal load line, being displaced some 10–20 tons towards higher loads. It is interesting to note that the reverse transition loads observed for both pyrophyllite and the filled resin tetrahedra are very similar (curve D), and that the characteristics for the filled

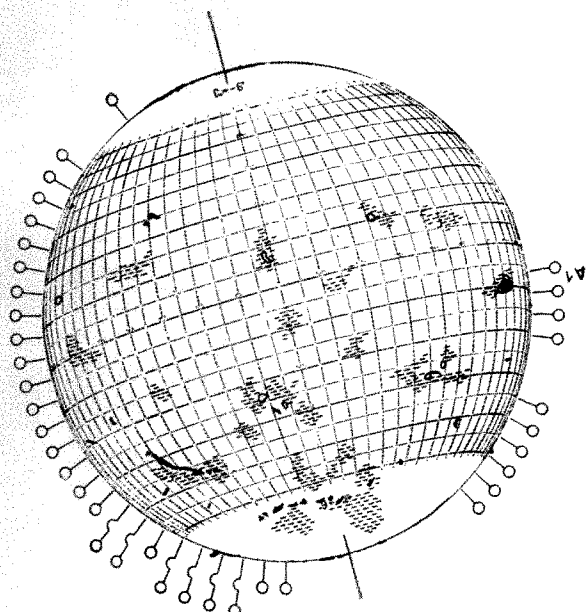


Fig. 3. Solar map (by courtesy of the Fraunhofer Institut). Hatched areas indicate shredded and weak plagues; bordered areas represent continuous plagues

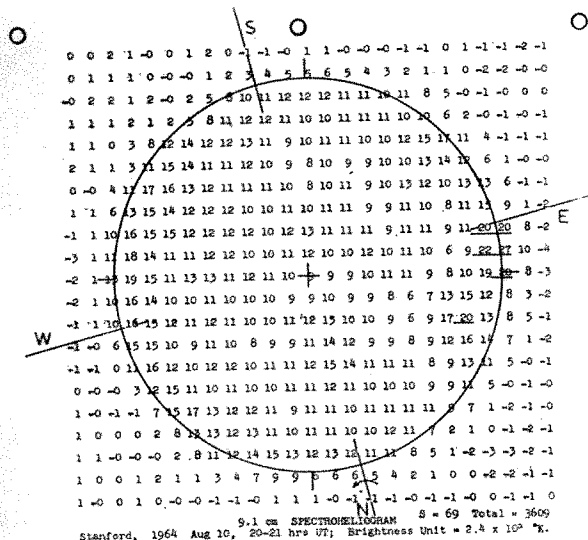


Fig. 4. 9.1-cm spectroheliograph, 20–21 h U.T., August 10, 1964. (By courtesy of the Stanford Radio Astronomy Institute)

The photograph was obtained at a period of quiet Sun and does not show the activity visible on the previous X-ray photographs<sup>1</sup>; it thus has the advantage of limited X-ray activity and limited Ca K plage activity for comparison. The overall lower activity and the greater sensitivity of the apparatus also reveals the limb brightening which was not apparent in the first photograph.

Further detailed analysis of the photograph is being undertaken, in particular in relation to X-ray flux measurements and detailed correlation.

I would like to thank, for most helpful discussions, all members of the Space Research Group at the University of Leicester, who are under the leadership of Prof. E. A. Stewardson, in particular Dr. K. A. Pounds, and also the workshop staff of the Department, who, under the direction of Mr. R. Cox, produced the camera. Mr. David Watson of the Department was responsible for the flight arrangements at Woomera.

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<sup>1</sup> Blake, R. L., Chubb, T. A., Friedman, H., and Unzicker, A. E., *Astrophys. J.*, **137**, 3 (1963).

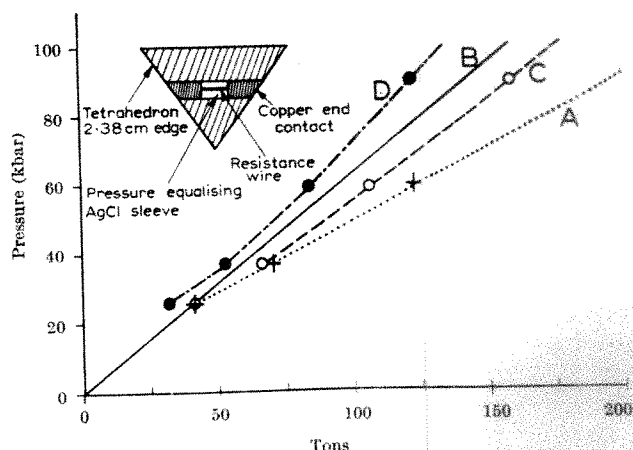


Fig. 1. Load/pressure calibration curves. Inset, tetrahedron with face/face configuration. A, Pyrophyllite tetrahedra; B, nominal pressure line; C, filled epoxide tetrahedra; D, reverse transitions



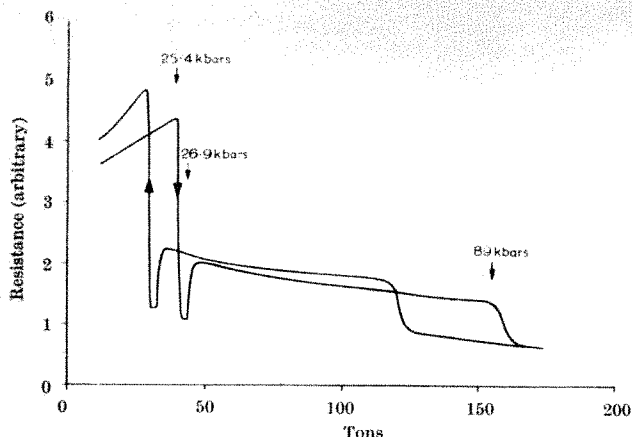


Fig. 2. Resistance of bismuth specimen versus anvil load, for filled resin tetrahedron: transitions marked at 25.4 kbars, 26.9 kbars and 89 kbars

tetrahedra are now symmetrical about the nominal load line.

With the improved load/pressure characteristic, the 89 kbar bismuth transition<sup>5</sup> is easily accessible. Fig. 2 shows a typical variation of resistance of bismuth with pressure, and it may be noted in passing that we have not detected any discontinuity in slope which could be reconciled with the volumetric transitions reported by Bridgman at ~44 kbars and ~64 kbars (ref. 6). (The bismuth used in our experiments was 99.999 per cent pure.)

There is little doubt that pressures over 100 kbars can thus be attained without much difficulty in the tetrahedral apparatus; further, this new material should be of value in other types of gasketing ultra-high-pressure apparatus since its 'pressure equalizing properties' appear to be superior to those of pyrophyllite. Two possible disadvantages may be noted. First, at pressures  $\geq 75$  kbars violent explosions of the test cell have sometimes been encountered; these appear to be associated with too rapid a rate of loading. Secondly, experiments have not yet been performed at very high temperatures, and the filled resin may well turn out to be inferior to pyrophyllite in mechanical and electrical properties under such conditions.

We thank Miss A. Thake for carrying out many of the experiments, and the Plastics Department of Standard Telecommunication Laboratories, Ltd., in particular K. A. Pettican, for the special techniques used in producing the filled resins.

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<sup>4</sup> To be published.

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### Effect of Temperature on the Pre-breakdown Pulses of Conduction Current in Insulating Oils

It has been previously<sup>1,2</sup> reported that the pre-breakdown conduction current pulses in liquid dielectrics are very sensitive to test conditions and the composition of the test liquid. It has been found that a convenient way of investigating these pulses is to record them as a double

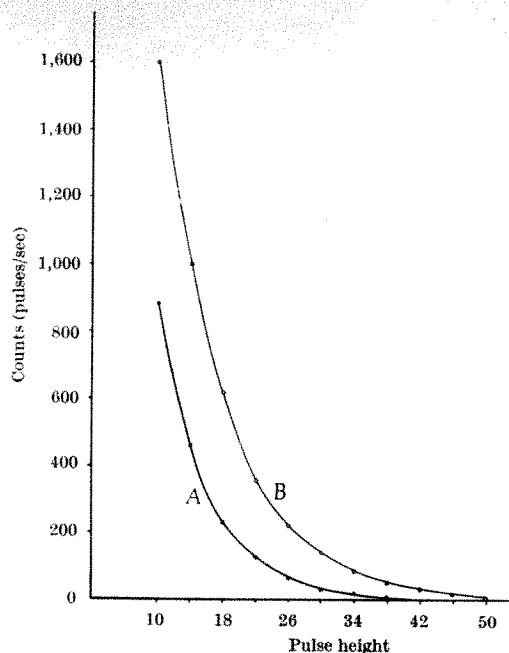


Fig. 1. Result of pulse height analysis. A, at 20° C; B, at 50° C

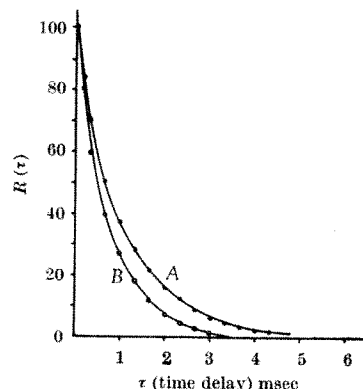


Fig. 2. Autocorrelation functions. A, at 20° C; B, at 50° C

trace on a high-speed magnetic tape (speed 75 in./sec). By using two play-back heads which can be displaced relative to each other it is possible to obtain the autocorrelation function of the random pulses. To reduce distortion and interference, frequency modulation before recording and demodulation during play-back were used.

The results here illustrate the statistical analysis and, as an example, show how the conduction current pulses are affected by a change in the temperature of the test liquid. This consisted of liquid paraffin, supplied by British Drug Houses, which was degassed at a pressure of  $10^{-3}$  mm mercury and filtered through a sintered glass filter of porosity No. 5 (average pore diameter  $1 \times 10^{-6}$  metre). Before the tests the sample was stress conditioned, that is, a stress of 450 kV/cm was applied for 2 h. Spherical nickel electrodes of 5 mm diameter with a gap spacing of  $125 \times 10^{-6}$  metre were used.

Fig. 1 shows the result of the pulse height analysis for an applied stress of 450 kV/cm. Curve A was obtained for a temperature of the liquid of 20° C and curve B for a temperature of 50° C. The corresponding autocorrelation functions for the two cases are given in Fig. 2. Using the method of Prony<sup>3</sup> for curve fitting, the following analytical expressions involving the sum of two exponentials were derived for the autocorrelation functions:

$$\text{Curve A } R(\tau) = 92.5e^{-912\tau} + 7.5e^{-4196\tau}$$

$$\text{Curve B } R(\tau) = 79.9e^{-1118\tau} + 20.1e^{-4187\tau}$$

The Fourier transform of these autocorrelation functions gives the power spectra according to the well-known Wiener-Khinchin relation. These spectra are shown in Fig. 3. For both the curve fitting and the computation of the power spectra a digital computer was used.

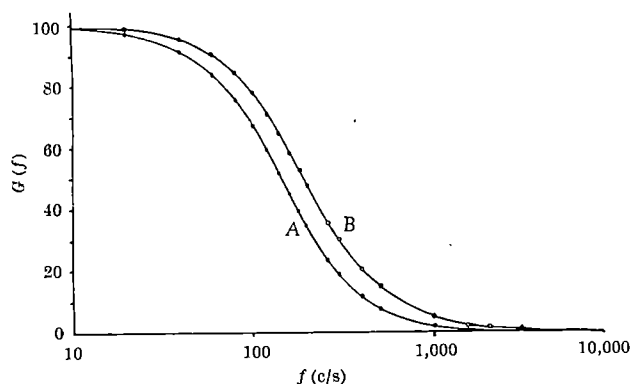


Fig. 3. Power spectra. A, at 20° C; B, at 50° C

It can be seen from Fig. 1 that the distribution of the pulse height is exponential, which seems to indicate that a multiplication process of the charge carriers takes place in the liquid. This figure also shows that increasing the temperature increases the current fluctuations. This is probably due to the resultant decrease in viscosity which in turn facilitates the motion of charge carriers in the bulk of the liquid. An inspection of the plots for the autocorrelation functions of Fig. 2 shows that no hidden periodicities are present in the random fluctuations. As can be seen from the power spectra of Fig. 3, the frequency-range of these fluctuations lies in the very low audio-frequency range. From Fig. 3 it is also clear that increasing the temperature results in an increase in the relative magnitude of the energy for all frequencies.

The experiments have shown that, as a rule, the effect of increasing the temperature or the applied stress is also to increase the relative magnitude (that is, the energy content) of the high-frequency components of the spectrum.

I thank Prof. M. W. Humphrey Davies for providing the facilities to carry out this work, and Dr. H. Tropper for his advice.

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### Electrostatic Switching of One-dimensional Superconductors

LITTLE<sup>1</sup> has speculated on the possibility of synthesizing an organic superconductor; for an example he considers a macromolecule consisting of an axial long-chain 'spine' of alternately doubly and singly bound carbon atoms with a complex resonating aromatic dye type of side-chain attached to every alternate carbon atom. He argues that it is possible for the virtual oscillation of charge in this type of side-chain to provide a sufficiently attractive interaction with electrons moving in the spine for superconductivity to result. He suggests that since this is essentially an electronic oscillation coupling rather than a phonon-coupled interaction, a very high transition temperature may result and room-temperature superconductivity may be possible; the biological implications of this are, of course, of great significance.

Ferrell<sup>2</sup> has since questioned these predictions on the grounds that the macromolecule is essentially a one-dimensional superconductor and that restricting the BCS Hamiltonian for the interaction to one dimension must introduce compressional modes of vibration to satisfy the requirements of gauge invariance. He suggests that these modes may dominate in one dimension and prevent the establishment of the long-range order required for superconductivity. The effect of these compressional modes will be determined by the side-chain structure and the interactions between an electron travelling along the macromolecule's spine and sites in the side-chain. Little has chosen a well-screened side-chain in order to reduce these interactions. It is to be hoped that a suitable side-chain, which will allow room-temperature superconductivity, can be found.

The type of macromolecule required is essentially a long-chain organic polymer. Little considers that this might be cross-coupled to produce a web-like structure; however, it appears that preferential orientation of the molecules along the macromolecule axis would be relatively simple to synthesize by the 'nylon' spinning technique, and if coupling between molecules can then be arranged—and this is quite feasible for some organic polymers—a long thread of superconductor could be produced. The ends of a thread might also be cross-linked to form a loop.

The superconducting thread would display a strong anisotropy; it would be superconducting along its length, corresponding to the molecular axis, but normal to this direction the types of organic molecules envisaged would probably be good electrical insulators. Magnetic field effects would be very different along and normal to the molecular axis, but it is of even greater significance that it would be possible to maintain an electric field in the superconductor.

The effect of an electric field applied across a superconducting thread will be to distort the screening effects of the side-chain and to modify the wave functions of the side-chain given by Little; the side-chain interaction will be altered and superconductivity will eventually be destroyed.

There is, therefore, the possibility of electrostatically switching a one-dimensional superconductor into its normal resistive state as well as the customary magnetic switching effects.

The matrix elements of the interactions considered by Little are in the range of 1 eV. The order of magnitude of perturbing voltage required to destroy these interactions and produce switching will probably be a small percentage of a matrix element of interaction, perhaps 10 mV. The chain diameter of the macromolecule considered by Little is 30 Å, so that the critical electric field would be of the order of 33,000 V/cm—this could be very convenient for electronic switching applications; much more so than magnetic switching in all probability, since this would probably require extremely high magnetic fields.

An even more interesting speculation is that the sort of switching voltages required, if small superconducting memory loops of this type exist in the brain, would be quite consistent with the sorts of voltage signals known to exist in the nervous system. The type of organic macromolecular superconductor Little has suggested might provide a memory system for the brain, and interrogation or storage could be performed by electrostatic switching with voltages compatible with the nervous system. Further speculation suggests, by comparison with associative cryoelectric computer memories, that interrogation could be non-destructive.

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# Recoil Effects of Neutron Reactions in Sodium Bromate

THE chemical effects following radioactive neutron capture and isomeric transition of the nuclei of bromine atoms in bromates have been studied by a number of investigators<sup>1</sup>. The present communication describes our investigation of the chemical distribution of radioactive bromine atoms arising from  $^{79}\text{Br}(n,2n)^{78}\text{Br}$  and  $^{79}\text{Br}(n,\gamma)^{80}\text{Br}$  reactions in crystalline sodium bromate. The effects of the irradiation and dissolution temperatures were investigated and the recoil effects of both nuclear reactions were compared.

Powdered sodium bromate was irradiated with fast neutrons produced by the  $^3\text{H}(d,n)^4\text{He}$  reaction, or with slow neutrons moderated in paraffin blocks. The irradiated salt was dissolved in water and the bromine species of lower oxidation states were separated from bromate by extraction with carbon tetrachloride containing a small amount of molecular bromine, or by precipitation of bromide as silver bromide. The radioactivity of  $^{78}\text{Br}$  (half-life, 6.4 min) or  $^{80}\text{Br}$  (half-life, 18 min) was measured by means of a Geiger-Müller counter or an NaI scintillation counter.

The results are summarized in Table 1. The mean values of repeated determinations are shown together with the standard deviations.

Table 1. THE RETENTION OF RADIOBROMINE ATOMS FOLLOWING  $(n,2n)$  AND  $(n,\gamma)$  REACTIONS IN SODIUM BROMATE

Temperature Irradiation	Room temperature Dissolution	$^{79}\text{Br}(n,2n)^{78}\text{Br}$ Solvent extraction (per cent)	$^{79}\text{Br}(n,2n)^{78}\text{Br}$ Precipitation (per cent)	$^{79}\text{Br}(n,\gamma)^{80}\text{Br}$ Solvent extraction (per cent)	$^{79}\text{Br}(n,\gamma)^{80}\text{Br}$ Precipitation (per cent)
Room temperature	Room temperature	$12.4 \pm 0.4$	$11.5 \pm 0.4$	$12 \pm 1$	$11 \pm 1$
$0^\circ\text{C}$	$0^\circ\text{C}$	$11.2 \pm 0.5$	$11.5 \pm 0.6$	$12 \pm 1$	$12 \pm 1$
$-196^\circ\text{C}$	$0^\circ\text{C}$	$9.4 \pm 0.3$	$8.9 \pm 0.5$	$10 \pm 1$	$10 \pm 1$
$-196^\circ\text{C}$	$-20^\circ\text{C}$	$9.1 \pm 0.4$			

\* Dissolved in a eutectic mixture of lithium chloride and water.

The retention values of  $^{80m}\text{Br}$  and  $^{82}\text{Br}$  in slow neutron irradiation of solid sodium bromate have been reported by several authors<sup>2-6</sup>. The results range from 4 to 25 per cent depending on the conditions of irradiation and separation. Jach and Harbottle reported that, when alkali bromate was irradiated at dry-ice temperature, nearly the same retention values were obtained for  $^{80m}\text{Br}$  and  $^{82}\text{Br}$  recoil atoms from  $(n,\gamma)$  reactions, whereas an isotopic effect appeared as the irradiated salts were annealed at  $200^\circ\text{C}$ . Our results for  $^{80}\text{Br}$  are in good agreement with the initial retention values for  $^{80m}\text{Br}$  and  $^{82}\text{Br}$  obtained by them.

Although it is generally observed that the retention is sensitive to irradiation and dissolution temperatures, our attempts at low-temperature irradiation and dissolution resulted in no marked decrease of the retention values. This confirms the results reported by previous workers in the case of  $^{80m}\text{Br}$  and  $^{82}\text{Br}$  recoil atoms from  $(n,\gamma)$  reactions in potassium bromate<sup>7</sup>.

Comparison of recoil effects in  $(n,\gamma)$  and  $(n,2n)$  reactions has been attempted by several investigators. In all alkyl iodides that have ever been investigated almost the same retention values have been reported for both reactions<sup>8,9</sup>. On the other hand, different retention values have been observed in propyl bromides<sup>10</sup>, molecular iodine dissolved in various hydrocarbons<sup>11</sup>, and cobalt complexes<sup>12</sup>. In sodium bromoacetate, the retention of  $^{80m}\text{Br}$  atoms in  $(n,2n)$  reaction has been found to be very similar to that of  $^{82}\text{Br}$  atoms in  $(n,\gamma)$  reaction, whereas  $^{80m}\text{Br}$  atoms arising from  $(n,\gamma)$  reaction have showed somewhat different retention values<sup>13</sup>. Larger recoil energy involved in the  $(n,2n)$  reactions and the annealing effect of recoil protons have been suggested as the possible cause of such differences.

The data presented in this communication indicate that in sodium bromate there exists no difference, within experi-

mental errors, between the retentions for both reactions. Although no theoretical or experimental estimate of the recoil energy of  $(n,2n)$  reactions is as yet available, it would be reasonable to presume that the incident fast neutron or the outgoing two neutrons impart far larger recoil energy to the atom than the capture  $\gamma$ -quanta emitted in  $(n,\gamma)$  reactions. It is noteworthy in an experimental estimate of recoil mechanism that in certain compounds, such as alkyl iodides and sodium bromate, the retention seems to remain unaffected by a large change in recoil energy.

Further work is in progress on other bromates.

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<sup>1</sup> See, for example: Apers, D. J., Dejehe, F. G., van Outryve d'Ydewalle, B. S., Capron, P. C., Jach, J., and Moorhead, E., *Radiochim. Acta*, **1**, 193 (1963).

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## GEOPHYSICS

### Depth of the Mohorovičić-discontinuity under the North Sea Basin

DURING this summer and autumn refraction seismic experiments were carried out on the North Sea on a line with azimuth  $150^\circ$  starting from a position  $54^\circ 40' \text{N}$  and  $3^\circ 20' \text{E}$ . The so-called velocity-depth method was adopted in which listening ship and shooting ship are in mirror position with regard to the starting point. In this way the effect of a possible tilt of the layers is eliminated. This has the advantage that no need exists for shooting a reversed profile.

The preliminary results are listed in Table 1.

Velocity	Thickness	Depth
1,500 m/sec	40 m	40 m
1.85 km/sec	2.2 km	2.2 km
3.0 km/sec	1.0 km	3.2 km
6.15 km/sec	26.5 km	30 km
8.3 km/sec		

Velocity-depth profile at  $54^\circ 40' \text{N}$ ,  $3^\circ 20' \text{E}$ .

The Conrad-discontinuity did not show up in the first arrivals. The heaviest charge was somewhat less than 3,000 kg TNT at a distance between the ships of 210 km. In all, 46 shots were made. More than half of them had a purely experimental character. In fact, quite a few charges were fired to find the appropriate charge-distance relation for this region. The experiments were carried out on ships of the Royal Netherlands Navy. On all the trials H.Neth.M.S. *Cerberus* acted as the shooting ship. The explosives used were surplus depth charges.

The measurements form part of a larger programme to investigate the relation between sedimentary thickness and the position of the Moho. This first result seems to



confirm the conclusion<sup>1</sup> drawn from gravity data, that in general the mass deficiency of the North Sea Basin sediments is compensated isostatically.

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## GEOLOGY

### Bauchite: a New Variety in the Quartz Monzonitic Series

THE name 'bauchite' is proposed for a coarse plutonic fayalite-bearing rock which was first described by me from Bauchi in northern Nigeria<sup>1</sup>. I share the reluctance of modern petrologists to propose new variety names for rocks. There are, however, cases in which a variety name is justifiable to imply a significant physical, chemical or petrological characteristic not adequately conveyed by the general or group name. The case of charnockite is an example: to have simply described a charnockite as a hypersthene granite would never have conveyed the petrological peculiarities of the rock.

Variety names may therefore be justified if the rock to be named satisfies the following conditions: (1) the rock has a fairly large occurrence covering a few square miles, or in the case of dykes if they are found widespread over a few square miles; (2) it is found in two or more localities in similar petrological settings; (3) it possesses certain invariable physical, mineralogical or chemical characteristics by which it is distinguished from other rocks; (4) these invariable characteristics are the result of unusual petrogenetic history of the rock and are found in all specimens from different localities without exception.

When the fayalite quartz monzonitic rock of the Nigerian basement complex was considered in the light of these criteria, it was found necessary to propose a special name for it. This rock, which has been fully described by me<sup>1</sup>, is usually very coarse grained with the microcline perthite averaging 3 cm by 2 cm. It is of peculiar dark brownish-green colour—much too dark for its mineralogical composition. This colour is invariable, and is its most distinguishing physical characteristic. In general appearance, it bears resemblance to larvikite, but it is composed of perthite, plagioclase, fayalite, pyroxene, amphibole, quartz and ore. Myrmekitic intergrowths of plagioclase and quartz are widespread around the margins of the perthite. In view of the extreme coarseness of the rock, conventional methods of modal analysis were found useless. Using enlarged photographs of large stained hand specimens<sup>2</sup>, the variation in the perthite:plagioclase:quartz ratios suggests that the rock, belonging to the quartz monzonitic series, varies from the quartz syenite to adamellite.

The rock is mineralogically distinguished by the presence of fayalite commonly associated with ferrohastingsite, with the latter invariably developed as a mould round the plagioclase and the fayalite developed inside the quartz grains. Chemically, the rock is characterized by high FeO and very low MgO.

The rock is now known to be fairly widespread in Nigeria. Around Bauchi town a single occurrence covers about 20 square miles. Other bodies, each as large as this, occur to the south and west of Bauchi. Large outcrops of the rock have recently been found in three other widely scattered localities in northern and western Nigeria.

In all these localities the rock is found to occur surrounded by coarse granite of very similar texture with

a gradational contact relationship, marked in the field only by a sudden change of colour from the light colour of the granite to the dark green colour of the bauchite. Petrographic investigation reveals that the rock often shows a fringing zone of charnockite towards its contact with the granite, as hypersthene takes the place of fayalite around the contact areas. This relationship as well as the greenish colour common to both suggest some petrogenetic affinity between charnockite and bauchite. The latter cannot, however, be described as charnockite as hypersthene is not generally found with the fayalite in this rock.

Table 1. CHEMICAL COMPOSITION OF FAYALITE QUARTZ-MONZONITE, BAUCHI

	Norms	
	1 (%)	2 (%)
SiO <sub>2</sub>	67.96	70.59
Al <sub>2</sub> O <sub>3</sub>	14.77	13.90
Fe <sub>2</sub> O <sub>3</sub>	0.98	1.17
FeO	2.88	2.30
MgO	0.41	0.30
CaO	2.37	2.08
Na <sub>2</sub> O	3.25	3.30
K <sub>2</sub> O	5.75	5.55
H <sub>2</sub> O <sup>+</sup>	0.36	0.33
H <sub>2</sub> O <sup>-</sup>	0.16	0.14
TiO <sub>2</sub>	0.38	0.33
P <sub>2</sub> O <sub>5</sub>	0.12	0.15
MnO	0.04	0.05
BaO	0.44	0.13
Total	99.87	100.32
		Q
		Or
		Ab
		An
		Wo
		En
		Fs
		Mt
		Il
		Ap

1, ME 101, fayalite quartz-monzonite, Kobi Hill, Bauchi.

2, ME 222, fayalite quartz-monzonite, Kofar Wombai Hill, Bauchi. Both analyses by the Colonial Geological Surveys (Mineral Resources Division), London.

Norm calculations made by me, after Barth (1951, pp. 79-81).

I have been engaged in the investigation of the rock, and from a consideration of the structural and petrological characteristics of the rock have concluded that the bauchite originated by an emanation into granite of ferrous iron rich fluid or magma<sup>3</sup>. The green colour of charnockite and bauchite is believed to be due to impregnation of feldspars with ferrous iron during the emanation. Fayalite was crystallized as a result of reaction between quartz and the emanation; and ferrohastingsite by reaction with plagioclase feldspars. The physical factors that determine whether fayalite or hypersthene will crystallize are not clear to me, but in view of the relationship of charnockite as fringe to bauchite, it may very well be temperature.

The occurrence of high-level intrusive fayalite-bearing granitic rock in the younger granite province of Nigeria<sup>4</sup> may be of some significance. Here, the fayalite-bearing rocks occur as ring or polygonal dykes associated with most of the ring-complexes of this province and vary from syenite to granite porphyry. These rocks are also invariably green but usually of lighter shade. The exact relationship of these to the rock in this account is not yet clear.

In conclusion, the name bauchite is used to denote a dark greenish fayalite-bearing rock of quartz syenite to adamellite mineralogical composition which was first described from Bauchi town in northern Nigeria<sup>1</sup>. Its salient and diagnostic characteristics are: (1) The feldspar of the rock, a micropertthite, is dark green in colour and the quartz is brownish-green with resinous lustre. (2) Fayalite occurs in association with amphibole and is commonly arranged in zones. The amphibole is commonly moulded around the plagioclase and the fayalite is usually in a quartz matrix. (3) The rock occurs in plutonic setting commonly fringed by charnockite and surrounded by a plutonic granite.

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## METALLURGY

## Influence of Impurities on the Oxidation of Fe-26Cr Alloys

Most research on the oxidation of alloys has been done using commercial materials. In recent years, however, there has been a great deal of effort to work with pure alloys in order to obtain a better understanding of the mechanism of oxidation of these materials<sup>1</sup>. This laboratory has been investigating the oxidation of Fe-26Cr alloys and comparing the results with those obtained using stainless steels. This communication describes the high-temperature oxidation behaviour of three vacuum-melted alloys and one commercial steel. The compositions of the materials are given in Table 1.

Table 1

	C	Mn	Si	Ni	Cr
Fe-Cr	0.022	0.003	0.02	0.02	26.2
Fe-Cr-Si	0.022	0.004	0.55	0.02	26.2
Fe-Cr-Mn	0.012	1.00	0.01	0.02	25.7
Type 446	0.18	0.75	0.86	0.32	25.9

The specimens were first mechanically polished to produce a flat surface, and electro-polished in perchloric-acetic acid to remove surface contamination. They were then annealed at 1,100° C in argon, re-electropolished, and finally etched to remove the film left after electropolishing. Oxidation was carried out in a stream of dry oxygen. Continuous weighings were made on an automatic balance.

Typical weight-gain/time curves at 1,090° C are shown in Fig. 1. It can be seen that the Fe-26Cr and Fe-26Cr-0.5Si pure alloys oxidize at a similar rate and appreciably more slowly than the commercial alloy and the pure alloy containing manganese.

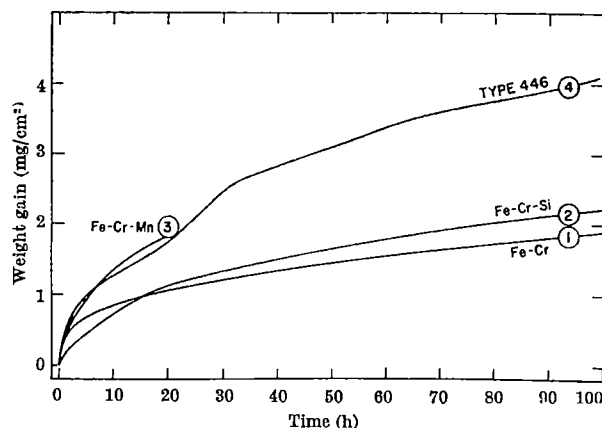


Fig. 1. Oxidation of Fe-26Cr, Fe-26Cr-0.5Si, Fe-26Cr-1 Mn and type 446 in 1 atm. dry oxygen at 1,090° C

After these oxidation runs the samples were examined metallographically and the scales analysed by X-ray diffraction and spectrographic analysis. Typical cross-sections of the oxidized specimens are shown in Fig. 2. The only oxide phase formed on the binary Fe-Cr alloy was  $\alpha$ - $\text{Cr}_2\text{O}_3$  containing a small amount of iron. (Pure chromium formed a similar single-phase scale under comparable conditions<sup>2</sup>.) The scales formed on the other three alloys are quite different. In the Fe-Cr-Si ternary, the silicon oxidizes preferentially to form a silica sub-scale under a layer of ' $\text{Cr}_2\text{O}_3$ ' indistinguishable from the oxide on the Fe-Cr binary. The reason the oxidation rate is not affected is because a continuous layer of silica does not form. The protectiveness of the ' $\text{Cr}_2\text{O}_3$ ' layer remains unchanged. In the Fe-Cr-Mn ternary, the Mn also oxidizes preferentially but forms an Mn-Cr spinel as a continuous layer at the expense of some of the ' $\text{Cr}_2\text{O}_3$ '. No scale section of the commercial steel is shown but, because of its manganese content, it forms a similar scale of spinel

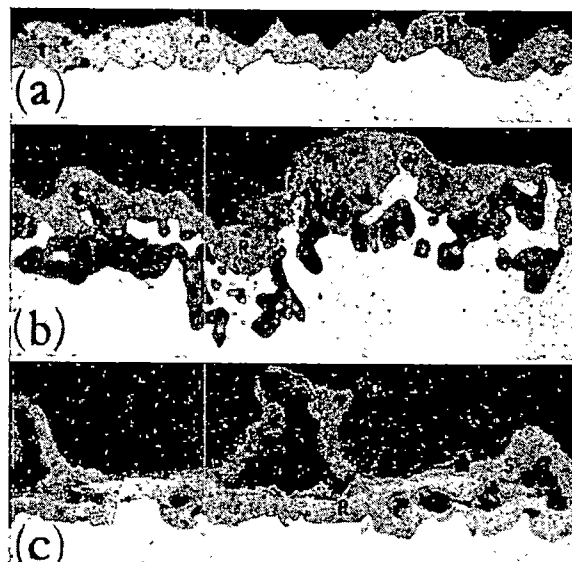


Fig. 2. Metallographic sections through oxide layers formed at 1,090° C. Wrinkled oxide and rugged metal surface are caused by compressive stress that develops continuously in the thickening oxide. R, rhombohedral  $(\text{Cr,Fe})_2\text{O}_3$ ; S,  $\text{MnO.Cr}_2\text{O}_3$  spinel; X, cristobalite silica. (a) Fe-26Cr alloy (curve 1).  $(\text{Cr,Fe})_2\text{O}_3$  is only phase formed. ( $\times$  c. 375). (b) Fe-26Cr-0.5Si (curve 2). Islands of silica have formed below  $(\text{Cr,Fe})_2\text{O}_3$  layer. ( $\times$  c. 750). (c) Fe-26Cr-1 Mn (curve 3). Light grey oxide is  $(\text{Cr,Fe})_2\text{O}_3$ ; darker grey is  $\text{MnO.Cr}_2\text{O}_3$  spinel ( $\times$  c. 750).

and ' $\text{Cr}_2\text{O}_3$ ' layers. Silica occurs again as a sub-scale. The high oxidation rate of the two alloys containing manganese is the result of the presence of the  $\text{MnO.Cr}_2\text{O}_3$  spinel.

These results indicate that manganese is a deleterious additive to FeCr alloys from the point of view of high-temperature oxidation. The addition of 0.5 per cent silicon does not improve oxidation resistance. It would appear that the investigation of the pure alloys is not only useful for elucidation of the oxidation mechanisms but may also lead to the development of materials with higher oxidation resistance. This investigation indicates that the pure binary alloy is the most oxidation resistant. Further work is in progress.

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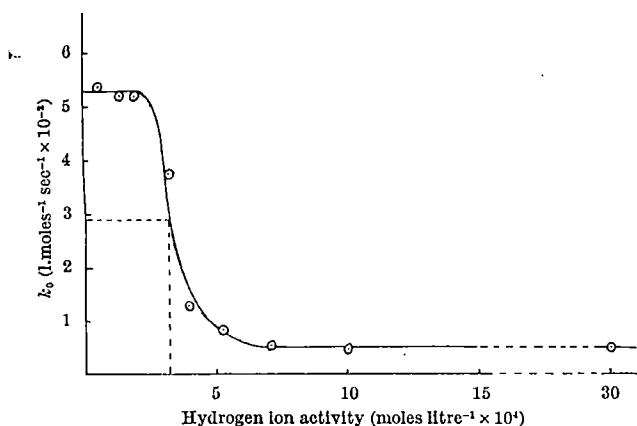
<sup>1</sup> Wood, G. C., *Corrosion Sci.*, 2, 173 (1962).

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## CHEMISTRY

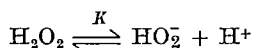
## Hydrolysis of Ferrous Ions: a Kinetic Method for the Determination of the Fe(II) Species

WE have recently shown<sup>1</sup> that the increase in the rate of the reaction of Fe(II) with hydrogen peroxide obtained by the addition of anions,  $\text{X}^{n-}$ , such as  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{P}_3\text{O}_9^{3-}$  and  $\text{HP}_3\text{O}_9^{2-}$ , is due to the species  $\text{FeX}^{2-n}$  reacting more rapidly with hydrogen peroxide than the hexa-aquo-ferrous ion. Following Hardwick's single observation<sup>2</sup> in perchlorate media that the rate at  $[\text{H}^+] = 5.3 \times 10^{-4}$  M is slightly greater than at lower pH, we have extended our investigation to higher pHs. We find that the observed bimolecular rate constant  $k_0$  in perchlorate media under nitrogen remains constant over a wide range of acidity, and then changes quickly over a narrow pH range to a higher limiting value which remains unchanged when the acidity is decreased further. Such a plot of  $k_0$

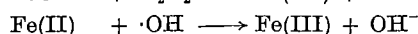
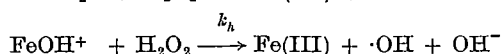
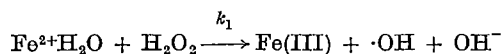
Fig. 1. Plot of  $k_0$  against hydrogen ion activity at  $\mu = 1.0$  and  $25^\circ \text{C}$ 

against  $[\text{H}_3\text{O}^+]$  is shown in Fig. 1, and resembles curves usually associated with acid-base reactions, for example, a titration curve.

The possibility that this curve is caused by the influence of the dissociation:



can be ruled out:  $K$  calculated at ionic strength  $\mu = 1.0$  and  $25^\circ \text{C}$  from the available data<sup>3</sup> equals  $5.4 \times 10^{-12}$  moles litre<sup>-1</sup>, and this dissociation obviously occurs at a higher  $p\text{H}$  than the rapid rise in  $k_0$  in Fig. 1. However, comparison with the effects of other anions<sup>1</sup> suggests that this curve is due to the conversion of the hexa-aquoferrous ion into a species with a positive charge  $< 2$ , which then reacts more rapidly with hydrogen peroxide. The constancy of  $k_0$  at high  $p\text{H}$  suggests that only one such species exists, probably  $\text{FeOH}^+$ , with the mechanism:



where  $\text{Fe}^{2+} + \text{H}_2\text{O}$  and  $\text{FeOH}^+$  have their co-ordination spheres completed with water molecules and

$$K_h = [\text{FeOH}^+][\text{H}_3\text{O}^+]/[\text{Fe}^{2+} + \text{H}_2\text{O}]$$

The lower limiting value of  $k_0$  is equated with  $k_1$ , and the higher limiting value with  $k_h$ . The rate of formation of ferric ions would be given by:

$$\begin{aligned} \frac{d[\text{Fe(III)}]}{dt} &= 2k_0[\text{Fe(II)}][\text{H}_2\text{O}_2] \\ &= 2k_1[\text{Fe}^{2+} + \text{H}_2\text{O}][\text{H}_2\text{O}_2] + 2k_h[\text{FeOH}^+][\text{H}_2\text{O}_2] \end{aligned}$$

where  $[\text{Fe(II)}]$  is the total concentration of ferrous ions in solution. It can be shown that:

$$k_0 = \frac{k_1[\text{H}_3\text{O}^+] + k_h K_h}{[\text{H}_3\text{O}^+] + K_h}$$

and for the condition that  $[\text{H}_3\text{O}^+] = K_h$ ,  $k_0 = (k_1 + k_h)/2$ . Therefore,  $K_h$  can be found from Fig. 1, being equal to  $[\text{H}_3\text{O}^+]$  where  $k_0 = (k_1 + k_h)/2 = 290$  l.moles<sup>-1</sup> sec<sup>-1</sup>. At this value of  $k_0$  the hydrogen ion activity  $\alpha_{\text{H}^+} = 3.2 \times 10^{-4}$ ; an approximate value for the activity coefficient of perchloric acid  $f_{\pm}$  in dilute solutions in 1 M  $\text{NaClO}_4$  can be computed<sup>4</sup> from the geometric mean of the activity coefficients of 1 M  $\text{HClO}_4$  and 1 M  $\text{NaClO}_4$ , the latter values being interpolated from the available experimental data<sup>5,6</sup>. In this way at  $\mu = 1.0$  and  $25^\circ \text{C}$   $K_h = \alpha_{\text{H}^+}/f_{\pm} = 4.3 \times 10^{-4}$  moles litre<sup>-1</sup>. As expected for such an ion-neutral

molecule reaction<sup>1</sup>,  $k_h$  is independent of ionic strength:  $K_h$  is found to vary slightly with  $\mu$ .

$K_h$  has been determined by several workers by measurement of  $[\text{H}_3\text{O}^+]$  produced by ferrous ion in conductivity water or water approaching neutral point. These values are collected in Table 1 and vary widely among themselves. Some are measured in the presence of a precipitate of ferrous hydroxide, and some use ferrous chloride solutions where ferrous is known to complex<sup>1</sup>: the last two measurements quoted in Table 1 are virtually by the same experimental procedure and yet the results differ considerably. The value found kinetically in this present work is very much greater than any of those of the previous workers derived by  $p\text{H}$ -difference. The kinetic method differs from the other methods in two respects: the quantities determined are really  $[\text{Fe}^{2+} + \text{H}_2\text{O}]$  and  $[\text{FeOH}^+]$  (cf. the other anions<sup>1</sup>) and not  $[\text{H}_3\text{O}^+]$  as in the other determinations; the hydrolysis is controlled by the addition of acid to the system. We have re-determined  $K_h$  by  $p\text{H}$ -difference (a) with ferrous perchlorate in conditions of added acid (b) in the same way as the previous workers by dissolving ferrous perchlorate in conductivity water. In all the calculations the observed hydrogen ion activities were converted to  $[\text{H}_3\text{O}^+]$  using the approximate value for activity coefficient at  $\mu = 1.0$  as described in the kinetic measurements here. Table 2 shows that (a) produces a value of  $K_h$  independent of total added ferrous ion concentration, and the average value of  $K_h = 4.8 \times 10^{-4}$  is in excellent agreement with that produced here by the kinetic method under the same conditions. The value of  $K_h$  by (b) varies with  $[\text{Fe(II)}]$  and is of the same order as that found by previous workers under their conditions. The concentration of ferric ions in these solutions was found to be negligible using the thiocyanate method.  $p\text{H}$  was measured using a glass electrode and the expanded scale of a Pye Dynacap direct-reading instrument. These results indicate that the hydrolysis appears to be inhibited at the higher  $p\text{H}$ : here the ratio  $[\text{H}_3\text{O}^+]$  produced/ $[\text{Fe(II)}]$  at equilibrium is much lower than at the lower  $p\text{H}$ . This suggests that, at the higher  $p\text{H}$ , a hydrolysed species (probably not  $\text{FeOH}^+$ ) removes hexa-aquoferrous ions into a less hydrolysable form, possibly a polymer of a basic cation type: this may account for the turbidity which is always produced under these conditions.

Under the controlled conditions with added acid used by us, the agreement between the two methods, kinetic and  $p\text{H}$ -difference, together with the constancy of  $k_0$  at

Table 1.  $K_h$  BY PREVIOUS WORKERS FROM  $p\text{H}$ -DIFFERENCE

Authors	$K_h$ moles litre <sup>-1</sup>	Comments
Leussing and Koltthoff <sup>7</sup>	$5 \times 10^{-3}$	From solubility product at $25^\circ \text{C}$ : $\text{FeCl}_2 + \text{NaOH}$
Gayer and Wootner <sup>8</sup>	$6.8 \times 10^{-4}$	$\mu = 0.50$ at $25^\circ \text{C}$ : $\text{FeCl}_2 \cdot 2\text{H}_2\text{O}$ + conductivity water
Lindstrand <sup>9</sup>	$1.2 \times 10^{-4}$	$\text{Fe}(\text{ClO}_4)_2$ + conductivity water at $20^\circ \text{C}$
Hedström <sup>10</sup>	$3.2 \times 10^{-10}$	$\text{Fe}(\text{ClO}_4)_2$ soln. approaching neutral point at $\mu = 1.0$ and $25^\circ \text{C}$
Bolzan <sup>11</sup>	$1.6 \times 10^{-7}$	$\text{Fe}(\text{ClO}_4)_2$ soln. approaching neutral point at $\mu = 1.0$ and $25^\circ \text{C}$

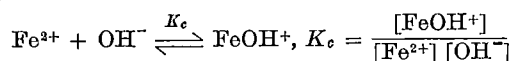
Table 2. VARIATION WITH  $[\text{Fe(II)}]$  OF  $K_h$  DETERMINED BY  $p\text{H}$ -DIFFERENCE AT  $\mu = 1.0$  AND  $25^\circ \text{C}$ 

$[\text{Fe(II)}]$ M (total)	$K_h$ moles litre <sup>-1</sup>
Method (a)	
$5.00 \times 10^{-3}$	$3.5 \times 10^{-4}$
$4.00 \times 10^{-4}$	$6.4 \times 10^{-4}$
$1.00 \times 10^{-3}$	$5.3 \times 10^{-4}$
$1.00 \times 10^{-3}$	$4.0 \times 10^{-4}$
Method (b)	
$1.00 \times 10^{-3}$	$2.2 \times 10^{-3}$
$1.04 \times 10^{-4}$	$1.3 \times 10^{-3}$
$9.98 \times 10^{-4}$	$2.0 \times 10^{-3}$
$1.05 \times 10^{-3}$	$1.2 \times 10^{-3}$
$1.00 \times 10^{-1}$	$1.1 \times 10^{-7}$



high  $pH$ , suggest that only one equilibrium, equation (1), exists here. However, the average value of  $K_h$ ,  $4.6 \times 10^{-4}$  moles litre $^{-1}$ , is much higher than the values of  $K_h$  recorded for some other bi-positive cations of the first transition series<sup>12</sup>. Although the crystal field stabilization for the hexa-aquo cation  $M^{2+}$  over that for  $MOH^+$  is probably less for  $Fe(II)$  than the other cations in the series except  $Mn(II)$ , this difference is not likely to be great enough to account for the large value of  $K_h$  for  $Fe(II)$ . However, these values of  $K_h$  for the other cations have been determined by experiments involving the measurement of  $pH$ -difference in a similar manner to that used by the previous workers for ferrous ions<sup>7-11</sup>.

$K_c$  for the ion association:



is found to be  $2.0 \times 10^{10}$  using  $K_w = 1.007 \times 10^{-14}$  at  $25^\circ C$  and  $f_{H^+} f_{OH^-} / a_{H_2O} = 0.537$  at  $\mu = 1.0$  and  $25^\circ C$  as found for solutions containing  $NaCl$  (ref. 13). This is approximately  $10^8$  times bigger than the association constants<sup>1</sup> of  $Fe^{2+}$  with  $Cl^-$ ,  $SO_4^{2-}$ ,  $P_3O_9^{3-}$  and  $HP_3O_9^{2-}$ . A hydrolytic species, such as  $FeOH^+$ , is most certainly an inner sphere complex<sup>14</sup> and the others may therefore possibly be of the outer sphere type: alternatively, the high  $K_c$  for  $FeOH^+$  may be due to a high covalent contribution in the bonding.

One of us (M. A. S.) holds a Colombo Plan scholarship.

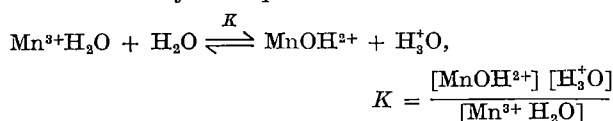
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## Hydrolysis of the Manganic Ion

RECENTLY the kinetics of the reactions of manganic perchlorate in aqueous solution have become the subject of investigation, but so far the species of  $Mn(III)$  present have not been identified. From a spectrophotometric investigation of manganic perchlorate solutions prepared electrolytically, we find maxima in the region of 203–220  $m\mu$  and at 470  $m\mu$  and a minimum of 350  $m\mu$ . The results of a detailed examination of such solutions at  $25^\circ C$  in the region 300–550  $m\mu$  with acidity varying between 0.10 M and 3.70 M  $HClO_4$  at an ionic strength  $\mu = 4.0$  maintained by the addition of manganous perchlorate (which also stabilizes the manganic ion) can be interpreted in terms of only one equilibrium:



where  $Mn^{3+} + H_2O$  is the hexa-aquo ion and  $MnOH^{2+}$  the hydroxopenta-aquo ion having extinction coefficients  $\epsilon_1$

and  $\epsilon_2$ , respectively. The observed extinction coefficient  $\epsilon$  should follow:

$$\epsilon([H_3O^+] + K) = \epsilon_1[H_3O^+] + \epsilon_2K \quad (1)$$

and rearranging:

$$\left(1 - \frac{\epsilon_1}{\epsilon}\right)[H_3O^+] = \frac{K\epsilon_2}{\epsilon} - K \quad (2)$$

When  $\epsilon_1/\epsilon$  is sufficiently  $< 1$ , a plot of  $[H_3O^+]$  against  $1/\epsilon$  at a fixed wave-length should be approximately linear. Such plots are found to be linear up to about 1.0–1.5 N  $HClO_4$ ; examples are shown in Fig. 1. From this plot at each wave-length an approximate value for  $K$  is found from the intercept, and then from the slope an approximate value for  $\epsilon_2$ ; by substitution of these values back in equation (1) an approximate value for  $\epsilon_1$  is obtained. This is then used in the full equation (2), and  $(1 - \epsilon_1/\epsilon) \times [H_3O^+]$  plotted against  $1/\epsilon$  for each wave-length for the whole acidity range 0.10–3.70 N  $HClO_4$ : fine adjustment is made to  $\epsilon_1$  to give the best straight line in each case over the whole acidity range, thus giving an accurate value for  $\epsilon_1$ , and accurate values for  $K$  and  $\epsilon_2$  from the intercept and slope respectively.

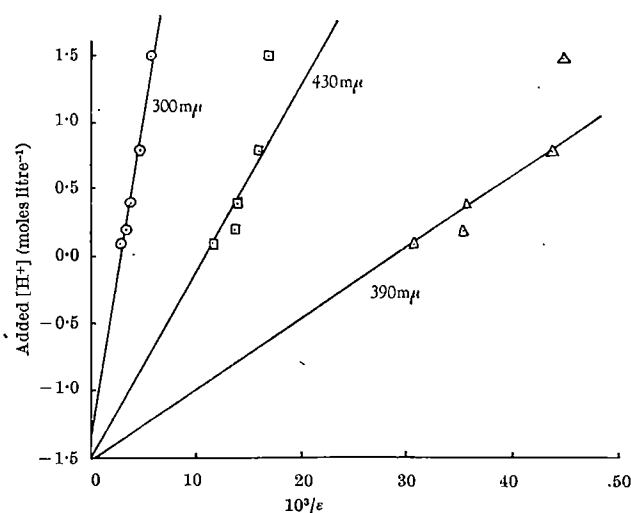


Fig. 1. Plots of  $[H_3O^+]$  against  $1/\epsilon$  for various wave-lengths at  $\mu = 4.0$  and  $25^\circ C$

The spectra for the hexa-aquo ion and the hydroxopenta-aquo ion both have a maximum of 470  $m\mu$  and a minimum of 350  $m\mu$ :  $\epsilon_1 = 52$  and  $\epsilon_2 = 165$  at 470  $m\mu$  and  $\epsilon_1 = 0.50$  and  $\epsilon_2 = 28$  at 350  $m\mu$ :  $\epsilon_1$  and  $\epsilon_2$  both have a maximum in the region 203–225  $m\mu$  with  $\epsilon \sim \epsilon_1 \sim \epsilon_2 \sim 4 \times 10^3$ , but the exact wave-length of the maximum is dependent on conditions. From the analysis of the results at fifteen wave-lengths over the range 300–550  $m\mu$ ,  $K = 0.88 \pm 0.04$  at  $\mu = 4.0$  and  $25^\circ C$ . From similar analyses at 300  $m\mu$  and 470  $m\mu$  over the temperature range  $1^\circ$ – $35^\circ C$  it was found for  $\mu = 4.0$  that  $\Delta H = 4.8 \pm 0.8$  kcal mole $^{-1}$  and  $\Delta S = 15.7 \pm 2.6$  cal deg $^{-1}$  mole $^{-1}$ , with  $\Delta G$  at  $25^\circ C = 123$  cal mole $^{-1}$ . Solutions were used immediately after preparation and the total  $[Mn(III)]$  was determined by oxidizing  $Fe(II)$  to  $Fe(III)$  in perchlorate media,  $[Fe(III)]$  being estimated spectrophotometrically at 260  $m\mu$ . No interference from polymers of  $Mn(III)$  could be detected: Beer's law is obeyed by the solutions of manganic perchlorate at both the low and the high end of the acidity range. While this work was in progress approximate values for  $K$  were published: Diebler and Sutin<sup>1</sup> give  $K \sim 5$  and Fackler and Chawla<sup>2</sup> give  $K \sim 1.5$ , both at  $\mu = 6.0$  and  $23^\circ C$ . Although Diebler and Sutin used aged solutions of  $Mn(III)$  which contain polymers, these approximate values support the conclusion from the accurate value by

us that the hexa-aquamanganic ion is considerably hydrolysed even in a very high concentration of acid. This value of  $K$  is high compared with those for the other tripositive ions of the first transition series (following communication). The crystal field stabilization of the hexa-aquo ion over the hydroxopenta-aquo species will be smaller for Fe(III) than Mn(III), so this effect alone cannot control the hydrolysis as  $K$  for Mn(III)  $\gg$  Fe(III) ( $K = 10^{-3}$  at  $\mu = 3$  and  $25^\circ\text{C}$  (ref. 3)).

One of us (G. D.) thanks the Department of Scientific and Industrial Research for the award of a maintenance grant.

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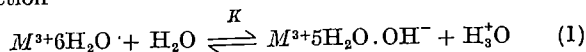
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### Hydrolysis of the Tripositive Ions of the First Transition Series: a Discussion of the Heats and Entropies for the Dissociation of the First Proton

THERMODYNAMIC data are now available for the reaction



for all members of the first transition series which form a reasonably stable hexa-aquo ion  $M^{3+}$  in solution except titanous. These are collected in Table 1 for perchlorate media, where possible at ionic strength  $\mu = 1.0$ : the equilibrium constant  $K = [MOH^{2+}][H_3O^+]/[M^{3+}]$  at  $25^\circ\text{C}$  is also included, the value for titanous at  $\mu = 1.0$  being interpolated from the data of Pecsok and Fletcher<sup>6</sup>. As the first hydrolysis product  $MOH^{2+}$  must be an inner sphere complex<sup>7</sup>, changes in  $\Delta H$  for reaction (1) along the first transition series might be expected to arise from changes in the difference between the crystal field stabilizations produced by  $H_2O$  and  $OH^-$ . The crystal field splitting is greater for  $H_2O$  than  $OH^-$  and in general should result in stabilization of the hexa-aquo ion: this stabilization is absent for the  $t_{2g}^3e_g^2$  arrangement, and therefore  $K$  for the tripositive ions in the first transition series should have a maximum at ferric ion. However, Table 1 shows clearly that this is not the case, indicating that the influence of differences in crystal field stabilization on reaction (1) must be small. In fact, the spectral data<sup>8</sup> for the rhodium(III) complexes  $Rh(NH_3)_5H_2O^{3+}$  and  $Rh(NH_3)_5OH^{2+}$ , where the wave-lengths of maximum absorption are  $31,600\text{ cm}^{-1}$  and  $31,200\text{ cm}^{-1}$ , respectively, suggest that the difference in stabilization produced by  $H_2O$  and  $OH^-$  will be small enough to neglect when considering changing influences on reaction (1) along a transition series.

Mn<sup>3+</sup> has the largest value for  $K$  at  $25^\circ\text{C}$ , and the next largest values are for Co<sup>3+</sup> and Ti<sup>3+</sup>. V<sup>3+</sup> and Fe<sup>3+</sup> are hydrolysed to the same extent and have nearly

identical values for  $\Delta H$  and  $\Delta S$ . Cr<sup>3+</sup> is much less hydrolysed than V<sup>3+</sup> and Fe<sup>3+</sup>, and this is due to a smaller entropy change as  $\Delta H \sim 10\text{ kcal mole}^{-1}$  for all three ions. The high value of  $K$  for Mn<sup>3+</sup> is due to the value for  $\Delta H$  being less than those for V<sup>3+</sup> and Fe<sup>3+</sup>. For Co<sup>3+</sup>  $\Delta H \sim 10\text{ kcal mole}^{-1}$ , and the value of  $\Delta S > 20\text{ e.u.}$  is responsible for  $K$  being higher than the value for V<sup>3+</sup> or Fe<sup>3+</sup>. As Co<sup>3+</sup> is the only ion in Table 1 which is supposed to be in a low spin state this will be considered separately later.

The entropy change is always positive in going from left to right in reaction (1), indicating a general decrease in restriction: this is probably due to the partial neutralization of the overall change in the hydroxopenta-aquo ion from the adjacent  $OH^-$  with a consequent decrease in restriction imposed on the solvent molecules, even though the number of species bearing a positive charge has increased. However, changes in this positive  $\Delta S$  for reaction (1) along a transition series might be expected to be related to changes in the metal-ligand distances within the hydrated cations themselves. As the ion-ion attraction of  $M^{3+}$  for  $OH^-$  will be greater than the ion-dipole attraction of  $M^{3+}$  for  $H_2O$ , the metal-ligand distance for  $OH^-$  should be shorter than for  $H_2O$ , causing the hydroxopenta-aquo ion itself to have a lower entropy than the hexa-aquo ion: any screening of this attraction should therefore have a proportionately greater effect on the ion-ion interaction of the  $OH^-$  tending to increase the positive entropy change  $\Delta S$ . Therefore, filling of the  $t_{2g}$  levels, where screening is low, should result in a progressive shortening of the metal-ligand distance for  $OH^-$  relative to  $H_2O$ , thereby decreasing the positive entropy change for reaction (1). Table 1 shows that  $\Delta S$  decreases in going from V<sup>3+</sup>( $t_{2g}^3$ ) to Cr<sup>3+</sup>( $t_{2g}^3$ ), and the general decrease in  $K$  from Ti<sup>3+</sup>( $t_{2g}^2$ ) through V<sup>3+</sup> to Cr<sup>3+</sup> is probably due to this effect. This greater reduction in the entropy of the hydroxopenta-aquo ion relative to the hexa-aquo ion should be a maximum with the  $t_{2g}^3$  arrangement of Cr<sup>3+</sup>. With Mn<sup>3+</sup>, the next electron goes into an  $e_g$  level, increasing the screening, which should thereby increase the entropy of the hydroxopenta-aquo ion more than the hexa-aquo ion with a consequent increase in  $\Delta S$  for reaction (1); and the introduction of another electron into an  $e_g$  level with Fe<sup>3+</sup> should similarly cause a further increase in  $\Delta S$ . Table 1 confirms that  $\Delta S$  varies in this way, with a minimum at Cr<sup>3+</sup>.

As stated here, a feature of reaction (1) in this series is the constancy of  $\Delta H$  at  $\sim 10\text{ kcal mole}^{-1}$  except for Mn<sup>3+</sup>. The electronic arrangement in the  $3d$  orbitals for Mn<sup>3+</sup> is one electron short of the highly stable half-filled  $d$  orbitals in the high spin arrangement  $t_{2g}^3e_g^2$ . The ionization potentials<sup>9</sup> clearly show that this half-filled arrangement possesses high stability in the gas phase for the first transition series, and this appears also to be true in solution: for the  $M^{3+}-M^{2+}$  couple in solution, the  $t_{2g}^3e_g^2$  form is the more stable where it occurs, for example, Mn<sup>2+</sup> more stable than Mn<sup>3+</sup> and Fe<sup>3+</sup> more stable than Fe<sup>2+</sup>. Mn<sup>3+</sup> could attain this stable structure by delocalization of electrons on the ligand through orbital overlap. As the  $OH^-$  ion is more polarizable than the  $H_2O$  molecule, such a delocalization by  $sp^3-d$  overlap would tend to be greater in the hydroxopenta-aquo ion than in the hexa-aquo ion: the extra stability of the former species would result in a lower positive value for  $\Delta H$ , as found for the manganic ion. The effect will not be present in Fe<sup>3+</sup> which already has the  $t_{2g}^3e_g^2$  arrangement, and Cr<sup>3+</sup>( $t_{2g}^3$ ) is more stable in solution than Cr<sup>2+</sup>( $t_{2g}^3e_g^2$ ). The delocalization may also result in a shortening of the metal-ligand distance for  $OH^-$  which would contribute to a small extent to a lower  $\Delta S$  for reaction (1) for Mn<sup>3+</sup>. Of the  $M^{3+}$  ions in Table 1, Jahn-Teller distortions arising from the  $d_{z^2}$  and  $d_{x^2-y^2}$  orbitals might be expected only for Mn(III), probably with four short and two long metal-

Table 1

Hexa-aquo ion $M^{3+}$ : arrangement of the $3d$ electrons	$K$ at $25^\circ\text{C}$ moles litre <sup>-1</sup>	$\Delta H$ kcal mole <sup>-1</sup>	$\Delta S$ cal deg <sup>-1</sup> mole <sup>-1</sup>	$\mu$	Ref.
Ti <sup>3+</sup> $t_{2g}^2$	$1.0 \times 10^{-3}$	—	—	1.0	
V <sup>3+</sup> $t_{2g}^3$	$2.0 \times 10^{-3}$	10	20	1.0	1
Cr <sup>3+</sup> $t_{2g}^3$	$1.1 \times 10^{-4}$	9.4	13.5	1.0	2
Mn <sup>3+</sup> $t_{2g}^3e_g^2$	0.88	4.8	15.7	4.0	3
Fe <sup>3+</sup> $t_{2g}^3e_g^2$	$1.66 \times 10^{-3}$	10.2	21	1.0	4
Co <sup>3+</sup> $t_{2g}^6$	$1.35 \times 10^{-2}$	10	25	1.0	5

ligand bonds. To influence reaction (1), the distortion with the  $\text{OH}^-$  present must produce greater stability: however, relative changes in metal-ligand distances for  $\text{H}_2\text{O}$  and  $\text{OH}^-$  which might result appear from the above discussion to influence only  $\Delta S$  and not  $\Delta H$ .

As mentioned earlier,  $\text{Co}^{3+}$  is a special case, being supposed to have a low spin arrangement, whereas all the other tri-positive ions exist in the high spin state. The  $t_{2g}^6$  arrangement with low screening should be comparable with  $\text{Cr}^{3+}$  with a low value for  $K$  arising from a small positive  $\Delta S$ , but although  $\Delta H = 10 \text{ kcal mole}^{-1}$ ,  $\Delta S > 20 \text{ e.u.}$ , resulting in a value of  $K_{\text{Co}^{3+}} > K_{\text{Fe}^{3+}}$ . However, this value of  $\Delta S = 25 \text{ e.u.}$  has a large possible deviation<sup>8</sup> of  $\pm 7 \text{ e.u.}$ , which could bring  $\Delta S$  slightly less than  $20 \text{ e.u.}$  as expected for this electronic arrangement.  $\text{Co}^{3+}$  is assigned to the low spin state on the basis of its diamagnetism<sup>10</sup>, although this could be explained by  $\text{Co}^{3+}$  existing as a dimer in the condition of the magnetic measurements: there is kinetic and spectroscopic evidence<sup>11</sup> that  $\text{Co}^{3+}$  does dimerize, and this is favoured by the high concentrations used in the magnetic measurements. If dimerization is responsible for the diamagnetism and monomeric  $\text{Co}^{3+}$  has the  $t_{2g}^4 e_g^2$  arrangement,  $\Delta S$  might well be expected  $\sim 20 \text{ e.u.}$  (allowed by the error) comparable with  $\Delta S$  for  $\text{V}^{3+}$  and  $\text{Fe}^{3+}$ . However, the balance between high and low spin is obviously delicate for the cobaltic ion, and the crystal field splitting of  $\text{OH}^-$  may be just small enough in this case to ensure that the hydroxopentaaquo ion is in the high spin state, even if the monomeric hexa-aquo ion is in the low spin state. From the foregoing discussion of entropies, reaction (1) going from  $\text{Co}^{3+}(t_{2g}^6)$  to  $\text{CoOH}^{2+}(t_{2g}^4 e_g^2)$  should result in a lengthening of the metal-ligand distances with  $\Delta S$  having a high positive value, possibly  $> 20 \text{ cal deg}^{-1} \text{ mole}^{-1}$ .

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### Crystal-Molecular Structure and Magnetic Properties of $\text{Cr}_3(\text{CH}_3\text{COO})_6 \cdot 3\text{H}_2\text{O}$

As part of a programme concerning magnetic exchange interaction in relatively small molecules we have largely completed an X-ray diffraction investigation of the crystal structure of  $\text{Cr}_3(\text{CH}_3\text{COO})_6 \cdot 3\text{H}_2\text{O}$ . Compounds of this type have long been thought to be trimeric and have previously been formulated as  $\text{M}_3(\text{CH}_3\text{COO})_6(\text{OH})_2 \cdot 3\text{H}_2\text{O}$  (ref. 1). We have investigated both the chromium ( $\text{M} = \text{Cr}^{\text{III}}$ ) and iron ( $\text{M} = \text{Fe}^{\text{III}}$ ) complexes and find that they are isostructural, and that the cation is trimeric and of a form suggested earlier<sup>2</sup>. We conclude, however, that for these compounds ( $\text{M} = \text{Cr}^{\text{III}}$  or  $\text{Fe}^{\text{III}}$ ) the correct formulation is  $\text{M}_3(\text{CH}_3\text{COO})_6 \cdot 3\text{H}_2\text{O}$ .

Crystals of  $\text{Cr}_3(\text{CH}_3\text{COO})_6 \cdot 3\text{H}_2\text{O}$  are orthorhombic, space-group  $P2_12_12$  with  $a = 13.7 \text{ \AA}$ ,  $b = 23.2 \text{ \AA}$ ,  $c = 9.2 \text{ \AA}$  and  $Z = 4$  molecules per unit cell. Atom co-ordinates have been determined from Patterson and Fourier techniques based on about 1,800 independent observed reflexions and have been partially refined by least squares

methods. The  $R$  factor at the present stage of refinement is 0.16.

While there is no constraint imposed by crystal symmetry the complex ion has  $3/m$  point group symmetry; the chromium atoms lie at the apices of an equilateral triangle of edge  $3.28 \text{ \AA}$  and each is octahedrally co-ordinated by oxygen atoms. The three octahedra share a common vertex which is coplanar with, and at the centre of, the triangle of chromium atoms. A single oxygen atom occupies this shared vertex; the vertex of each octahedron *trans* to the shared vertex is occupied by a water molecule. The four remaining vertices of each octahedron about the chromium atoms are occupied by four oxygen atoms, one from each of four different carboxyl groups. The carboxyl groups thus form bridges between adjacent vertices of adjacent octahedra. The co-ordination of the metal atoms and the bridging arrangement are illustrated in Fig. 1. At the present stage of refinement the observed Cr-O distances range from  $1.9 \text{ \AA}$  to  $2.1 \text{ \AA}$  with a mean value of  $2.0 \text{ \AA}$ .

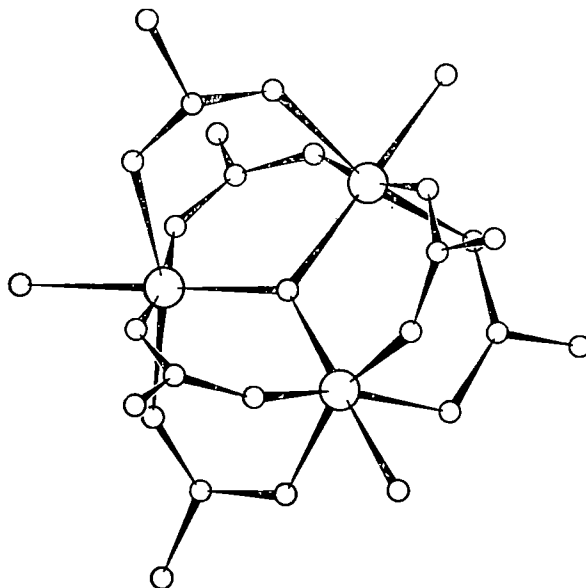


Fig. 1. Stereochemistry of the trinuclear ion  $[\text{Cr}_3(\text{CH}_3\text{COO})_6 \cdot 3\text{H}_2\text{O}]^+$

The magnetic susceptibility of this compound has been reported over a very wide range of temperature ( $-0.35^\circ \text{K}$ – $300^\circ \text{K}$ ; refs. 3 and 4) and values for the range  $80^\circ \text{K}$ – $300^\circ \text{K}$ , which have been obtained in this laboratory<sup>8</sup>, confirm the earlier work. The complex is a model one for the investigation of magnetic exchange in a trinuclear system and its magnetic behaviour has been explained by earlier workers by means of the spin Hamiltonian<sup>3,4,6,7</sup>:

$$H = J_0(S_1 \cdot S_2 + S_1 \cdot S_3) + (J_0 + J_1)S_2 \cdot S_3$$

where  $S_1$ ,  $S_2$  and  $S_3$  are the spins of the three chromium atoms (each  $3/2$ ), and  $J_0$  and  $J_1$  are the exchange integrals. The calculated values are  $J_0 = 30^\circ$  and  $J_1 = 7.5^\circ$  but the evaluation of  $J_1$  is rather uncertain. The observed trigonal symmetry of the cation requires  $J_1 = 0$ , which value is reasonably consistent with the magnetic data but is not consistent with the heat capacity of the compound at very low temperature<sup>6</sup>. It is not unlikely, however, that a very small amount of magnetic exchange between different trimeric units, which is suggested by the magnetic results<sup>4</sup>, may account for the heat capacity data.

For the isostructural iron complex the unit cell dimensions are  $a = 13.0 \text{ \AA}$ ,  $b = 23.0 \text{ \AA}$ ,  $c = 9.0 \text{ \AA}$ . This compound also shows magnetic exchange and  $J_0$  has been reported from earlier results<sup>6</sup> to be about  $40^\circ$ . The magnetic results obtained in this laboratory<sup>8</sup> both for the



iron and for the chromium complexes will be reported elsewhere as will the complete structural details for the chromium complex.

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### Cathode-ray Polarography with Rapid-dropping Mercury Electrodes

LINEAR sweep cathode-ray polarography with the dropping mercury electrode has developed in two distinct lines: single sweep polarography<sup>1-3</sup> in which the applied potential change is impressed on the electrodes once in the lifetime of each mercury drop, and multisweep polarography<sup>4-6</sup> in which it is applied several or many times. The latter is electronically the simpler, but the former possesses distinct advantages and is in more general use. In order to ensure that the drop surface area does not change appreciably during the electrode reaction, it is necessary, in the case of single-sweep working, to synchronize the application of the potential with the latter part of drop life. In earlier instruments this is achieved by using a multivibrator with fixed 'rest' and 'sweep' periods and arranging for the sharp decrease in current, which occurs when a drop detaches, to trigger the instrument back to its 'rest' condition while growth of the next mercury drop takes place. Later instruments<sup>7,8</sup> use a more satisfactory arrangement by which the drop is detached by the application of an electromechanical pulse to the electrode capillary at the conclusion of the potential sweep.

Recently we have investigated a new method of cathode-ray polarography using dropping mercury electrodes of very short drop time. Using a transistorized timing unit and drop controller, described elsewhere<sup>9</sup>, we have been able to achieve fast drop rates with accurate control. Electrodes with drop times as short as 0.08 sec have been produced in this way and these have been used in conjunction with a Southern Analytical cathode-ray polarograph. Polarographic reductions at an electrode of this type do not produce the characteristic cathode-ray polarographic wave (Fig. 1); but instead a step is obtained similar to that of conventional polarography. A typical example of this is shown in Fig. 2.

Cathode-ray-type waves arise from a combination of two processes. When the applied potential reaches a value equal to that of the characteristic reduction potential of the reacting species, all that species in the immediate vicinity of the mercury drop surface is 'stripped out' of solution (or reacted at the drop surface) by a process that is not diffusion-controlled and not governed by the Ilkovic equation. It gives rise to an initial current of a magnitude greater than the limiting diffusion current. However, once this process is complete the reaction can only continue if the depleted layer around the mercury drop is replenished with the reacting species. Under polarographic conditions this can only take place by the normal processes of diffusion, so that the current falls to the limiting diffusion current, or in practice, often to a value below this. The wave shown in Fig. 1 therefore results.

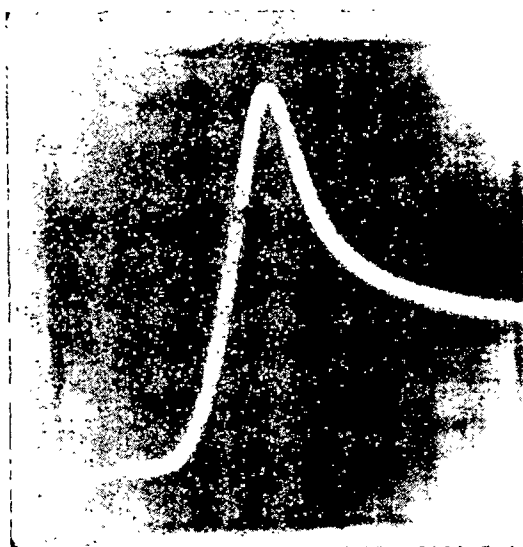


Fig. 1. Linear sweep (single sweep) cathode-ray polarograph. 1  $\mu$ g lead/ml. in M potassium chloride. Sensitivity factor  $1 \times 10^3$ . Start potential, 0.35 V

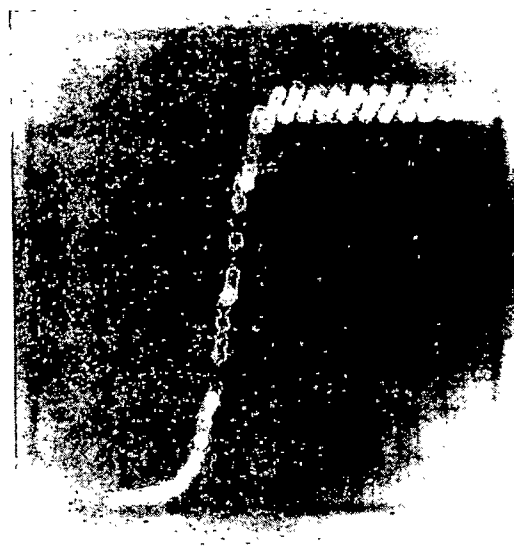


Fig. 2. Linear sweep cathode-ray polarograph; rapid-dropping mercury electrode. 1  $\mu$ g lead/ml. in M potassium chloride. Dropping mercury electrode drop time, 0.08 sec; sensitivity factor  $1.5 \times 10^3$ ; half-wave potential 0.42 V versus saturated Ag/AgCl electrode

Both 'stripping' and 'limiting diffusion' processes are operating in any polarographic reduction, but a high ratio of rate of change of applied potential to drop time favours the appearance of the stripping peak. The undamped, high-speed response of the cathode-ray tube is also a factor in the presentation of this wave-form. In conventional polarography the ratio is reversed. The ratio of drop time to rate of change of applied potential is high, so that the 'stripping' peak is suppressed and the conventional step appears. The damped indicating or recording system also makes some contribution to this. This interpretation on the basis of potential sweep rate-drop time ratios is supported by the work of Smith<sup>10</sup>, who has produced cathode-ray-type waves with a conventional polarograph by using dropping mercury electrodes with drop time in some cases as long as 8 min. Such drop times bear a ratio to the rate of change of applied potential similar to that of conventional polarography. Cathode-ray-type waves also result when mercury pool micro-electrodes are used.

In the work recorded here the total sweep time used was 2 sec for a change of 0.5 V (0.25 V per second), so that 25 drops form, and are detached, during the sweep period. This establishes a ratio similar to that of conventional polarography, so that waves of 'conventional' shape would be expected. The results of our investigations are considered to add further support to the foregoing interpretation.

This type of polarography has a number of advantages, especially those connected with the rapidity of the polarographic determination. A reversible polarographic wave of conventional shape is produced on the screen in less than 2 sec, which should lead to many applications to kinetic investigations and high-speed polarography for industrial control. There is a loss in sensitivity compared with single sweep cathode ray polarography<sup>11,12</sup>, due to the very small area of the 0.08-sec mercury drops used. The sensitivity obtained in practice is, however, much greater than that calculated, presumably due to stirring at the drop surface caused by the pulse required to detach the drop. The detachment of such small drops was found to require a comparatively large amount of energy. Our experiments have shown that the sensitivity obtainable is at least equal to a conventional polarograph.

High-sweep linear sweep cathode-ray polarography of this type may be carried out with any linear sweep cathode-ray polarograph by the addition of a suitable drop control system. The later Southern Analytical polarographs are particularly suitable for this, since a control relay is already incorporated in the electrode stand. It is only necessary to disconnect this relay from the instrument synchronization circuit and to connect it to a drop timer of suitable period. Modification of the control circuits of the above instrument to give variable drop times might not be difficult and is worth consideration. Instruments of this complexity are, however, not strictly necessary, since the essential requirements are only a simple saw-tooth generator of suitable period, a drop timer and electrode stand with a drop-control relay and a cathode-ray oscilloscope.

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### $\beta$ -Sorption Complexes of Hydroxy-salts

MANY lamellar substances form interlamellar sorption complexes with polar molecules. These may be  $\alpha$ -complexes, in which the molecules lie flat on the sorbent layers, or  $\beta$ -complexes, in which the molecules are upright with respect to the layer<sup>1</sup>. Such complexes have been examined with graphitic acid<sup>2,3</sup>, montmorillonite<sup>4</sup>, and nickel cyanide<sup>5</sup>.

In the present communication, an investigation is reported of the  $\beta$ -type interlamellar complexes formed by hydroxy-salts of zinc<sup>6</sup> with straight chain amines. The substances used were hydroxysulphate (HS) 4/1, HS 3/1, hydroxychloride (HC) 6/1, HC 4/1, and hydroxychlorosulphate (HCS) 5/1/2.

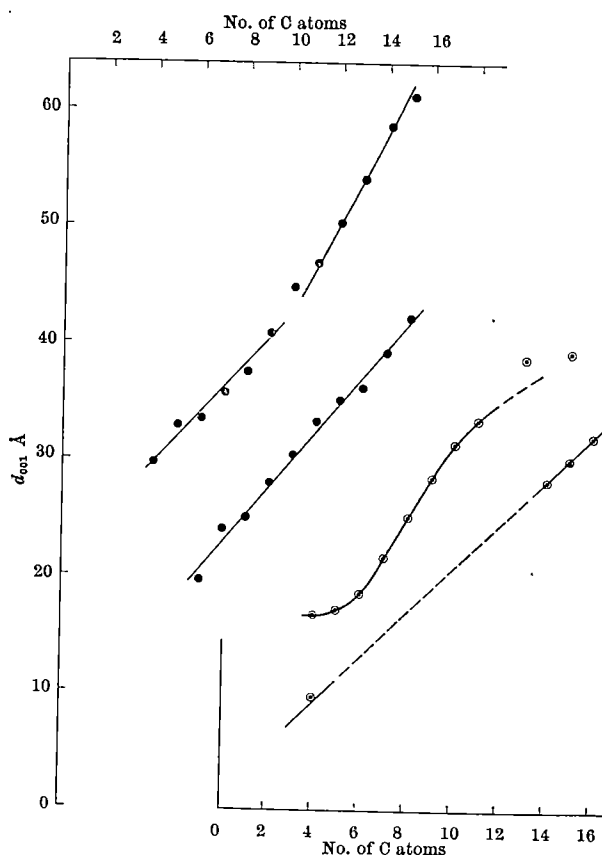


Fig. 1. Spacing variations for HS 4/1 (on left, top scale of C numbers) and HC 6/1 (on right, bottom scale). In both cases, substance dried at room temperature. ●, HS 4/1; ○, HC 6/1.

The results are influenced by the method of drying the hydroxy-salt, and we report results for the substances dried at 60° C and at room temperature. As usual, the results are shown in the form of graphs giving spacing as a function of number of C atoms in the chain.

Taking first the results for the substance dried at room temperature, the first four substrates show very similar tendencies, for 4–8 C atoms. The HCS gives a smaller slope, due perhaps to the fact that this substance is more complex<sup>7</sup>. The similarity of the slopes is attributed to the fact that the shorter-chain amines are highly polar, and their sorption energy more than compensates for the inter-layer interaction energy; thus small differences in the lattice energies of the substrates are not shown up. Examples of curves are shown in Fig. 1.

The second part of the graphs, for molecules having more than 8 C atoms, shows more marked differences. The basal spacing of the most basic substrates, HS 4/1 and HC 6/1, has a steeper slope than that given by the less basic substances, HS 3/1 and HC 4/1.

This may be due to the fact that the latter two compounds have a more ordered structure than the first two, and so a greater inter-layer energy (that is, a lower energy state). Further, the polarity of the longer-chain amines is less, the sorption is thus less energetic, and differences appear between the substrates.

With the hydroxysulphates, two types of spacing appear, possibly corresponding to different inclinations of the molecules. This phenomenon has not been observed with the hydroxychlorides, except for isolated cases. The HCS gives irregular results, with three spacings in some cases.

With the salts dried at 60° C (Fig. 2), all the graphs follow the same trend up to 8 C atoms. Beyond this point, a separation occurs. The HS 4/1 gives a completely distinct graph. In all cases, the slope of the line is less the more crystalline the compound is, and the less basic.

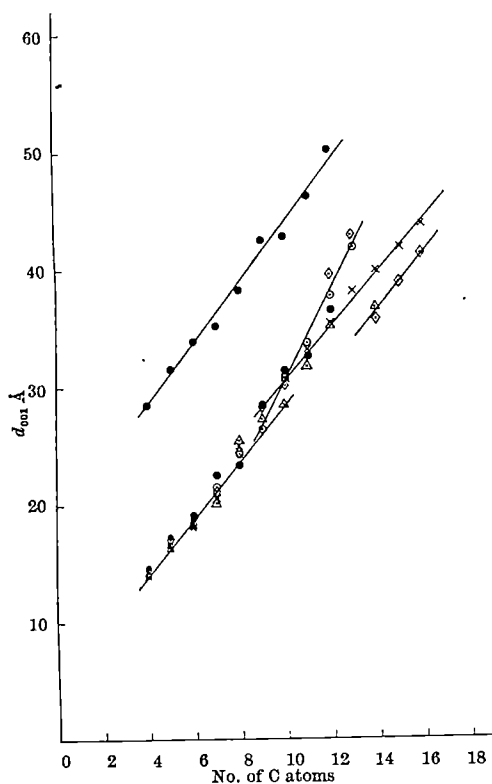


Fig. 2. Spacing variations for substances dried at 60° C. ●, HS 4/1; ×, HS 3/1; ○, HC 6/1; △, HC 4/1; ◇, HCS 5/1/2

From the slope of the graphs, the orientation and quantity of molecular layers can be deduced. In general, the slope is of the order of 2.3–2.9 Å/C atom, indicating two erect layers of molecules; but for certain substances, HC 6/1, HS 4/1 (at room temperature) and HCS (at 60° C), a slope of 3.5–3.9 is found, possibly due to the existence of three erect layers.

With alcohols, the hydroxy-salts form  $\beta$ -complexes with difficulty, and only in isolated cases. Aromatic substances (anthranilic acid, hippuric acid, naphthol yellow and others) give large-spacing  $\beta$ -complexes.

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## RADIATION CHEMISTRY

### Short-lived Radicals in Irradiated Glycine

SEVERAL investigators have used the electron spin resonance technique to measure the numbers of stable free radicals induced by ionizing radiation in amino-acids<sup>1–4</sup>. The observation that at doses in the M.rad region the radical yield does not increase linearly with the absorbed dose but tends towards saturation was explained by Rotblat and Simmons<sup>4</sup> as being due to a

back reaction between stable and unstable radicals, but as their nature was still in some dispute at that time no details of the mechanism were attempted. The availability of new evidence concerning the radical species has now prompted further investigation of this problem.

If the electron spin resonance measurements can be made only after the irradiation has been completed, it is not possible to observe radicals with life-times less than about 10 sec at room temperature. In some cases, however, it may be possible to stabilize such radicals by irradiating at low temperatures, and this has been done by Henriksen for frozen aqueous solutions of glycine<sup>5</sup>. He was thus able to show that the water component gave rise to OH radicals plus an unidentified species, while the glycine gave rise to  $^+NH_3CHOO^-$  and  $-CH_2COO^-$ . Very recently the use of carbon-13 has enabled this proposal to be confirmed for pure glycine<sup>6</sup>. It seems likely that these were the radicals detected by ultra-violet absorption during the pulse radiolysis of glycine solutions at room temperature<sup>7</sup>.

Since these organic radicals retain their specific orientation within the molecule after their formation<sup>8</sup>, it seems unlikely that they can move at all in the dry powder. Hence the reactive transients must be the unobserved fragments, namely,  $NH_3^+$  (complementary to  $-CH_2COO^-$ ) and  $\dot{H}$  (complementary to  $^+NH_3CHOO^-$ ). Ammonia formed from the neutralization of  $NH_3^+$  was not detected at room temperature, but it was observed when the powder was heated to 120° C (ref. 9). This effectively eliminates  $NH_3^+$  as the attacking radical as it could not be sufficiently mobile at room temperature. It therefore seems most probable that hydrogen atoms are the reactive species in the solid material. If this is true, then not only can each  $\dot{H}$  attack and neutralize one of the observable radicals, but it can also react with another  $\dot{H}$  to form molecular hydrogen which should then be detectable after irradiation of glycine. Accordingly an experiment was devised to test this hypothesis.

A sample consisting of 1 g of dry 'AnalaR' grade glycine was evacuated to  $5 \times 10^{-5}$  torr in a glass tube and sealed. It was then irradiated with 50 M.rads using the 15-MeV electron linear accelerator at St. Bartholomew's Hospital, London. Afterwards the sample tube was connected to a mass spectrometer (Associated Electrical Industries Type MS 10) tuned to mass number 2, and the vacuum seal opened. A large positive reading was obtained, from which it was concluded that about  $3 \times 10^{-7}$  g, corresponding to about  $10^{17}$  molecules, had been present. A check with unirradiated glycine was completely negative, while a check on an empty glass sample tube given 50 M.rads showed a signal the amplitude of which was just a few per cent of that from the glycine. It was thus confirmed that hydrogen is a product of irradiated glycine, and accordingly it is concluded that atomic hydrogen is the reactive, short-lived radical previously postulated<sup>4</sup> to account for the shape of the yield-dose curves for stable radicals.

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## BIOPHYSICS

Inhibition of Cell Division in *Escherichia coli* by Electrolysis Products from a Platinum Electrode

In an investigation of the possible effects of an electric field on growth processes in bacteria, we have discovered a new and interesting effect. In *E. coli*, the presence of certain group VIIIb transition metal compounds in concentrations of about 1–10 parts per million of the metal in the culture medium causes an inhibition of the cell division process. The bacteria form long filaments, up to 300 times the normal length, which implies that the growth process is not markedly affected.

Experiments were performed in a continuous culture apparatus in a chamber of special design. The nutrient medium was the chemically defined 'C' medium of Roberts *et al.*<sup>1</sup> used with 1 g of glucose/l. and 0.026 g/l.  $MgCl_2$ . The chamber was maintained at  $37^\circ \pm 0.1^\circ C$ . *E. coli* B and K-12 were used to inoculate the chamber. Compressed air was bubbled through a glass frit bubbler. Two half-cylindrical platinum mesh electrodes were built into the chamber. Once the bacterial population reached a steady state (about 24 h) as determined by turbidity measurements, it maintained its constant value ( $\pm 2$  per cent transmission for a 5-fold dilution of the effluent) for a period of 10–14 days. All experiments were performed within 10 days after inoculation.

The resistance of the chamber was approximately 6 ohms. An audio oscillator was the source of sinusoidal voltages of 50–10<sup>5</sup> c/s. This was fed into a conventional audio power amplifier and then to the platinum electrodes. The voltage and current were monitored with a dual beam oscilloscope.

On turning the electric field on at 1,000 c/s, 2 amp (peak to peak) through the chamber, the turbidity began to decrease after 1 h. (Platinum was chosen for the electrode material because of its well-known chemical inertness, and 1,000 c/s was chosen to eliminate electrolysis effects and electrode polarization. As we will show, both are mistaken ideas which led, via serendipity, to the effects described in this communication.) After 2 h, 'washout' of the bacteria was imminent and the voltage was turned off. After 8 h the population density had returned to its previous value. This process could be repeated. Microscopic examination of the effluent from the chamber showed that the *E. coli* ceased dividing (disappearance of 'waists') within 1–2 h after the current was turned on, and began to elongate. Within a few hours, all bacteria were in the form of long filaments, the length of which increased rapidly with time. For 1–2 h after the removal of the voltage, the cells continued to increase in length. Thereafter, however, the fairly uniform appearance of the bacteria under the phase contrast microscope changed. The darker material segregated into normal sized segments over the full length of the filament, leaving small, light grey gaps between segments. 'Waists' began to form in the gaps, and cell division occurred shortly thereafter over the length of the filament.

A frequency of the applied voltage of 500 c/s is very effective in causing filamentous growth. The efficiency decreases as the frequency increases, until at 6,000 c/s no filaments can be seen and no change in turbidity occurs over 24 h of application. From 6,000 c/s to 10<sup>5</sup> c/s no effects can be detected within 24-h trials. Increasing the current at a given frequency increases the size of the filaments appearing within a given time and causes a faster decline of the turbidity. We have found that oxygen is required to cause the effect; with nitrogen or helium bubbled through the cell, no effect of the electric field can be detected at all.

A number of physical and chemical agents are known to cause filamentous growth, that is, inhibit cell division but not growth. Among these are dyes such as methylene blue<sup>2</sup> and penicillin<sup>3</sup>, near ultra-violet irradiation<sup>4</sup>, transfer to an unaccustomed medium, osmotic pressure changes, temperature changes<sup>5</sup>, and magnesium deficiency or excess<sup>6</sup>.

We have, with a variety of tests, eliminated the possibilities that ultra-violet light, temperature, pH, and magnesium concentration are involved in this effect of the electric field. We have also found that adaptive mechanisms and mutation effects are not involved.

It is possible that a chemical change is produced in the medium by electrolysis and that these new chemical products are the causative agents. To test this the nutrient was pumped into one chamber with electrodes in it (electrolysis chamber) before pumping it into a second chamber without electrodes which had been inoculated (bacterial chamber). The electric current was passed through the electrolysis chamber. No electric current was present in the bacterial chamber. If a new chemical species is created in the electrolysis chamber and it has a sufficiently long life it will appear in the bacterial chamber. The test conclusively showed that one or more long-lived new chemical species was created by the electric current and is responsible for the bacterial elongation. It caused elongation if oxygen was present in the electrolysis chamber regardless of what gas was bubbled through the bacterial chamber. Helium bubbled through the electrolysis chamber produced no elongation in the bacterial chamber. The active agent was not an insoluble gaseous product, since transferring the gas bubbling through the electrolysis chamber into the bacterial chamber caused no effect, if the medium was not also transferred.

A suspicion that an oxidizing agent was being generated by electrolysis was investigated by using a potassium iodide–starch test solution. Ordinary medium gave no reaction. The electrolysed medium gave a definite positive test—the blue colour developing after about 5 min and going through yellow and orange intermediate states. The time course of the development of the concentration of this new agent in the electrolysed medium was strikingly parallel to the time course of the elongation processes. The concentration was proportional to the electric current. It decreased as the frequency increased from 500 to 6,000 c/s, at which frequency it was not detectable. All these measurements strongly implicate this new chemical as the causative agent.

Given the chemically defined 'C' medium, a number of oxidizing agents are conceivably created during electrolysis. The following ions or molecules were looked for with appropriate sensitive qualitative tests:  $ClO^-$ ,  $ClO_2^-$ ,  $ClO_3^-$ ,  $ClO_4^-$ ,  $H_2O_2$ ,  $NH_2OH$  and  $S_2O_8^{2-}$ . None of these was detected. Some oxidizing agents were tested by inoculation directly into the bacterial continuous culture chamber. These were:  $H_2O_2$ ,  $NH_2OH$ ,  $ClO^-$ ,  $N_2O$  (gas),  $NO$  (gas) and  $NO_2^-$ —all in appropriate quantities below that at which killing occurred. None of these chemicals caused elongation.

Each component of the 'C' medium was then made up in an appropriate concentration and electrolysed for 6 amp-h. The resultant solution was tested with the potassium iodide–starch solution. Negative results were obtained with the following:  $PO_4^{3-}$ ;  $SO_4^{2-}$ ;  $PO_4^{3-} + \text{glucose}$ ;  $PO_4^{3-} + SO_4^{2-} + \text{glucose}$ ;  $Na_2SO_4$ ;  $Na_2CO_3$ . Positive tests were obtained with the following:  $(NH_4)_2SO_4$ ;  $(NH_4)_2CO_3$ ;  $NH_4Cl$  and chlorides. A faint positive response was obtained with  $NaCl$ . Only solutions with chlorides showed the intermediate yellow to orange to blue transitions. It is known that platinum electrodes can be attacked by an acidified chloride solution during electrolysis (to form  $[PtCl_6]^{2-}$ ) (ref. 7). We therefore suspected that a soluble platinum salt was the active agent. A solution of  $(NH_4)_2PtCl_6$ , when tested with the potassium iodide–

starch test, produced an exact duplication of the results with the electrolysed medium, if the platinum was present in a concentration of greater than 100 p.p.m. Inoculating the bacterial continuous culture chamber with a solution of  $(\text{NH}_4)_2\text{PtCl}_6$  so as to maintain a concentration in the chamber of Pt (IV) 10 p.p.m. over 2 h caused filaments to appear. This verified that the platinum salt was indeed an active agent. Quantitative tests for platinum IV in the electrolysed medium ( $\text{SnCl}_2$  test) showed that under our standard test condition (1,000 c/s, 3 amp) the steady-state concentration of the metal was 8 p.p.m. and was reached in about 2 h.

We have now tested a number of platinum and other group VIIIb compounds to determine the most effective anions and cations for this effect. The chemicals were inoculated so as to maintain a concentration of 8 p.p.m. of the metal in the nutrient medium of the cell for a period of 2 h. The effects fell into three categories: the bacteria were killed; there was no apparent change; a minimum of 20 per cent of the bacteria were forced into a filament form. These results are given in Table 1. The generalizations to be derived from these results are minimal. Platinum is not the only metal capable of inhibiting cell division, and in fact we have found that rhodium is as effective as platinum. We cannot at this time specify the relative effectiveness of the various metallic oxidation states or the effects due to various ligands and their spatial arrangements about the metal.

Table 1. EFFECTS OF GROUP VIIIb TRANSITION METAL COMPOUNDS ON BACTERIAL GROWTH (CONCENTRATIONS OF METAL IONS MAINTAINED FOR 2 H AT 8 P.P.M. IN THE CONTINUOUS CULTURE CHAMBER)

A. Caused bacterial death	B. Caused no change	C. Caused elongation
$\text{CoCl}_2$	$[\text{Co}(\text{NH}_3)_6]\text{Cl}_2$	$\text{K}^+, \text{NH}_4^+, \text{H}^+ - [\text{PtCl}_6]^-$
$(\text{NH}_4)_2\text{IrCl}_6$	$\text{K}_2\text{Ir}(\text{NO}_2)_6$	$(\text{NH}_4)_2\text{PtBr}_6$
$\text{NiCl}_2$	$[\text{Ni}(\text{NH}_3)_6]\text{Cl}_2$	$(\text{NH}_4)_2\text{PtI}_6$
$(\text{NH}_4)_2\text{OsCl}_6$		$[\text{Pt}(\text{en})_3]\text{Cl}_4$
$(\text{NH}_4)_2\text{PdCl}_6$	<i>Cis</i> and <i>trans</i> $[\text{Rh}(\text{en})_3]\text{Cl}_2\text{NO}_2$	$\text{RhCl}_3$
$[\text{Rh}(\text{NH}_3)_6]\text{Cl}_3$		$(\text{NH}_4)_2\text{RhCl}_6$
$\text{PdCl}_2$		$[\text{Ru}(\text{NH}_3)_6]\text{ClO}_4\text{Cl}$

We believe the evidence is fairly conclusive that various group VIIIb transition metal ions in concentrations of 1–10 p.p.m. can inhibit cell division in *E. coli* while not apparently interfering with growth. Many questions arise from this work that we are now attempting to answer, such as: What is the mechanism of action of these metal ions? Where is the locus of action in the bacterial cell? How does the effect of these metal ions relate to the actions of the other causative agents of filamentous growth—is there a weak link that all operate on? Can these metal ions inhibit cell division in other bacteria, or cells?

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## BIOCHEMISTRY

### Ethylene Production and Lipid Mobilization during Germination of Castor Beans

RECENT investigations<sup>1</sup> have shown that ethylene is evolved during the germination of oat seeds and *Penicillium digitatum* spores. This article reports investigations conducted with a germinating fatty seed, the castor bean (*Ricinus zanzibarensis*). Ethylene production and lipid levels were determined at various stages, and these values will be discussed with relation to the activity of the glyoxylate pathway enzymes<sup>2</sup> and the changes in mitochondria<sup>3</sup> during the germination of the seeds.

When castor beans (20 per run) were germinated under aseptic conditions at room temperature in the light, analysis for ethylene<sup>4</sup> showed that they were active producers of this gas, and that they exhibited several peaks in the rate of its evolution (Table 1). To minimize the uneven development, three days after wetting the seeds twenty of those showing signs of germination were selected for measurement of subsequent ethylene evolution. To determine the ethylene production during the first few days after wetting, separate gas collections were made from flasks containing twenty seeds, whether or not the seeds germinated. The first peak in ethylene production occurred on the third day after wetting of the seeds, and coincided with the cracking of the seed coat. This evolution may indicate the presence in the seed of preformed ethylene, the gas being forced out on hydration of the seed, or it may reflect an actual formation of the gas on activation of enzymatic or chemical systems during germination. The second peak, at nine to ten days, was about twice as great as the first, and coincided with the rapid elongation of the seedling after its emergence from the seed coat. The third peak was on the fourteenth and fifteenth days, at full separation of the leaves from the cotyledons and, like that of the tenth day, was at a period of very rapid growth. (The maximum value was 2.9 ml./h/seedling, compared with that from oats, about 0.2 ml./h/seedling.) Thereafter, rates of production of both ethylene and carbon dioxide decreased. A close correlation between the changes in rates of production of the two gases existed through the germination period.

Table 1. LIPID CONTENT AND PEAKS IN ETHYLENE PRODUCTION OF GERMINATING CASTOR BEANS

Days*	$\text{C}_2\text{H}_4$ (ml./24 h/bean)	Per cent ether soluble (dry weight basis)
3	22, 24	36.5
9–10	35, 39	12.5
14–15	68, 73	8.4

\* After wetting of seeds.

Results for ethylene are given for 2 separate runs.

A rapid breakdown of ether-soluble material began between the third and fourth days, and by the tenth day the lipids had reached a low level (Table 1), as observed by a number of other workers. Carpenter and Beevers<sup>5</sup> have reported that isocitritase, one of the key enzymes of the glyoxylate cycle, shows maximum activity in germinating castor beans at the time of most rapid fat breakdown (the sixth day after wetting), and that it then declines in activity as the lipid degradation decreases. Since we found ethylene production to be at a maximum during this later stage, it would appear that the functioning of the glyoxylate pathway does not enhance ethylene evolution. That operation of the complete pathway is not essential to the production of ethylene is evident from the fact that the gas is produced by animal tissues<sup>6,7</sup>, which apparently do not possess all the glyoxylate cycle enzymes.

In our earlier<sup>8</sup> work with sub-cellular particles from tomatoes we showed that the ethylene-producing system was activated by disintegration of the particles, and pointed out the possible relationship of this to the reported<sup>9</sup> disintegration of mitochondria as fruits ripen. Akazawa and Beevers<sup>3</sup> found that mitochondria from the endo-

sperms of castor beans, during the first five days of germination, increased in amount and in  $q_{01}$  values and had P/O ratios greater than 2; three days later all these values were markedly lower. In 9–10 days the endosperm tissue had completed a cycle of activation, growth and ageing, and it is possible that destruction of mitochondria with ageing is reflected in the high ethylene values.

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### Inhibition of Intestinal Amino-acid Transport by Sugars

GALACTOSAEMIA, an inborn defect of galactose metabolism, is known to be associated with derangements in the renal tubular transport of amino-acids<sup>1</sup>. It is of interest that some of the features of this disease, including amino-aciduria, can be reproduced in rats by feeding them a diet containing 30 per cent galactose<sup>2</sup>. Recently, Segal *et al.*<sup>3</sup> have shown that the uptake of certain amino-acids by rat kidney slices can be inhibited *in vitro* by galactose and fructose at relatively low concentrations (5–6 m molar), while at a higher concentration (16–8 m molar) glucose also exerts some inhibitory effect. Since in the mammalian organism many of the transport processes of the kidney and the small intestine appear to be similar we have examined the effect of sugars, especially galactose, on the transport of amino-acids by the small intestine of the rat.

Male Sprague-Dawley rats weighing 120–150 g were fasted overnight. Six everted segments from the distal jejunum and proximal ileum, each measuring 3–5 mm, were incubated under 95 per cent oxygen and 5 per cent carbon dioxide in 5 ml. of Krebs-Ringer bicarbonate buffer (pH 7.4). Each amino-acid was examined at a concentration of 5 mmoles/l. The specific activity was 0.02  $\mu\text{C}/\mu\text{mole}$  except in the case of hydroxyproline where it was 0.1  $\mu\text{C}/\mu\text{mole}$ . All amino-acids were in the L form (except glycine) and were uniformly labelled with carbon-14 except for hydroxyproline which was labelled with tritium at the 5 position. The sugars when added

to the medium were present at a final concentration of 28 mM. After incubation for 60 min, the segments were removed, blotted, and boiled for 5 min in 2 ml. of water. The transfer of amino-acids was expressed as the distribution ratio of amino-acid in intracellular fluid to that in the medium as described by Rosenberg *et al.*<sup>4</sup>. Extracellular fluid volume was measured with <sup>14</sup>C-inulin and total water content of the segments by drying to constant weight at 110° C.

The results are shown in Table 1. Glucose, xylose, and ribose caused no inhibition of amino-acid transport while galactose caused a significant depression in the transport of alanine ( $P < 0.001$ ), valine ( $P < 0.001$ ), glycine ( $P < 0.001$ ) and hydroxyproline ( $P < 0.02$ ). Lysine transport was inhibited at a  $P$  value of  $< 0.5$  and while not statistically significant it should be noted that the transport of lysine in the absence of hexose was low and thus any depression of its transport would be difficult to demonstrate. It will be seen that fructose at the same concentration also inhibited alanine ( $P < 0.001$ ) and hydroxyproline ( $P < 0.001$ ) uptake but the transport of other amino-acids was not inhibited.

We also examined the effects of feeding Sprague-Dawley rats a diet containing 30 per cent galactose for 2–3 months. All the galactose-fed rats developed cataracts. The litter-mate controls were fed the same food but without added galactose. Intestinal slice experiments were carried out as above and distribution ratios calculated. No sugars were added to the incubation medium. In the galactose-fed rats there was a significant depression in the intestinal transport of all the amino-acids tested (Table 2).

The *in vitro* inhibition of amino-acid transport by galactose, and sometimes by fructose, in the gut of the rat cannot be explained by a water shift. There was no significant difference between the <sup>14</sup>C-inulin space in the segments incubated without added sugar and in those incubated in the presence of any of the sugars tested. Recently, Newey and Smyth<sup>5</sup> reported that galactose inhibited and glucose enhanced the transfer of glycine and methionine in everted rat gut sacs. In contrast to these authors we did not observe any enhanced transfer of amino-acids in the presence of glucose, and, in addition, we found some inhibition with fructose. Our results with intestinal slices are therefore more in keeping with those reported by Segal *et al.* with kidney cortex slices<sup>3</sup>. Like Newey and Smyth<sup>5</sup> we did find that when galactose transport was inhibited by phlorrhizin, the observed influence of galactose on amino-acid transport was prevented.

The results in rats fed 30 per cent galactose show that these animals develop a defect in amino-acid transport in the intestine as well as in the kidney. This *in vivo* defect involved amino-acids of three different transport

Table 1. THE EFFECT OF SUGARS ON THE *in vitro* TRANSPORT OF AMINO-ACIDS BY RAT INTESTINAL SLICES

Addition to medium*	Distribution ratios†				
	L-Alanine	L-Valine	Glycine	L-Lysine	Hydroxy-L-proline
None	4.9 ± 1.0 (16)	4.1 ± 0.3 (11)	2.0 ± 0.3 (8)	1.3 ± 0.2 (6)	2.0 ± 0.3 (8)
D-Glucose	4.1 ± 0.5 (6)	3.8 ± 0.4 (6)	2.0 ± 0.3 (6)	1.2 ± 0.3 (6)	2.0 ± 0.2 (4)
D-Galactose	2.7 ± 0.7 (8)	2.8 ± 0.8 (9)	1.3 ± 0.3 (9)	0.9 ± 0.09 (6)	1.5 ± 0.4 (8)
D-Fructose	2.9 ± 0.7 (11)	4.1 ± 1.0 (9)	2.6 ± 0.4 (4)	1.2 ± 0.2 (7)	1.5 ± 0.2 (4)
D-Xylose	5.4 ± 0.4 (4)	3.8 ± 0.4 (5)	2.0 ± 0.04 (2)	1.2 ± 0.07 (3)	2.8 ± 0.02 (3)
D-Ribose	5.0 ± 0.7 (3)	3.7 ± 0.3 (6)	1.9 ± 0.3 (5)	1.1 ± 0.07 (3)	3.0 ± 0.2 (3)

\* Sugars were present at a final concentration of 28 m molar. † Ratios shown are means ± S.D. with the number of determinations in parentheses.

Table 2. AMINO-ACID TRANSPORT BY THE JEJUNAL SLICES FROM RATS FED 30 PER CENT D-GALACTOSE FOR 2–3 MONTHS

Intestinal slices	Distribution ratios*				
	L-Alanine	L-Valine	Glycine	L-Lysine	Hydroxy-L-proline
Control	4.0 ± 0.6 (6)	4.2 ± 0.9 (6)	2.3 ± 0.5 (6)	1.6 ± 0.4 (6)	1.9 ± 0.3 (6)
Galactose-fed	2.8 ± 0.8 (6)	2.3 ± 0.3 (6)	1.5 ± 0.7 (6)	1.3 ± 0.3 (6)	1.3 ± 0.5 (6)
$P$	< 0.01	< 0.01	< 0.05	< 0.05	< 0.05

\* Ratios shown are means ± S.D. with the number of determinations in parentheses.



'groups' and is also observed *in vitro* when galactose is added to intestinal slices. In view of the fact that glucose and galactose are mutually inhibitory with regard to their transport across the intestine, competitive inhibition of amino-acid transport by galactose seems unlikely as one would then expect glucose to inhibit as well. One could argue that either the transport of the hexoses or their phosphorylation in the intestinal cell might result in a drop in the mucosal levels of adenosine triphosphate (ATP). We measured ATP levels in intestinal slices of rats fed 30 per cent galactose and in controls. The mean ATP level in the galactose-fed rats was 0.492  $\mu$ moles/g and in the controls 0.637  $\mu$ moles/g ( $P < 0.05$ ). While these results are significant and of interest they do not allow us to conclude that the decrease in ATP levels is primarily involved in the impairment of amino-acid transport. Since phosphorylation of galactose and fructose occurs in the intestine and since phosphorylated sugars may inhibit enzymes such as phosphoglucomutase<sup>1</sup>, the accumulation of hexose phosphates may also be a factor in the derangement of amino-acid transport.

The precise mechanism for the inhibiting effect of galactose on amino-acid transport remains to be determined. However, on the basis of the investigations presented, it would appear likely that in the human disorder of galactosaemia there may be a defect in the transport of amino-acids not only in the kidney but also in the intestine.

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### Inhibitory Effect of Progesterone on the Activity of Human Myometrial Actomyosin Adenosinetriphosphatase

In the previous communication<sup>1</sup> from this laboratory it was noted that progesterone in concentrations of more than 10  $\mu$ g (and up to 50  $\mu$ g tested) per mg wet weight human non-pregnant myometrial tissue inhibits the human myometrial ATPase activity on an average of 27.3 per cent ( $P < 0.001$ ) based on fifteen experiments. On the other hand, oestradiol in fourteen cases in similar concentrations, as for progesterone showed only an average reduction of 5.8 per cent ( $P < 0.01$ ) on ATPase activity<sup>2</sup>.

In order to make sure that this inhibitory effect of progesterone is not entirely on the non-actomyosin fraction of the homogenate, actomyosin was extracted using 0.5 M potassium chloride and ATP according to the procedure described by Csapo<sup>3</sup>. The ATPase activity of the human myometrial actomyosin was determined according to the procedure described before<sup>1</sup>. The amount of inorganic phosphorus liberated/mg nitrogen actomyosin/min at 37°C was taken as a measure of ATPase activity. The results of eleven non-pregnant and three pregnant human samples are shown in Table 1.

Table 1

Number of cases	Average $\mu$ moles of phosphorus liberated/mg N <sub>2</sub> actomyosin/min incubation		Average reduction in phosphorus liberated ( $\mu$ moles)
	Control	Progesterone 7.46/ mg N <sub>2</sub> actomyosin	
11 non-pregnant	0.092	0.074	0.018 (19.6%)*
3 pregnant	0.047	0.034	0.013 (27.6%)
* $t = 5.13$			
$P < 0.001$			

In conclusion it can be said, based on the results described, that progesterone has a definite inhibitory effect on the human myometrial actomyosin ATPase activity.

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### Binding of Thyroxine and Triiodothyronine by Heart and Pituitary Proteins

THE presence of thyroxine-binding activity in extracts of muscle tissues<sup>1</sup> and in extracts of mouse thyrotropic and mammotrophic pituitary tumours<sup>2</sup> has been demonstrated by observing the migration of radioactive thyroxine with the proteins in question during electrophoresis on paper at pH 8.6. The work recorded here on the relative affinity of water-soluble human heart and pituitary proteins for thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) made use of an independent binding assay.

The tissues were selected at autopsy, which was performed no later than 2 h after death. Every effort was made to obtain heart muscle and pituitary tissue that seemed to be unaffected by disease. (Death in all cases was associated with cancer.) The tissues were chilled immediately after collection, and were kept at 4°C throughout preparation. Pooled samples were used in all experiments.

Protein fractions were prepared by homogenizing small pieces of water-washed tissues from which visible fat and fibre had previously been removed. About 5 ml. of water was used per g of tissue homogenized. Insoluble material was separated by centrifugation and discarded.

In order to remove any T<sub>4</sub> or T<sub>3</sub> bound to the tissue proteins, each supernatant of about 50 ml. was dialysed at 4°C in a cellulose casing against two 6-l. changes of distilled water, one 6-l. change of 0.05 M acetic acid (pH 3.0), and another two 6-l. changes of distilled water. Acetic acid was used in the dialysis procedure in order to enhance the separation of T<sub>4</sub> and T<sub>3</sub> from the proteins.

Some material precipitated during dialysis and this material was removed by centrifugation, leaving the water-soluble proteins in the supernatant. The latter was dried by lyophilization. Approximately 1 g of protein was recovered from each 100 g of heart muscle, and up to 10 mg from each pituitary gland.

Before any binding investigation was made, each tissue preparation was tested for the presence of iodine by use of the ceric sulphate-arsenious acid reagent<sup>3</sup>, and was found to be negative. The tissue preparations were checked for contamination with serum proteins by electrophoresis on paper at pH 8.6 in the presence of radioactive T<sub>4</sub>. No radioactivity was found in the inter- $\alpha$  or albumin zone, and this result was interpreted as proof of the absence of serum proteins.

$T_4$  and  $T_3$  binding by human heart muscle, human pituitary tissue, and human serum albumin was measured by peroxidase assay<sup>4</sup>.

Several differences in the binding of thyroid hormones by heart muscle and pituitary tissue were shown. Like human serum albumin (Table 1), a given weight of heart protein (Table 2) had a higher affinity for  $T_4$  than for  $T_3$ . On the other hand, pituitary protein (Table 3) had a higher affinity for  $T_3$  than for  $T_4$ . Since tissue protein preparations were heterogeneous, no comparison of per cent bound hormone per mg of tissue is valid. It is evident, however, that pituitary protein binds no more than 70 per cent of either hormone. Muscle and human serum albumin are similar as to extent of binding of either hormone for higher protein levels.

Table 1. THYROXINE ( $T_4$ ) AND TRIIODOTHYRONINE ( $T_3$ ) BINDING BY HUMAN SERUM ALBUMIN (H.S.A.)

H.S.A. (mg)	Free*		Bound*		% Bound	
	$T_4$	$T_3$	$T_4$	$T_3$	$T_4$	$T_3$
0.05	0.0340	0.0500	0.0160	0.000	32	0
0.2	0.0300	0.0460	0.0200	0.004	40	8
0.5	0.0270	0.0280	0.0230	0.022	46	44
1.0	0.0155	0.0250	0.0305	0.025	61	50
2.0	0.0100	0.0210	0.0400	0.029	80	58
3.0	0.0065	0.0180	0.0435	0.032	87	64
4.0	0.0041	—	0.0459	—	92	84
5.0	0.0015	0.0080	0.0485	0.042	97	80
6.0	—	0.0010	—	0.049	—	—

\* Micromoles.

Table 2. THYROXINE ( $T_4$ ) AND TRIIODOTHYRONINE ( $T_3$ ) BINDING BY THE HUMAN HEART MUSCLE (H.H.M.)

H.H.M. (mg)†	Free*		Bound*		% Bound	
	$T_4$	$T_3$	$T_4$	$T_3$	$T_4$	$T_3$
0.5	All	0.045	None	0.005	0	10
0.75	All	0.041	None	0.000	0	18
1.0	0.047	0.046	0.003	0.004	6	8
1.25	0.035	0.040	0.015	0.010	30	20
1.5	0.0245	0.0365	0.0255	0.0135	51	27
1.75	0.0230	0.033	0.0270	0.017	54	34
2.0	0.0110	0.03	0.0390	0.02	98	40
2.5	0.0020	0.027	0.0480	0.023	96	46

\* Micromoles.

† mg: mg of lyophilized powder and not protein nitrogen.

Table 3. THYROXINE ( $T_4$ ) AND TRIIODOTHYRONINE ( $T_3$ ) BINDING BY THE HUMAN PITUITARY

Pituitary (mg)†	Free*		Bound*		% Bound	
	$T_4$	$T_3$	$T_4$	$T_3$	$T_4$	$T_3$
0.2	0.0490	0.0450	0.0010	0.0050	2	10
0.5	0.0490	0.0370	0.0010	0.0130	2	26
1.0	0.0385	0.0330	0.0115	0.0170	23	34
2.0	0.0210	0.0190	0.0290	0.0310	58	62
3.0	0.0170	0.0130	0.0330	0.0370	66	74

\* Micromoles.

† Lyophilized powder and not protein nitrogen.

Human serum albumin is a relatively pure protein, and its association constant and number of binding sites for the thyroid hormone have been evaluated by several investigators. Such information on heart and pituitary protein must await the purification of the binding protein. From the tables it is probably safe to conclude that these tissues differ in their relative affinities for  $T_4$  and  $T_3$  in the present *in vitro* assay system. It cannot yet be stated whether these observations have genuine physiological significance, or merely represent non-specific binding properties of the proteins under investigation.

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## Biological Activity of Degradation Products of Indolepyruvic Acid

Stowe and Thimann<sup>1</sup> reported that they had isolated indolepyruvic acid from maize endosperm by paper chromatography. They used the methods of ammoniacal chromatography. However, Bently<sup>2</sup> found that under these conditions synthetic indolepyruvic acid breaks down into a number of compounds. Moreover, in a recent paper by Kaper and Veldstra<sup>3</sup>, it was shown that both synthetic and isolated indolepyruvic acid break down when chromatographed in a basic solvent. Srivastava<sup>4</sup> has also obtained results similar to that reported by Kaper and Veldstra.

Kaper and Veldstra identified some of the degradation products using chemical and physical methods. The purpose of the work recorded here was to identify the degradation products by the use of biological methods.

The *Avena* first internode assay of Nitsch and Nitsch<sup>5</sup> and the *Avena* curvature assay of Went<sup>6</sup> were used to test the biological activity of the indolepyruvic acid breakdown products. The two tests afore-mentioned were also used for bioassaying the synthetic compounds indoleacetic acid (IAA), indoleglycollic acid (obtained from Prof. A. W. Galston, Yale University) and indolepyruvic acid.

At the beginning of the investigation two spots were thought to be biologically active. However, later experiments showed that if the chromatogram was allowed to develop for about 12 h, only one spot showed biological activity. The active spot on the indolepyruvic acid chromatogram (solvent, isopropanol : ammonia : water, 10 : 1 : 1) corresponded with spot indoleacetic acid in earlier work<sup>3</sup>. A second spot (identified as spot *D* by Kaper and Veldstra) thought to be active was later found to be inactive, owing its activity to the closeness of indoleacetic acid. Standard curves under similar conditions were run for synthetic indoleglycollic acid, indoleacetic acid and indolepyruvic acid using the *Avena* first internode test. The indoleglycollic acid showed no ability to induce growth, whereas indolepyruvic acid and indoleacetic acid did (Table 1). A chromatogram of indolepyruvic acid, developed in an acid solvent (acetic acid : water), was tested with the *Avena* first internode test ( $R_F$  0.17). The activity of indolepyruvic acid is somewhat less than that of indoleacetic acid as stated previously by Went<sup>6</sup>. The results obtained with the *Avena* curvature test were primarily the same as those observed in the *Avena* first internode test (Table 2). Using the *Avena* curvature test, only one spot on the chromatogram promoted curvature, namely, indoleacetic acid. Synthetic indoleglycollic acid did not induce growth. Thus, if the spot thought (by Kaper and Veldstra) to be indoleglycollic acid is that acid, then indeed it would be inactive.

This evidence, together with the earlier physical investigations with paper chromatography and ultra-violet spectrophotometry<sup>7</sup>, suggests that spot *D* is indoleglycollic acid on the basis of its negative biological activity. Excluding indoleacetic acid, the chromatogram contained

Table 1. BIOASSAY OF SYNTHETIC INDOLEACETIC ACID (IAA), INDOLEGLYCOLLIC ACID (IGA) AND INDOLEPYRUVIC ACID (IPA) WITH THE *Avena* FIRST INTERNODE TEST

Compounds	Conc. (molarity)	Increased growth (mm)
IAA	$5.71 \times 10^{-6}$	3.9
IAA	$5.71 \times 10^{-7}$	3.5
IAA	$5.71 \times 10^{-8}$	2.6
IAA	$5.71 \times 10^{-9}$	1.9
Control	—	1.2
IGA	$5.23 \times 10^{-6}$	1.3
IGA	$5.23 \times 10^{-7}$	1.4
IGA	$5.23 \times 10^{-8}$	1.4
IGA	$5.23 \times 10^{-9}$	1.3
Control	—	1.0
IPA	$5.15 \times 10^{-6}$	2.8
IPA	$5.15 \times 10^{-7}$	2.4
IPA	$5.15 \times 10^{-8}$	1.7
IPA	$5.15 \times 10^{-9}$	1.3
Control	—	1.5

Table 2. A COMPARISON OF INDOLEACETIC ACID (IAA), INDOLEGLYCOLLIC ACID (IGA) AND INDOLEPYRUVIC ACID (IPA) ON GROWTH BY THE USE OF THE *Avena* CURVATURE TEST

Compound	Conc. (molarity)	Degrees curv.
IAA	$5.71 \times 10^{-7}$	23
IAA	$2.85 \times 10^{-7}$	13
IAA	$1.42 \times 10^{-7}$	0
IGA	$5.23 \times 10^{-6}$	0
IGA	$5.23 \times 10^{-7}$	0
IGA	$5.23 \times 10^{-8}$	0
IPA	$5.15 \times 10^{-6}$ *	15
IPA	$5.15 \times 10^{-7}$ †	15
IPA	$5.15 \times 10^{-8}$ †	0
IPA	$5.15 \times 10^{-7}$ †	0
IPA	$5.15 \times 10^{-8}$ *	0

\* Indolepyruvic acid put directly into hot agar.

† Agar blocks soaked in buffer containing indolepyruvic acid.

no other factors which caused growth of *Avena* first internodes or curvature of *Avena* coleoptiles. Therefore, it may be concluded that indoleacetic acid is the only biologically active spot on the indolepyruvic acid chromatogram, developed in a basic solvent, and by analogy spot D is indoleglycollic acid.

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## PHYSIOLOGY

### Effect of Thyroid Deficiency in the Ewe on Lamb Viability

THE importance of normal thyroid function in maintaining pregnancy in women has been known for some time, and thyroid medication in some cases of threatened abortion has proved successful<sup>1,2</sup>.

Investigations of the role of the thyroid gland during pregnancy in domestic animals have given conflicting results, the most recent reporting both healthy and weak lambs from thyroidectomized ewes<sup>3</sup>. The ability of thyroid remnants to regenerate into normal active tissue, and also the possibility of supernumerary thyroid tissue, always casts some doubt on the actual total absence of thyroid tissue in surgically thyroidectomized ewes.

In the present investigation, 12 ewes were subjected to a sham operation in which the thyroid was left in place, and a further 12 ewes were thyroidectomized. After oestrous cycles had been recorded in both groups for several months<sup>4</sup>, the ewes were mated and pregnancy was allowed to proceed to parturition. During pregnancy <sup>125</sup>I-uptake was determined to detect any residual thyroid tissue. A month after parturition the thyroidectomized ewes suffered severely during cold weather and 3 died and were examined *post mortem*. Later in the winter of 1962 (southern hemisphere) a further 4 thyroidectomized ewes died of general debility and infection. The remainder were slaughtered and all were thoroughly examined *post*

*mortem*. Only one sham-operated ewe died during this period.

The results of the *post mortem* investigations of thyroid tissue, and the lambing and lamb viability data are given in Table 1.

It is apparent from these results that thyroid deficiency in the ewe has severely reduced both the pre- and post-natal viability of the lambs, despite the presence of an apparently adequate thyroid in the lamb itself;  $0.59 \pm 0.15$  g as compared with  $0.53 \pm 0.19$  g in lambs from sham-operated ewes.

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### Evidence that Calcium activates the Contraction of Actomyosin by overcoming Substrate Inhibition

It is believed that muscle contracts or relaxes depending on the concentration of calcium in the sarcoplasm: when the concentration rises to some required level the muscle contracts; when it falls below this level the muscle relaxes<sup>1-4</sup>. The sarcoplasmic reticulum, which can take up calcium actively, seems to play a major part in controlling the level of calcium available to the contractile protein<sup>5-7</sup>.

Actomyosin, isolated from muscle as a gel, retains some of its contractile properties. When exposed to ATP and magnesium the translucent gel shrinks and becomes more dense and opaque—it 'superprecipitates'<sup>8</sup>. Superprecipitation, like muscular contraction, seems to need trace amounts of calcium.

However, the need for calcium in superprecipitation is apparent only at high concentrations of ATP. At low concentrations of ATP, some superprecipitation has been observed even in the presence of ethylene glycol *bis* (2-amino-ethylether)-N,N'-tetraacetic acid (EGTA), which chelates most of the calcium<sup>9,10</sup>. On this basis, Weber and Herz expressed the view that calcium is probably not a direct reactant in superprecipitation<sup>9,10</sup>. Our results support this conclusion, and show that calcium activates superprecipitation at high concentrations of Mg-ATP by inactivating an inhibitory reaction between Mg-ATP and the protein.

Superprecipitation has been measured as a decrease in protein volume, determined after centrifugation. This method is slow and is not able to give continuous measurements of a single fast reaction. A better method measures the process as an increase in optical density<sup>11</sup>. The procedure described briefly in the legend of Fig. 1 and in detail elsewhere<sup>12</sup> is rapid and reproducible; it records automatically and continuously the increase in optical density of an actomyosin suspension.

Calcium had two different effects on superprecipitation, depending on the concentration of ATP. At low concentrations of ATP, calcium decreased the extent of superprecipitation. At high concentrations of ATP, calcium

Table 1. LAMBING DATA FROM THYROIDECTOMIZED AND SHAM-OPERATED EWES

	No. of ewes	No. of lambs	Lambs dead at birth	Lambs dying within one week	Lambs surviving	Average weight of lambs (kg)
Thyroidectomized ewes lacking thyroid tissue	9	6	4	2	—	$2.3 \pm 0.2$ *
Thyroidectomized ewes with regenerated thyroid tissue	3	4	2	—	2	$2.3 \pm 0.4$
Sham-operated ewes	12	8	—	1	7	$2.5 \pm 0.3$

\* Average weight does not include the weights of two prematurely aborted lambs.



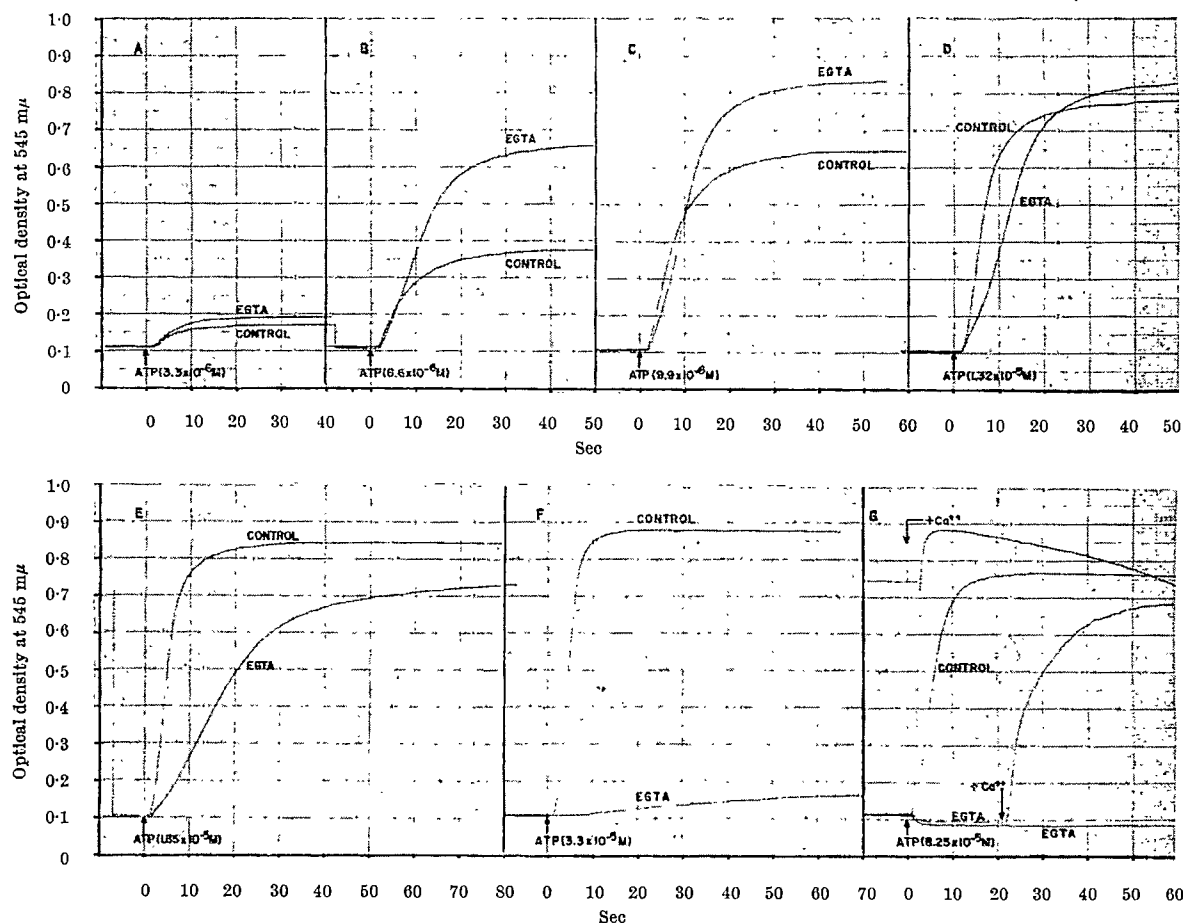


Fig. 1. Effects of ATP concentration on superprecipitation of actomyosin gel with and without EGTA. Twelve ml. of an actomyosin suspension, in the sample cell of a Zeiss spectrophotometer, was stirred continuously by a magnetic bar at the bottom of the cell. Temperature was held constant by circulating water through the cell holder. ATP was added from a microburette through polyethylene tubing. The increase in optical density (2.0 cm light path) that occurred during superprecipitation was recorded continuously on a Photovolt recorder. Conditions: 0.12 mg actomyosin gel per ml.; 0.03 M KCl; 0.005 M MgCl<sub>2</sub>; 0.06 M *tris*, pH 7.4; 25° C. Initial concentrations of ATP are indicated; ATP was hydrolysed while the optical density changed; there was no change without ATP hydrolysis. An optical density of 0.9–1.0 indicates complete superprecipitation. The optical density change stops short of this value when the added ATP is completely hydrolysed. When used, EGTA, 0.002 M in the reaction mixture, was added 30 sec before ATP. CaCl<sub>2</sub>, 0.002 M in the reaction, was added as indicated. Natural actomyosin was extracted from rabbit muscle according to the method of Szent-Györgyi<sup>18</sup>. The gel contained some calcium. All other reagents were treated to remove calcium (for example, with Chelex resin).

increased the extent and rate of superprecipitation. We see these different effects of calcium in the records of Fig. 1. There is normally some calcium in the gel; so the control curves show the response to ATP with calcium; the curves marked EGTA show the response when this calcium is chelated. At low concentrations of ATP, EGTA increased the extent of superprecipitation (Fig. 1, frames A–C). At higher concentrations of ATP the situation was reversed (Fig. 1, frames D–G). Now, with EGTA, the substrate inhibited superprecipitation—the higher the ATP concentration the greater the inhibition. At  $8 \times 10^{-5}$  M ATP (Fig. 1G, bottom curve) there was no superprecipitation at all. As shown on the record, calcium overcame this inhibition immediately and allowed rapid superprecipitation. In the control the calcium was sufficient to allow rapid superprecipitation at high concentrations of ATP; the substrate inhibition that did occur was reversed by adding more calcium (compare the top two curves of Fig. 1G).

The records of Figs. 1 and 2 were obtained at 0.005 M magnesium chloride. When we changed the magnesium concentration we saw two different effects on superprecipitation, again depending on ATP concentration. For example, when the ATP concentration was low, then increasing the level of magnesium increased the rate and extent of superprecipitation. When the ATP concentration was high enough to inhibit superprecipitation (in the absence of calcium) then the addition of more magnesium enhanced this inhibition. Thus, magnesium

appears to act together with ATP in both reactions: that which causes superprecipitation, and that which inhibits superprecipitation in the absence of calcium.

By masking some of the protein SH groups, *p*-chloromercuribenzoate (*p*-CMB) virtually stopped the inhibitory reaction without seriously inhibiting superprecipitation. This selective inactivation of the inhibitory reaction closely resembles the action of calcium; in fact, when the protein was treated with *p*-CMB, calcium was no longer required for superprecipitation at high concentrations of Mg-ATP.

To obtain this effect, we added *p*-CMB (2.0 μmoles per 100 mg of protein) to the reaction mixture 20 sec before addition of ATP. Depending on the concentration of ATP, this treatment had two different effects on superprecipitation with EGTA: (1) high concentrations of ATP, which completely inhibited untreated protein (Fig. 2, curve C), now caused rapid superprecipitation (Fig. 2, curve B)—*p*-CMB blocked the inhibitory reaction; (2) low concentrations of ATP, which did not inhibit untreated protein (Fig. 2, curve D), now caused superprecipitation at a somewhat slower rate (Fig. 2, curve E)—*p*-CMB actually interfered with superprecipitation, but to a relatively small extent.

When the same amount of *p*-CMB was added after ATP it did not substantially reverse substrate inhibition; larger amounts of *p*-CMB, after ATP, did somewhat better, but the effect was limited because these higher levels also seriously inhibit superprecipitation.

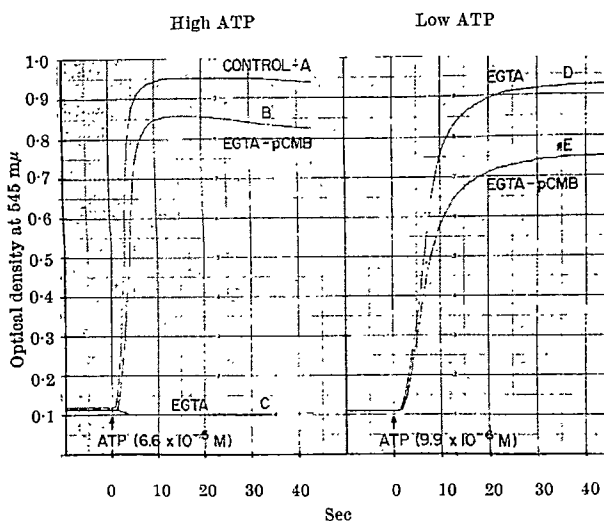


Fig. 2. Effects of *p*-CMB on superprecipitation at high and low concentrations of ATP. The conditions of the reaction and the procedure for measuring optical density are given in the legend of Fig. 1. Curves marked *p*-CMB; 2.0  $\mu$ moles of *p*-CMB per 100 mg of actomyosin, added 20 sec before ATP. Curves marked EGTA; 0.002 M EGTA in the reaction mixture, added 30 sec before ATP. Initial ATP concentrations are indicated; ATP concentration changes during the reaction as described in the legend of Fig. 1.

Apparently, ATP protects the protein against certain reactions with *p*-CMB—perhaps by directly masking some SH group(s), perhaps by changing the conformation of the protein—this has been observed before<sup>13-15</sup>.

In all these experiments, we measured ATP hydrolysis during superprecipitation, using the standard Fiske-Subbarow method for determining inorganic phosphate. Without calcium (with EGTA) high concentrations of Mg-ATP inhibited hydrolysis as well as superprecipitation; calcium or *p*-CMB overcame the inhibition of hydrolysis when they overcame the inhibition of superprecipitation. However, calcium or *p*-CMB in the same amounts did not significantly affect hydrolysis at lower concentrations of Mg-ATP.

We conclude that high concentrations of Mg-ATP block superprecipitation and ATP hydrolysis by binding to an inhibitory site on the gel; this site (distinguished from the active site for superprecipitation) can be selectively inactivated by *p*-CMB or Ca. Proteins other than actomyosin may be involved in the inhibition of superprecipitation by Mg-ATP (refs. 16-18); these proteins are probably present in the gel we use, so the inhibitory sites we modify with *p*-CMB may not be on actomyosin molecules, *per se*. In any event, calcium and *p*-CMB appear to inactivate these inhibitory sites without seriously inhibiting the active sites for superprecipitation; thus these agents allow superprecipitation at concentrations of ATP that otherwise inhibit, for example,  $10^{-4}$  M. The concentration of ATP in muscle is probably as high as  $5 \times 10^{-3}$  M (ref. 19)—this is enough to inhibit completely the superprecipitation of actomyosin gel in the absence of calcium; so it seems that calcium may allow contraction in muscle by overcoming substrate inhibition of actomyosin.

It has been known for some time that the ATPase activity of actomyosin can be activated by *p*-CMB (refs. 14, 20); and this activation is seen only at high concentrations of ATP (ref. 20). Moreover, it has been known for a long time that ATP plays a dual part in the actomyosin system: it relaxes (inhibits or clears) at high concentrations and causes contraction (superprecipitation) at low concentrations<sup>8,21</sup>. It has already been reported by Tonomura and Yoshimura<sup>20</sup> and by Murayama and Gergely<sup>15</sup> that *p*-CMB-treated actomyosin superprecipitates at high concentrations of ATP which ordinarily cause clearing. Metal chelators enhance the clearing effect of ATP, and Weber and Herz showed that this is

probably caused by the removal of some calcium from the protein<sup>9,10</sup>.

The data outlined in this communication confirm and extend many reports in the literature. Taken together, we consider that this information forms a cogent argument for the major contention of this communication: that calcium activates contraction of actomyosin by overcoming inhibition caused by high levels of Mg-ATP (refs. 9 and 10), and that *p*-CMB does the same; but neither calcium nor *p*-CMB directly activates the superprecipitation reaction. As shown here, these agents actually inhibit the process; their activating effects are apparent only when the concentration of Mg-ATP is high enough to inhibit seriously actomyosin contraction—and this appears to be the case in muscle.

These results will be reported in detail in a subsequent paper. The work was supported by research grant 6276 and by a research career development award to H. M. L., both from the United States Public Health Service. We thank Mr. Michael Proper for his assistance.

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### A Comparison of <sup>3</sup>H- and <sup>14</sup>C-Glucose Metabolism in the Intact Rat

DURING the course of using glucose-6-<sup>3</sup>H in an investigation of glucose turnover in rats, apparent values were obtained for the size of the body glucose pool, the glucose space, and the rates of glucose production and utilization, which were obviously too large when compared to similar work<sup>1,2</sup> using glucose labelled with carbon-14. The possibility was considered that the tritium label on carbon six did not represent the entire glucose molecule, thereby giving inaccurate values for the foregoing parameters of carbohydrate metabolism.

The following experiments were performed in order to ascertain whether or not glucose-6-<sup>3</sup>H represents the entire glucose molecule. Permanent indwelling polyethylene cannulae were inserted into the aorta and vena cava of Sprague-Dawley rats using the method of Popovic *et al.*<sup>3</sup>. Several days later, when the rats had returned to their pre-operative weights, a mixture containing D-glucose-U-<sup>14</sup>C and D-glucose-6-<sup>3</sup>H was administered intra-arterially to the unanaesthetized animals in the post-absorptive state. Serial samples of venous blood were taken at intervals between 30 and 130 min after isotope administration and specific activities of the plasma glucose with respect to both carbon-14 and tritium were determined.

As expected, the specific activities of the plasma glucose decreased with time, presumably because of the

production of new glucose. However, as can be seen in Table 1 under Exp. 1, the  $^3\text{H}$ :carbon-14 isotope ratio did not remain constant but decreased with time, indicating that the tritium was lost more rapidly than the carbon-14. This decreasing ratio provides evidence that the metabolism of the glucose chain cannot be followed quantitatively by a tritium tracer on carbon-6. The mechanisms which could account for this excess loss of tritium include the operation of a tritium isotope effect, cleavage of the carbon chain followed by resynthesis of glucose, or cleavage of the (6)—C—H bond of glucose.

Table 1. THE  $^3\text{H}$ : $^{14}\text{C}$  ISOTOPE RATIOS OF PLASMA GLUCOSE FOLLOWING INTRA-ARTERIAL ADMINISTRATION OF CARBON-14 AND TRITIUM GLUCOSE MIXTURES INTO RATS

Exp.	Isotopic glucose administered	Time after isotope administration	$^3\text{H}$ : $^{14}\text{C}$	Per cent of $^3\text{H}$ lost relative to initial $^3\text{H}$ : $^{14}\text{C}$
1	Glucose-U- $^{14}\text{C}$ and glucose-6- $^3\text{H}$	0	8.3*	
		30	7.5	9.6
		60	6.5	12.2
		90	5.3	36.1
		110	4.2	49.4
2	Glucose-6- $^{14}\text{C}$ and glucose-6- $^3\text{H}$	0	13.9*	
		30	12.3	11.5
		60	11.7	15.8
		110	9.4	32.4
		130	8.4	39.6
3	Glucose-6- $^{14}\text{C}$ and glucose-6- $^3\text{H}$	0	7.5*	
		30	6.6	12.0
		60	6.2	17.3
		90	5.1	32.0
		110	4.7	37.3
		130	4.0	46.7

\* This represents the  $^3\text{H}$ : $^{14}\text{C}$  ratio of the labelled glucose before administration to the rat at time 0.

When tritium is used as a tracer in biological systems, experimental results can be markedly influenced by isotope effects which result in lower reaction rates for tritium transfer as compared to protium. However, our results show that the  $^3\text{H}$ :carbon-14 ratio of plasma glucose decreases with time, giving precisely the opposite result from that which would be expected from a tritium isotope effect<sup>4,5</sup>.

A change in the  $^3\text{H}$ :carbon-14 ratio could result from the formation of new glucose from three-carbon fragments derived from the administered doubly-labelled glucose. It is conceivable that tritium, but not carbon-14, could be lost from these fragments, possibly during the enolization step leading to formation of pyruvic acid. However, von Holt<sup>6</sup> has demonstrated that in non-fasted rats only 12 per cent of the plasma glucose is derived from glucose degradation products in 120 min.

Cleavage of the carbon-tritium bond could occur in an aqueous biological system, either by physical-chemical exchange or by an enzymatic hydrogen transfer system. The former possibility seems unlikely, since we established in the course of this investigation that the tritium label is stable during the synthesis of the glucosatriazole derivative used to isolate the plasma glucose. During preparation of this derivative the tritium-labelled glucose is in an aqueous medium for many hours under varying physical and chemical conditions including high temperatures (110°–140°).

In order to demonstrate that the excess loss of tritium was due to cleavage of a (6)—C—H bond rather than cleavage of the carbon chain itself, glucose labelled with carbon-14 at the sixth carbon was administered together with D-glucose-6- $^3\text{H}$ . As can be seen in Table 1, Expts. 2 and 3, the  $^3\text{H}$ :carbon-14 isotope ratio decreased with time at rates comparable to that of the experiment with D-glucose-U-carbon-14. This strongly suggests that loss of tritium is due to cleavage of the (6)—C—H bond.

One known metabolic reaction that involves cleavage of the (6)—C—H bond is the oxidation of glucose to glucuronic acid. However, the change in the  $^3\text{H}$ : $^{14}\text{C}$  ratio indicates that approximately 40–50 per cent of the glucose pool undergoes hydrogen exchange in 130 min. It is

highly unlikely that such extensive glucuronic acid formation occurs in this time.

The most likely explanation for our results appears to be a hydrogen transfer reaction involving the (6)—C- $^3\text{H}$  bond of glucose. Speculation can be made as to where in the organism such transfer takes place. The fact that the decrease in the  $^3\text{H}$ : $^{14}\text{C}$  ratio is found in the plasma glucose itself tends to rule out the intracellular space of extra hepatic tissues as the site of cleavage. It is well established that in the post-absorptive animal the liver is the source of plasma glucose. Once this plasma glucose enters extra-hepatic tissues, it is phosphorylated and cannot be released as glucose because of the absence of glucose-6-phosphatase in these tissues. However, it is possible that hydrogen exchange at carbon six could occur in the liver. The experiments of Bloom and Foster indicate that tritium is not removed from carbon-6 of intracellular glucose formed from glycerol 3- $^3\text{H}$ , 1,3- $^{14}\text{C}$  in rat liver slices and diaphragm *in vitro*<sup>7</sup>. In both tissues the  $^3\text{H}$ : $^{14}\text{C}$  ratio of glucose isolated from glycogen was equal to that expected from the tritium and carbon-14 content of the precursor glycerol. However, the results do not eliminate the possibility that hydrogen exchange occurs in the process of transfer of the glucose in and/or out of the liver cell. Removal of tritium from carbon-6 could possibly occur at the cell surfaces of other tissues as well.

In conclusion, these results indicate that glucose-6- $^3\text{H}$  cannot be used as a quantitative tracer for the glucose carbon chain in the intact rat. However, the loss of tritium observed in our experiments may reflect an important physiological event in glucose metabolism in the intact organism.

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### Sodium-Potassium Ion Exchange Equilibria for Fragmented Human Erythrocyte Ghosts

AN investigation of the calcium-potassium uptake of human erythrocyte ghosts demonstrated that their behaviour was qualitatively the same as a phospholipid-cholesterol membrane model<sup>1</sup>. The membrane model's behaviour can be explained by the presence of negative fixed charge due to a particular phospholipid, phosphatidyl serine<sup>2</sup>. Fragmented human erythrocyte ghosts behave as if they also possess negative fixed charge due to some chemical component or components. The work recorded here extends the analysis of the cation exchange properties of ghost fragments to the equilibrium between sodium and potassium at two concentrations of total salt.

Human erythrocyte ghosts were prepared, treated and analysed as described previously<sup>1</sup>. Some of the material was fragmented by freezing and thawing three times instead of sonication.



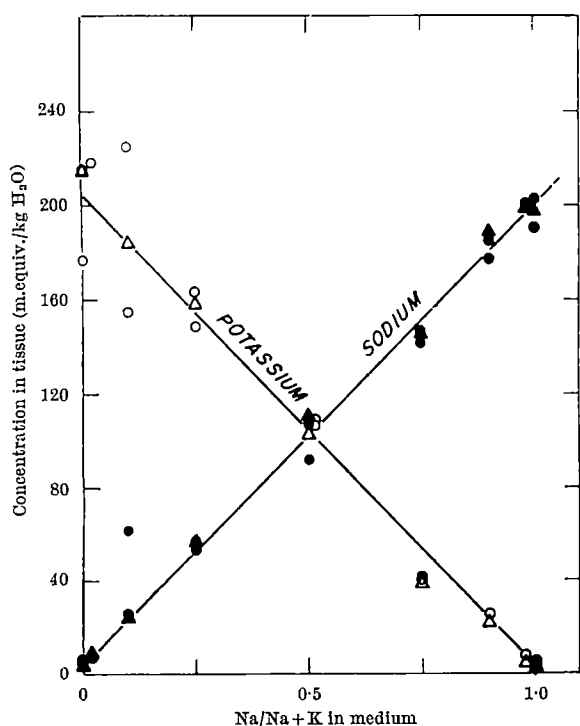


Fig. 1. Ion exchange equilibria for fragmented human erythrocyte ghosts in sodium-potassium chloride solutions of various sodium/potassium ratios. Total sodium plus potassium chloride 200 m.equiv./l. Each point represents a single sample.  $\circ$ , K, sonicated;  $\bullet$ , Na, sonicated;  $\Delta$ , K, freeze thawed;  $\blacktriangle$ , Na, freeze thawed

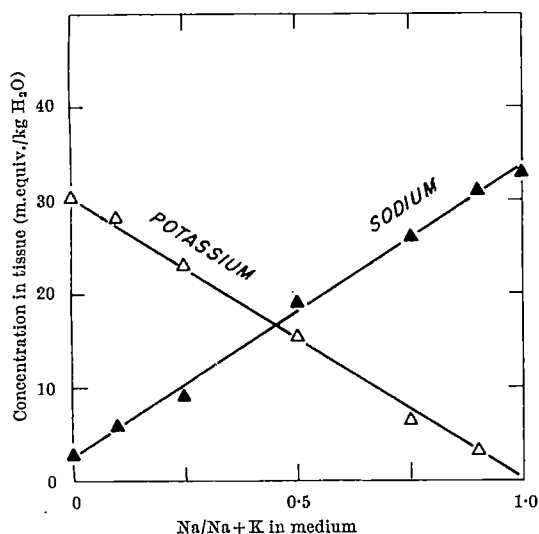


Fig. 2. Ion exchange equilibria for fragmented human erythrocytes ghosts in sodium-potassium chloride solutions of various sodium/potassium ratios. Total sodium plus potassium chloride 20 m.equiv./l. Each point represents a single sample.  $\Delta$ , K, freeze thawed;  $\blacktriangle$ , Na, freeze thawed

Figs. 1 and 2 show the ion exchange equilibria for fragments exposed to sodium and potassium in varying fractions at total concentrations of 200 and 20 m.equiv./l., respectively. If this figure is compared with similar data obtained for the phospholipid-cholesterol membrane model<sup>1</sup>, it can be seen that, again, qualitatively the same behaviour is observed in both systems. One important difference occurs when the results obtained are analysed in terms of estimated fixed charge concentration. (A value of 92 m.equiv./kg  $H_2O$  was obtained from calcium-potassium equilibria in the ghost fragments<sup>1</sup>.) If the ion equilibria is a non-selective Donnan equilibrium, the total ion content of the fragments should be approximately 250 m.equiv./kg  $H_2O$  at the higher total salt concentration

and 96 m.equiv./kg  $H_2O$  at the lower. The observed ion content of the fragments is considerably lower than this, suggesting that the remaining capacity of the fragments is taken up by another cation, probably hydrogen. This is supported by an examination of the binding of monovalent cations to ghost fragments after washing with sucrose solutions<sup>3</sup>. It was found that at least one-third of the total binding capacity was taken up by hydrogen ion at the pH of these experiments (pH 6.0-7.0) and a concentration of total sodium and potassium of 50 m.equiv./l.

The uptake of water by the fragments was independent of the sodium-potassium ratio but depended on the total concentration. In the 200-m.equiv./l. solutions, water uptake was  $7.31 \pm 0.22$  mg  $H_2O$ /mg dry tissue and in the 20-m.equiv./l. solutions water uptake was  $10.98 \pm 0.64$  mg  $H_2O$ /mg dry tissue.

The fragments show no selectivity between sodium and potassium. The results obtained did not depend on the method used for fragmenting the ghosts.

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### Mitochondrial Pyridine Nucleotides in the Oxidation of Isocitrate and Malate

CHAPPEL<sup>1,2</sup> has shown that the rate of uptake of oxygen by rat liver mitochondria, with isocitrate in the presence of malate, is greater than the sum of that observed when either substrate is present alone. Since the oxidation of both isocitrate and malate is linked to pyridine nucleotides, the composition of pyridine nucleotides during the oxidation of these substrates was measured.

Fluorescence assays<sup>3</sup> of intact mitochondria<sup>4</sup> demonstrated oxidation of reduced pyridine nucleotides as expected under aerobic conditions (Fig. 1). When ADP was added, the rate of oxidation was markedly enhanced<sup>3</sup>. The addition of malonate (to inhibit malate formation) was without effect on the rate of oxidation of reduced pyridine nucleotides. When isocitrate was added, there was a slight increase in reduction of the pyridine nucleotides which was markedly enhanced on the subsequent addition of malate until a state of anaerobiosis was reached, at which time almost complete reduction of the pyridine nucleotides was observed. Malate, when present without the prior addition of isocitrate, did not change the rate of pyridine nucleotide reduction.

Since fluorescence assays of suspensions of whole mitochondria do not determine which of the pyridine nucleotides is reduced, or whether the increase in fluorescence was due to fluorescence enhancement of bound pyridine nucleotides<sup>5</sup>, the composition of the extractable pyridine nucleotides was determined<sup>6</sup>.

In these experiments, mitochondria (approximately 6 mg protein/ml.)<sup>7</sup> were incubated at 25° C in the isotonic medium described in the legend to Fig. 1, to which 8 mM malonate had been added. The mixture (3.3 ml. in 25-ml. Erlenmeyer flasks open to the atmosphere) was stirred gently. At the end of 1 min 4 mM ADP was added, and at the end of 2 min 1-malate (3 mM) and *DL*-isocitrate (6 mM) were added. At the end of 4 min, acid and alkaline extracts of the pyridine nucleotides were prepared. In control experiments extractions were carried out on fresh

Table 1. MEAN CONCENTRATION OF RAT LIVER PYRIDINE NUCLEOTIDES (nmol/mg protein)

Mitochondrial preparation	NADH	NAD	NADPH	NADP	Total PN
Fresh ( $n=38$ )	$0.76 \pm 0.06$	$1.06 \pm 0.07$	$2.42 \pm 0.15$	$0.11 \pm 0.04$	$4.35 \pm 0.20$
Incubated ( $n=38$ )	$0.39 \pm 0.07$	$2.11 \pm 0.17$	$1.44 \pm 0.11$	$0.26 \pm 0.06$	$4.20 \pm 0.20$
Incubated (with malate) ( $n=10$ )	$0.46 \pm 0.15$	$2.21 \pm 0.14$	$1.03 \pm 0.08$	$0.77 \pm 0.17$	$4.58 \pm 0.26$
Incubated (with malate and isocitrate) ( $n=10$ )	$0.30 \pm 0.06$	$2.20 \pm 0.24$	$1.87 \pm 0.20$	$0.07 \pm 0.04$	$4.43 \pm 0.57$

Means are given with their *S.E.*

mitochondria and on mitochondria incubated for 4 min, in which one or both substrates were omitted. The results are summarized in Table 1.

Under the four conditions shown in Table 1 there was no apparent change in the mean total extractable pyridine nucleotide concentration, suggesting that there was no destruction of pyridine nucleotides. An expected decrease in reduced pyridine nucleotides was observed, however, under all three conditions of incubation, as compared with the fresh mitochondria. Consistent with the fluorimetric tracing there was a marked increase in reduced pyridine nucleotides in the presence of isocitrate and malate compared with that found with malate alone. This was attributable only to NADPH ( $0.84 \mu\text{moles/mg}$  of protein). Since only about one-third of the total pool of extractable NADP was reducible by isocitrate in the presence of malate, this would represent the maximum portion of the pool of mitochondrial NADPH capable of being oxidized by the electron transport chain under the conditions of these experiments.

When mitochondria were incubated with or without added substrate there was a decrease in the concentration of NADPH which was associated with an increase in the concentration of NAD. This could be due to the activity of a phosphatase such as ATPase. Polarographic examination of the mitochondria, carried out on all specimens, however, showed a clear increase in respiratory rate on the addition of ADP, and a return to the previous rate when all the added ADP should have been phosphorylated to ATP. This is evidence against the presence of a very active ATPase. On the other hand, only between 1 and 2  $\mu\text{moles}$  of NADPH/mg of protein were apparently dephosphorylated in our investigations. As the  $K_m$  of ADP for the electron transport chain, under these

conditions, is about  $56 \times 10^{-6} \text{ M}$  (ref. 8), these quantities may have been too small to be detected polarographically as evidence of presumptive ATPase activity.

Experiments in which isocitrate alone was the added substrate consistently showed a loss of about 30 per cent of the total pyridine nucleotides when compared with the unincubated specimen from the same liver, and therefore could not be evaluated.

The rate of NADPH oxidation was accelerated on the addition of malate to the mitochondria, resulting in a relatively high concentration of NADP. This was not seen in the fluorimetric tracing of the mitochondrial suspension. The absence of any significant change in that tracing may have been due to the presence of NADPH not bound to protein, allowing free NADPH to be oxidized by malate or one of its metabolites. This would be in agreement with the suggestion of Klingenberg<sup>9</sup> concerning the ability of malate to facilitate the release of bound pyridine nucleotides through oxidation by oxalacetate.

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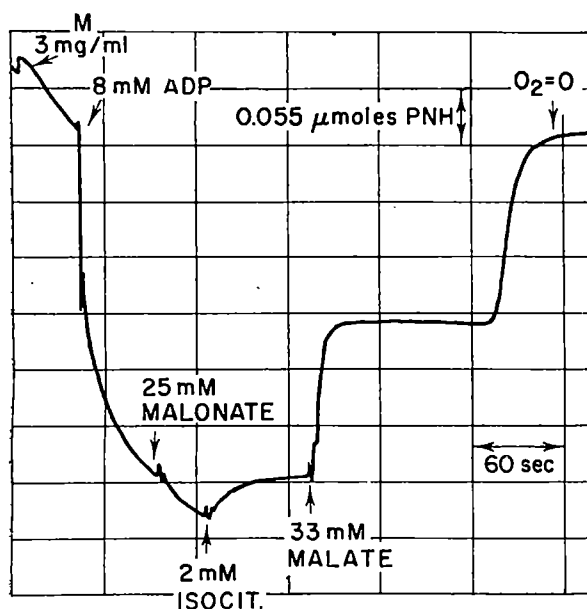


Fig. 1. Fluorimetric trace of a suspension of rat liver mitochondria. The incubation medium was 80 mM in potassium chloride, 10 mM in potassium phosphate buffer, pH 7.2, 10 mM in triethanolamine hydrochloride buffer, pH 7.2, and 5 mM in magnesium chloride, *M*, mitochondrial protein. (Experiment carried out with Dr. R. W. Estabrook and Miss J. Gonze)

### Cytochemical Localization of Ubiquinones in the Retina

A METHOD for the localization of ubiquinones was recently described by Tranzer and Pearse<sup>1</sup>. Subsequent work (to be published) has shown that a positive reaction *in vitro* is given not only by various ubiquinones (UQ 30, 45, 50) but also by members of the tocopherol series ( $\alpha$ ,  $\beta$ ,  $\gamma$ ). Under cytochemical conditions, however, the specificity of the reaction for ubiquinones is much higher and the two groups can usually be distinguished from each other. Vitamin A (alcohol) gives no reaction *in vitro*.

The method has been applied to the predominantly cone retina of the chick (White Sussex) and to the predominantly rod retinae of the albino and hooded rat, and the albino mouse (A strain). In some experiments dark- and light-adapted eyes have been used but the reaction has so far been carried out in the light only. The findings are similar in every case, the state of *in vivo* adaptation of the eye making no appreciable difference.

As shown in Fig. 1, moderate levels of UQ are present in the outer plexiform layer (OP), in the layer of visual

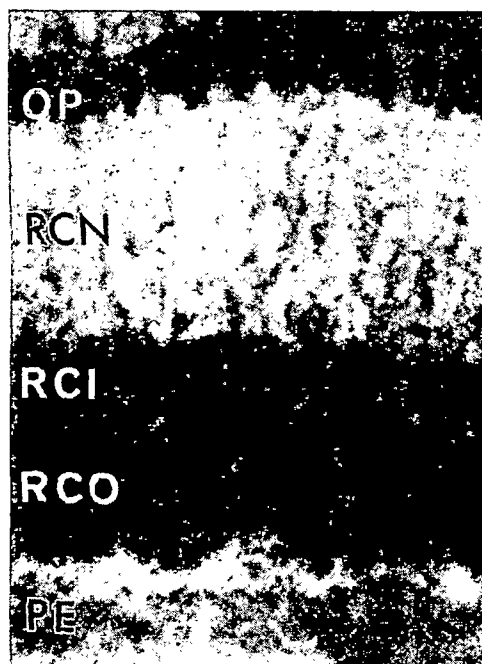


Fig. 1.  $5\mu$  cryostat section of albino rat retina. Shows distribution of ubiquinones in various layers. OP, outer plexiform; RCN, rod and cone nuclei; RCI, inner segment; RCO, outer segment; PE, pigment epithelium. ( $\times 570$ )

cell nuclei (RCN), and in the pigment epithelium (PE). A stronger reaction is present in the inner segments of the photoreceptors (RCI). In all the aforementioned regions the localization of UQ is intramitochondrial. In the outer segments of the photoreceptors (RCO) there is an intensely strong reaction which is necessarily non-mitochondrial. Quantitative investigations have not yet been carried out, but for a given incubation period the reaction in the outer segments is one of the strongest observed in mammalian or avian tissues.

There can be no doubt (from its solubility characteristics) that the UQ of the outer segments is dissolved in the lipid phase of the individual disks composing the outer segment. Their structure has been well described by Sjöstrand<sup>2</sup>. In this position UQ is perfectly situated to act as an electron sink; that is, to receive electrons from systems having a less positive redox potential.

During the period of dark adaptation electrons derived from the regeneration of rhodopsin might be transferred to the UQ of the disks. During either dark or light adaptation the outer segment might receive electrons from metabolic processes carried on by the mitochondria of the inner segment. The photoreceptor, with its disks partly loaded with ubiquinol (reduced UQ), might thus be regarded as a miniature capacitor.

The speculation can be completed by supposing that the stimulus provided by a photon striking and penetrating the outer membrane, and isomerizing a molecule of retinene in one of the disks, sets in motion the flow of electrons and thus an electric current. Since the flow of electrons would be from the outer segment to the inner segment the current would necessarily flow in the opposite direction, that is, into the outer segment. The negative  $\alpha$ -wave of the electroretinogram could be explained on the foregoing hypothesis.

Confirmation of the presence and concentration of UQ in the photoreceptors must be obtained by micro-dissection and micro-assay. If the essential premise outlined here is true, there will be a difference in the state of oxidation of UQ in dark- and light-adapted outer segments. The experiments described here failed to maintain the state of dark adaptation since, after removal of the eyes,

all work was carried out in air, and in the light. Ubiquinol, the reduced form of UQ, is rapidly oxidized by atmospheric oxygen if certain components of the respiratory chain are present (ubiquinol oxidase). If oxidation is prevented ubiquinol will reduce suitable tetrazolium salts directly and dark (anaerobic) incubation of the dark-adapted retina should reveal its presence if the level is sufficiently high. It should therefore be technically possible to make this demonstration and work is proceeding with this in view.

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## PHARMACOLOGY

### Neutralization of the Antibacterial Effect of Natural Coumarin Ostruthine by Vitamins K

THE effects of natural coumarin derivatives in the vegetable kingdom are well known, but their effect on micro-organisms has been little investigated up to now. We had earlier found that out of eighteen natural coumarins, for the greater part isolated from the family of *Daucaceae*, only ostruthine (6-geranyl-7-hydroxy-coumarin) caused a marked inhibition of the growth of gram-positive micro-organisms. Related phenolic coumarins, such as umbelliferone (7-hydroxy-coumarin), were ineffective<sup>1</sup>. The effect of ostruthine on the growth of the strain 'BS 270' of *Staphylococcus aureus* was not diminished by the addition of a surplus of glutathione, cysteine, thioglycolate, and 2,3-dimercaptopropanol<sup>2</sup>, that is, substances which possess the ability to react with the lactone ring of the coumarin molecule<sup>3</sup>. Thus, the explanation of the effect of ostruthine can be found in the presence of a side-chain of ten carbon atoms, rather than in the lactone grouping of the molecule.

We have now found it possible to diminish the inhibiting effect of ostruthine on the growth of the strain of *Staphylococcus aureus* 'BS 270' by vitamins K. We tested, on agar plates, with the technique of paper disks<sup>4</sup>, alcoholic solutions of ostruthine alone ( $1 \times 10^{-3}$  M;  $5 \times 10^{-4}$  M;  $2.5 \times 10^{-4}$  M;  $1 \times 10^{-4}$  M), and in a mixture with a double-molar surplus of vitamin K<sub>1</sub> (phylloquinone), vitamin K<sub>2</sub> (farnesyl-quinone), and vitamin K<sub>3</sub> (menadiolone). The effect of ostruthine was not considerably diminished in the mixture of vitamins.

On the other hand, interesting results were obtained in following up the consumption of oxygen in the washed cells of the bacterial culture. Ostruthine causes a strong inhibition of O<sub>2</sub> consumption which cannot be reversed by three-fold washing of the bacterial cells with a buffer solution. The effect of ostruthine alone and of the mixture solutions of vitamins K and other related compounds are given in Table 1.

From Table 1 it is seen that the effect of ostruthine on O<sub>2</sub> consumption can be neutralized by vitamins K<sub>1</sub> and K<sub>2</sub>, and, to a great extent, also by phytol alone.

Table 1. EFFECT OF OSTRUTHINE, VITAMINS K, PHYTOL, AND VITAMIN E ON THE OXYGEN CONSUMPTION IN *Staphylococcus aureus* 'BS 270'

Added Inhibitor	Activator	O <sub>2</sub> consumption as % of control		
		Inhibitor $1 \times 10^{-4}$ M	Activator $1 \times 10^{-4}$ M	Inhibitor + $2 \times 10^{-4}$ M activator
Ostruthine	Vitamin K <sub>1</sub>	20.3	104.2	120.0
Ostruthine	Vitamin K <sub>2</sub>	21.0	98.0	108.0
Ostruthine	Vitamin K <sub>3</sub>	23.7	110.0	21.7
Ostruthine	Phytol	30.8	100.0	82.4
Ostruthine	Vitamin E	22.3	101.6	100.9

The values were obtained by Warburg's method. O<sub>2</sub> consumption in controls = 100 per cent. Standard deviation  $\pm 2.6$  per cent. Main space of vessel in control: final volume 3 ml.; 3.6–4.0 mg dry weight of 18-h-old bacterial culture, 9 mg glucose, 0.05 M KH<sub>2</sub>PO<sub>4</sub>, 0.077 M NaCl, 1.5 per cent ethanol, 0.02 per cent albumin, pH 7.8, temperature 35° C. The bacteria were in contact with the respective substances for about 30 min before the beginning of measurements.



The same ability is also displayed by vitamin E ( $\alpha$ -tocopherol). Vitamin K<sub>1</sub> in a mixture with ostruthine brings about a strong increase in O<sub>2</sub> consumption. Vitamin K<sub>3</sub>, a substance without lipophile side-chain, does not neutralize the effect of ostruthine.

It is scarcely possible to elucidate the mechanism of protective action by the known antagonism of certain coumarins and K vitamins. This mechanism was proved only in coumarins with the hydroxy group on the fourth carbon, such as dicoumarol. Neutralization of the effect of ostruthine by means of the priority bond between the substance examined and the cell is not probable. This is borne out by our finding that the bacterial cells, exposed for 10 min to the effect of vitamin K<sub>1</sub> ( $2 \times 10^{-4}$  M), were, on separation by centrifugation, as sensitive to ostruthine as was the original culture. Similarly, the converse procedure, that is, the action of vitamin K<sub>1</sub> on the cells exposed to the effect of ostruthine, did not lead to any substantial diminution of inhibition. The protective action of vitamin K<sub>1</sub> thus sets in only when both substances are simultaneously added to the bacterial culture.

The explanation seems to be that the ostruthine molecules in the mixed solution with vitamin K<sub>1</sub>, and similarly with other effective substances, are oriented in a manner that renders it impossible for ostruthine with its lipophile chain to combine with the bacterial cells. This interpretation also accounts for the observation that weak neutralization of the effect of ostruthine takes place in the surface tests of alcoholic solutions on agar plates.

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### Identity of Ramycin with Fusidic Acid

RAMYCIN is an antibiotic isolated from the culture broth of a phycomycete, identified as *Mucor ramannianus*. This substance is active against Gram-positive bacteria, mainly *Staphylococci*. Its activity is, however, reduced in the presence of serum, and resistance develops rather quickly *in vitro*. The observation that ramycin prolonged the survival time of mice infected with *Streptococcus pyogenes* and *Staphylococcus aureus*, and was of low toxicity, indicated that this substance should be considered as a potentially useful antibiotic<sup>1</sup>.

Further characterization of the substance was desirable in order to classify it in one of the several groups of antibiotics. Ramycin was described as a colourless substance, containing only C, H and O, with an acid group having a *pK* of 4.6, an equivalent weight of 478 and containing one or more hydroxy groups and double bonds<sup>1</sup>.

Several groups of compounds can be excluded because ramycin contains no nitrogen. Its molecular weight and the fact that it shows only end absorption without characteristic bands in the ultra-violet also eliminate quinones, phenolic substances, and tetrone acids. For this reason, ramycin is likely to be an unsaturated acid of unusual molecular weight or a triterpenoid-steroid-like substance. The biological properties of several products of the last group indicated that it could belong to this class.

Ramycin was compared with several available steroid-like antibiotics. All products were chromatographed in a benzene-formamide system<sup>2</sup>, and the antibiotics were

located on the paper by bioautography on agar seeded with *Staphylococcus aureus*. Ramycin and fusidic acid presented the same *R<sub>F</sub>* value ( $0.30 \pm 0.05$ ), while cephalosporin *P*<sub>1</sub> and helvolic acid were different (*R<sub>F</sub>* relative to *R<sub>F</sub>* of ramycin or fusidic acid =  $1.35 \pm 0.05$  and  $2.6 \pm 0.1$ ). These results were confirmed by thin-layer chromatography on silica gel in the system toluene, acetic acid, and water 5 : 5 : 1 (ref. 3). All antibiotics could be separated by this method, except fusidic acid and ramycin, which had the same *R<sub>F</sub>* value. The colour reagents used for location of the products on the plates were a further aid in identification. All products gave a blue-grey colour after being sprayed with Folin-Ciocalteu reagent, and heated (100°, 10 min). Heating the plates which had been sprayed with antimony trichloride dissolved in chloroform gave different colours: ramycin and fusidic acid, wine red; cephalosporin *P*<sub>1</sub>, green; helvolic acid, no colour. As helvolic acid is less active than the other antibiotics it was necessary to use larger amounts for paper chromatography (3–4 µg instead of 1–2 µg) and thin-layer chromatography (100 µg instead of 50 µg) because helvolic acid gives a weaker colour with Folin reagent. The physical constants published for helvolic acid<sup>4–7</sup> and cephalosporin *P*<sub>1</sub> (refs. 8 and 9) confirm our observations that ramycin is different from these antibiotics. For the same reason, ramycin must be different from polyporenic acid *C*<sub>1</sub> (ref. 10), a product that was not available for direct comparison. The biological data published for ramycin and fusidic acid<sup>11</sup> are identical within the limits of error; some of the physical data (maxima of the infrared spectrum, *pK*) correspond, whereas others ( $[\alpha]_D$ , melting-point) present a small or marked difference<sup>12,13</sup>. For this reason new samples of ramycin were prepared from fresh cultures of *Mucor ramannianus*. These products were identical in all respects with fusidic acid, and were different from dihydrofusidic acid, the physical constants of which are closely related to those of the parent compound<sup>12,14</sup>. The discrepancy between the melting-points of the older and the newer samples can be ascribed to the presence of some impurities in the former (also revealed by thin-layer chromatography). It is also known that the melting-points and analyses of the substances of this group are often perturbed by solvent of crystallization<sup>4,6,9</sup>. We have also observed that the methyl esters of these products, obtained by reaction of the acids with an ethereal solution of diazomethane, could be examined by gas chromatography. The esters of fusidic acid and of ramycin (even the samples which showed the presence of some impurities on thin-layer chromatography) presented the same chromatogram (Table 1). Mass spectrographic investigations were also carried on the methyl esters of both antibiotics at Lilly Research Laboratories. Similar *m/e* peaks of 530 (molecular ion peak), 470, 461 and 452 were obtained in each case.

Table 1. Gas CHROMATOGRAPHY OF METHYL ESTERS OF FUSIDIC ACID AND RAMYCIN\*

Compound	Relative retention time
Cholic acid methyl ester	1.00
Ramycin methyl ester	1.79
Fusidic acid methyl ester	1.79
Dihydrofusidic acid methyl ester	1.64

\* The instrument was an argon chromatograph (Pye Instruments, Cambridge, England) modified to permit the injection of a sample dilution directly on top of the column through a silicone rubber septum. The column wall ahead of the column packing was kept at 300° and the detector cell at 250° by means of separate heating mantles. The column was a 130 cm x 4 mm i.d. glass tubing filled with acid-washed, silanized Gaschrom P<sub>1</sub> 100–120 mesh, coated with 0.75 per cent QF-1, a fluorinated silicone polymer of Dow-Corning Corp<sup>15</sup>.

We may conclude that fusidic acid, a steroid antibiotic with several new characteristics, is produced not only by *Fusidium coccineum*, but also by *Mucor ramannianus* and several cephalosporia<sup>15</sup>.

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## HAEMATOLOGY

### Electrophoresis of <sup>35</sup>S-labelled Material released from Clumping Platelets

RAT platelets contain a sulphated mucopolysaccharide<sup>1</sup> which, when subjected to paper electrophoresis and to paper chromatography, showed a mobility similar to chondroitin sulphate-A but unlike that of heparin<sup>1,2</sup>. The acid mucopolysaccharide (AMPS) content of human serum increased during the early phase of the blood clotting process<sup>3</sup>; thrombocytes were suggested as a possible source of this additional serum mucopolysaccharide. When thrombin was added *in vitro* to a suspension of <sup>35</sup>S-labelled pig platelets, up to 50 per cent of the radioactivity was lost from the platelets during clumping<sup>4</sup>. Electrophoretic identification of this released material has been attempted in the present investigation.

Platelets of six pigs ( $27.9 \pm 11.1$  kg) were labelled *in vivo* with sulphate-<sup>35</sup>S ( $1.80 \pm 0.18$  mc/kg body-weight) and separated from the blood components as described previously<sup>4,5</sup>. These platelets were resuspended either in a 1:1 or 1:3 mixture of imidazol buffer<sup>6</sup> and 0.85 per cent NaCl or in 0.85 per cent NaCl. Platelet clumping was induced in one-half of each platelet suspension ( $2.49 \times 10^6$  platelets/mm<sup>3</sup>) by the addition of 0.25 ml. 0.025 M CaCl<sub>2</sub> solution and 25 units of bovine thrombin (Parke Davis and Co.) per ml. platelet suspension and incubation at 37° C for 15 min. No calcium or thrombin was added to the other half of the suspension. Both halves of each suspension were centrifuged at 3,000 r.p.m. for 10 min. The platelet residues were digested with 0.5 N NaOH for 18 h at 0° C. Protein was precipitated from the supernatant fluids and from the digested residues with 1.0 ml. 60 per cent perchloric acid per 20 ml. volume and centrifuged at 3,000 r.p.m. for 15 min. Mucopolysaccharides were precipitated with 1.0 ml. 5 per cent phosphotungstic acid in 1 per cent HCl per 10 ml. of the perchloric acid supernatant for 18 h at 0° C. Samples were then centrifuged at 3,000 r.p.m. for 30 min; the precipitate was washed three times with acetone and dissolved in 0.5 ml. 0.1 N NaHCO<sub>3</sub>. Electrophoretic separation was carried out in a Shandon cell<sup>7</sup> using Oxoid strips (Consolidated Laboratories, Inc.). Strips were dried, stained with alcian blue and cut into 1-cm lengths for measurement of <sup>35</sup>S content.

The mean percentage of <sup>35</sup>S-labelled material released on treatment of the platelet suspension with calcium and thrombin was  $47.8 \pm 7.5$ . Phosphotungstic extracts of the supernatant from treated platelets and of both treated and untreated platelet residues characteristically showed three bands (Fig. 1a).

When compared with a control strip (AMPS) containing a mixture of chondroitin sulphate-A, hyaluronic acid and heparin, the mobility of band A was less than that of hyaluronic acid; the mobility of band B was similar to that of chondroitin sulphate. Band C moved further than any of the standard mucopolysaccharides. Extracts of the supernatant from the untreated platelet suspensions showed no stainable material. When standards were run simultaneously with the supernatant from clumping platelets (Fig. 1b), band A again moved less than did hyaluronic acid. Band B and chondroitin sulphate-A appeared to have the same mobility.

The distribution of <sup>35</sup>S on the electrophoretic strips is shown in Table 1 (means from four pigs). The total radioactivity of any strip was taken as 100 per cent and

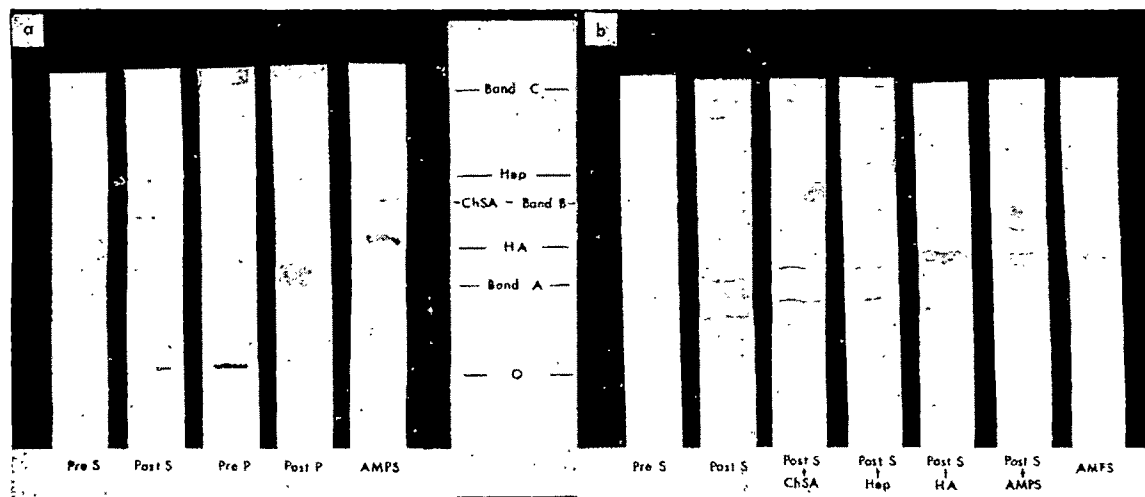


Fig. 1. Electrophoretic strips of phosphotungstic extracts of platelet supernatants and residues. Bands outlined in lead pencil. *a*, Pre S, supernatant before treatment of platelets with calcium and thrombin; Post S, supernatant after treatment of platelets; Pre P, platelets before treatment; Post P, platelets after treatment; AMPS, mixture of hyaluronic acid, chondroitin sulphate-A and heparin. *b*, Pre S, supernatant before treatment of platelets, with calcium and thrombin; Post S, supernatant from treated platelets, with and without added chondroitin sulphate-A (ChSA), heparin (Hep), or hyaluronic acid (HA).

Table 1. PERCENTAGE DISTRIBUTION OF <sup>35</sup>S ON ELECTROPHORETIC STRIPS

Extracts	Origin	Band A	Band B	Band C
Platelets before clumping	3.3	9.6	86.2	1.0
Platelets after clumping	1.8	9.3	77.7	11.3
Supernatants before clumping	14.3	32.6	56.6	0.0
Supernatants after clumping	1.7	11.1	85.5	1.7

the <sup>35</sup>S content of each band was expressed as a percentage of this total. The two important areas are the B bands from the platelets before clumping and from the supernatant after platelet clumping. Each of these two bands contains approximately 86 per cent of the <sup>35</sup>S-labelled material present on the strips. As band B appears to have the same electrophoretic mobility as chondroitin sulphate-A, and as the major sulphated mucopolysaccharide in rat platelets has been identified as chondroitin sulphate-A<sup>1</sup>, the <sup>35</sup>S released when pig platelets clump in a calcium-thrombin medium is believed to be in the form of chondroitin-<sup>35</sup>SO<sub>4</sub>-A.

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## HISTOLOGY

### Demonstration of Neutral Polysaccharides with Fluorescence Microscopy using Acridine Orange

ACRIDINE orange, a basic fluorochrome dye, stains the basophilic constituents of cells, such as nucleic acids (De Bruyn *et al.*<sup>1</sup>, Morthland *et al.*<sup>2</sup>, Armstrong<sup>3</sup>) and acid mucopolysaccharides (Hicks and Matthaei<sup>4</sup>, and Kuyper<sup>5</sup>), but aqueous solutions of this dye do not stain glycogen, paramylum and polysaccharides of the cyst wall of protozoa. All these polysaccharides can be stained with acridine orange after sulphation. Several sulphation techniques have been tried, and the best results have been obtained with chlorosulphonic acid in dry pyridine (Kramer and Windrum<sup>6</sup>). For the sulphation of glycogen 5 min treatment at 65° C is enough, whereas paramylum bodies and cyst wall require 10–15 min treatment at 70°–75° C.

**Procedure.** (1) Make smears of protozoa on clean slides, and dry them in air or fix in Carnoy for 15 min; bring the smears to water; (2) treat with chlorosulphonic acid in dry pyridine at 65°–75° C for 5–15 min; (3) wash in distilled water for 5 min; (4) stain with 0.1 per cent aqueous acridine orange for 5–10 min; (5) wash and mount the smears in distilled water, and seal the coverslip with paraffin; (6) examination of the preparations by ultra-violet fluorescence microscopy using a blue exciting filter (Jena BG 12) and yellow eyepiece filter shows intense red fluorescence of the sulphated polysaccharides.

This treatment with chlorosulphonic acid renders the polysaccharides acidic, with the result that they are able to bind basic fluorochrome acridine orange, and show intense red fluorescence in ultra-violet light. If stained after sulphation with other basic dyes like 0.5 per cent aqueous toluidine blue or 0.01 per cent aqueous azure A, these sulphated polysaccharides show metachromatic staining, which suggests a strongly acidic reaction. They remain negative to periodic acid-Schiff (PAS) stain.

This technique is based on the investigation of glycogen (PAS-positive, salivary amylase labile, non-metachromatic, coupled tetrazolium (CTZ) negative) in the cytoplasm of *Giardia intestinalis*, *Vorticella* sp., *Spirostomum* sp. and *Stylonychia* sp.; of paramylum bodies (PAS-negative, salivary amylase fast, non-metachromatic, CTZ-negative) of *Khawkinia* sp.; and of polysaccharides which in combination with proteins form the cyst-wall (PAS-positive, salivary amylase fast, non-metachromatic, CTZ-positive) of *Giardia intestinalis* and *Stylonychia* sp.

This investigation was carried out in the Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service, Columbia, S.C., and supported by an international postdoctoral research fellowship of the National Institutes of Health.

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### Thin Sections cut at Temperatures of –70° to –90° C

IN developing a satisfactory system for high-resolution autoradiography of soluble or unbound compounds<sup>1</sup>, we have investigated the possibility of frozen sectioning at low temperatures. At temperatures above –30° C adequate sections can be obtained for certain purposes, but in the temperature range commonly achieved in the standard laboratory cryostat, disruptive crystal formation limits the histological quality, and the thickness of the tissue section limits microscopic and autoradiographic resolution. At –20° C 4μ sections are difficult to prepare and the limit is about 2μ. Optimal conditions for avoiding disruptive ice crystal formation obtain in the range of vitreous ice at temperatures below –130° (refs. 2–4). Above –130° C both cubic and vitreous ice may be converted into the stable hexagonal phase during which the individual crystals can grow to 10μ and cause membrane disruption<sup>5</sup>. The transformation occurs rapidly above –70° C and is pronounced at –60° C. In order to utilize the technique of frozen sectioning for dry high-resolution autoradiography, a method is required which will reduce the risk of ice crystal formation and which will provide thin tissue sections in the range of 0.5μ to 1.0μ or lower.

The cryostat developed and modified for the work described herein (Harris Mfg. Co., Cambridge, Mass., Model 3L-2-075) is designed to maintain temperatures adjustable from –40° C to –85° C ± 1° C. It is equipped with an International Minot Custom Microtome (International Equipment Company, Needham Heights, Mass.) and an ultra-thin-sectioning attachment which is mounted in the freezing compartment (20 in. × 20 in. × 12 in.). A gear-rack adjustment was attached to the knife-holder in order to facilitate fine positioning of the knife. The cryostat is open-topped with an accessory stainless steel removable cover which has a 10 in. × 10 in. access port. The microtome is motor driven or hand operated from outside the cabinet. The open-top cryostat<sup>6</sup> has the advantage of easy access. The temperature may be maintained if excessive air movement is avoided in the cold chamber since air at –85° C has twice the density of air at room temperature. A side opening in the cold chamber permits attachment of a vacuum line for freeze drying within the cryostat. Thus, immediate drying of frozen



specimens without transfer from the cabinet avoids a rise in tissue temperature. The microtome can be lubricated with Molykote Z powder (The Alpha-Molykote Corp., Stamford, Conn.) applied in an absolute alcohol suspension.

The tissue of face area, about 1 mm<sup>2</sup>, is mounted on a specimen holder and quenched in liquid propane at about -180° C. It is then stored in a liquid nitrogen refrigerator. Either a steel or diamond microtome knife is used. Light-weight frozen-sectioning blades should be avoided. We have found that the standard microtome blade is satisfactory for preparing sections as thin as 0.5 µ. We used a knife with a bevel angle of about 30° and a clearance angle of about 25°.

The thickness of the section is mainly determined by the cutting temperature. Temperatures of -20° C are optimal for thick sections (more than 5.0 µ). Temperatures in the range of -70° C to -90° C restrict sectioning to less than 1 µ (liquid nitrogen in the cryostat cabinet lowered the temperature to -90° C). The optimal thickness varies not only with the temperature but also with the type of tissue. At temperatures lower than about -65° C sections thicker than 1 µ could not be cut due to crumbling. However, crumbling is avoided by thinner sectioning. At less than 1 µ rolling up sometimes is prevented by low cutting speeds, about 0.05 to 0.1 r.p.m. The sharpness of the knife and the clearance and bevel angle are very critical at low temperatures. Optimal conditions vary with the knife and the tissue. Sections are prepared under a dissecting microscope with a light beam directed on the cutting edge of the knife. The sections are manipulated and transferred with a fine camel's hair brush. The development of static charge can sometimes be helpful in this tissue transfer. Some loss in the handling of the tissue is to be expected.

A cryostat with variable temperature in the range we have used makes possible sectioning at 1.0 µ and below. Temperature may be the main factor controlling the choice of section thickness, and a cryostat with a range of -10° C to -90° C makes it possible to cut sections with a wide range of thickness. The technique of low-temperature frozen sectioning has been used successfully in the preparation of tissues.

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## PATHOLOGY

### 'Immuno-fluorescence' of Mast Cells

A PROPORTION of the mammary tumours induced in rats by oral administration of 9,10-dimethyl-1,2-benzanthracene (DMBA) have been found to undergo spontaneous regression<sup>1</sup>. Attempts to stimulate regressing tumours to grow again by hormonal means have been unsuccessful, and it appears possible that this regression may be due to an immunological mechanism<sup>2</sup>. If this is so it might be possible to demonstrate the presence of anti-tumour antibodies on tumour cells by using Coon's fluorescent antibody technique.

Portions of tumour were removed at biopsy and unfixed frozen sections of these were cut on a cryostat. They

were treated with highly purified rabbit anti-rat globulin which had been conjugated with fluorescein isothiocyanate and were then examined in blue violet light at about 420 mµ using a barrier filter with transmission of more than 450 mµ (lamp filter Chance OB 10 and eyepiece filter Wratten 15 + Chance OY 12).

We were not able to demonstrate the presence of fluorescent antibody on the tumour cells themselves, but large granular cells lying in the stroma of the tumour were found to fluoresce brightly. Further staining of the same sections with toluidine blue enabled us to identify these cells as tissue basophils or mast cells.

This phenomenon was observed in mast cells, both in growing and regressing tumours, in regional lymph nodes, in thymus and in spleen. The test of specificity carried out by attempted blocking with non-fluorescent antiserum was unsuccessful but, on the other hand, mast cells did not become fluorescent when treated with unconjugated fluorescein isothiocyanate as eosinophils did. Similar fluorescent staining of mast cells was observed, however, following treatment of cryostat sections with fluorescein conjugated antiserum bovine albumen. The work was carried out in the main on unfixed freshly frozen cryostat sections of tumour, but the effect was also noted after ethanol and acetone fixation in the cold. It was not observed with living cells suspended in Hanks's solution.

The evidence from our experiments does not favour an immunological explanation for the cause of spontaneous tumour regression; it suggests that the fluorescence we have found in mast cells is non-specific in nature.

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### Direct Agar Isolation of Mycoplasmas from Human Leukaemic Bone Marrow

THERE are no reports of the direct isolation of mycoplasmas from human leukaemic blood or blood-forming organs, although the direct isolation of mycoplasmas from the blood of a few septic patients has been claimed<sup>1-4</sup>. In the present investigation, mycoplasmas were isolated from broth cultures seeded with three bone marrow samples obtained on the same day from one of three children suffering from acute lymphoblastic leukaemia (Table 1).

The composition of the broth and agar medium used was that developed in this laboratory and previously used for the isolation of *Mycoplasma pneumoniae*, the aetiological agent of primary atypical pneumonia in man<sup>5-7</sup>.

Bone marrow samples were obtained by aspiration from the iliac crest after the overlying skin had been cleansed thoroughly with iodine and alcohol. The aspirates (0.2 ml.) were mixed directly with 3 ml. of broth containing 1:1,000 thallium acetate and the mixture incubated at 37° C. Although direct streaking of agar medium with the bone marrow specimens failed to yield

Table 1. THREE PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA WHOSE BONE MARROW ASPIRATES WERE TESTED FOR THE PRESENCE OF MYCOPLASMAS

Date of onset	Date of collection of bone marrow	Treatment prior to collection of bone marrow	Isolation of mycoplasmas from bone marrow
Sept. 1964	Oct. 7, 1964	None	+
July 1964	Oct. 12, 1964	X-ray, antimetabolites, steroids, transfusions	-
1961	Oct. 12, 1964	Antimetabolites, penicillin, steroids, transfusions	-

\* Isolated from each of 3 specimens collected on the same day.

mycoplasma colonies, mycoplasmas were isolated on agar from three broth cultures seeded with the bone marrow specimens obtained from one patient. The mixtures yielded colonies after incubation for 7 days at 37° C and continued to produce mycoplasma colonies aerobically when streaked on agar and at concentrations of 10<sup>4</sup> colony-forming units per ml. for a period of 90 days. The viability of mycoplasmas in a fluid medium kept for prolonged periods at 37° C is unique and probably distinguishes the strain from others.

Serological investigations were undertaken in an attempt to identify the isolate, called *N-1*, with the known species of mycoplasmas indigenous to man and animals. Growth inhibition tests indicated that *N-1* was not inhibited by antisera against the following species of human mycoplasmas: *M. hominis*, type 1; *M. hominis*, type 2; *M. pneumoniae*; *M. fermentans*; and *M. salivarium*. Growth of *N-1* was also not inhibited by antisera against the animal mycoplasmas, *M. mycoides*; *M. mycoides* var. *capri*; *M. gallisepticum*; *M. gallinarum* and three other strains pathogenic for fowl. The growth inhibition test did indicate that *N-1* is closely related to, or identical with, a new mycoplasma species which was first described in 1961 after isolation from tissue culture samples sent to this Institute for mycoplasma testing<sup>6</sup>. This strain was afterwards determined as belonging to a new mycoplasma serotype<sup>8</sup>. More recently, this serotype has been isolated from the human oral cavity and named *M. orale* by some<sup>9,10</sup> and *M. pharynges* by others<sup>11</sup>. Attempts to isolate *N-1* from the throat of the leukaemia patient were unsuccessful one month after the mycoplasmas were isolated from the bone marrow. Like *M. orale*, *N-1* does not ferment glucose.

*M. orale* probably includes a number of sub-species identifiable by more sensitive tests such as gel-diffusion and complement-fixation. One such sub-species has been reported<sup>12</sup> and, since *N-1* is not inhibited by antisera to this new sub-species, *N-1* may be a second sub-species of *M. orale*.

The successful isolation of mycoplasmas directly from the three samples from only one of the three investigated cases of leukaemia may have been due to the fact that this patient was the most recently diagnosed and was also not treated with drugs before collection of the bone marrow specimens. However, the possibility that *N-1* is a laboratory contaminant cannot be absolutely excluded even though mycoplasmas could not be isolated from: (1) two bone marrow specimens obtained from leukaemia patients receiving chemotherapy; (2) three bone marrow specimens obtained from patients not having malignant disease; (3) throat swabs of persons handling all specimens.

Most of the cytopathic agents isolated recently in tissue cultures exposed to human leukaemic material have been identified as mycoplasmas<sup>13-17</sup>. Furthermore, mycoplasmas have been isolated from tissue cultures inoculated with non-leukaemic human neoplastic tissue<sup>18,19</sup>. Although a few of the species isolated from human malignancies are known human serotypes, a number are clearly unrelated. The Negroni agent<sup>13</sup>, identified as a mycoplasma<sup>14</sup> and isolated from primary tissue cultures inoculated with human leukaemic bone marrow from ten patients, cannot be identified with any known human serotype and is also capable of fermenting glucose<sup>14</sup>. This latter property, in contrast to *N-1*, is shared only with the human mycoplasmas, *M. pneumoniae* and *M. fermentans*.

Because of the high rate of contamination of uninoculated cell lines with mycoplasmas the isolation of mycoplasmas from materials passed through serially passaged tissue cultures is difficult to interpret. This report indicates that mycoplasmas may be present in human leukaemic bone marrow and can be isolated directly in cell-free medium.

Mycoplasmas are the smallest free-living micro-organisms and may share all, or some, of the following properties with viruses: size, filterability, morphology in electron

microscopy, ether sensitivity, ability to haemagglutinate and to cause haemadsorption, interference with virus replication *in vitro*, lack of inhibition by many antibiotics, neutralization by homologous antisera and production of cytopathic effects<sup>6</sup>.

The mycoplasmas are very fastidious in their growth requirements and have metabolic properties which are known to be incompatible with the normal growth of mammalian cells *in vitro*. Most mycoplasma strains convert arginine to ornithine, not by way of arginase, but by a three-enzyme reaction sequence: the arginine dihydrolase system first described in the *Streptococcus*<sup>20,21</sup>. The depletion of arginine from cultures of mammalian cells infected with mycoplasmas results in overt cytopathic effects<sup>22-25</sup>. Interference with the metabolism of mammalian cells by these micro-organisms has also been explained by their possessing a pyrimidine nucleoside phosphorylase capable of cleaving thymidine and other deoxyribonucleosides<sup>16</sup>. Finally, evidence has been obtained indicating that metabolism of amino-acids, other than arginine, is altered in mammalian cells grown *in vitro* and contaminated with mycoplasmas<sup>22</sup>.

These properties make it interesting to speculate on the part which mycoplasmas may play in the aetiology of human leukaemia since presence of the micro-organisms in bone marrow may result in changes in the regulation of myelopoiesis.

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### Pre-erythrocytic Development of *Plasmodium berghei*

THE fate of the sporozoites of *Plasmodium berghei* in rodent hosts has been the subject of much research and speculation and has drawn the attention of malariologists from the early days of the discovery of this parasite<sup>1</sup>. For it was evident to all that this useful and easily accessible *Plasmodium*, an instrument of great promise in biological and medical research, could not achieve or claim true distinction so long as part of its life-cycle remained unmapped and obscure. Early attempts to elucidate the primary development of this parasite were, according to C. G. Huff, unsuccessful (personal communication). Investigations were greatly hampered by the necessity of obtaining sporozoites from wild-caught *Anopheles durenii* from the forest galleries of Katanga and in view of the early inability to infect laboratory-bred anopheline species under experimental conditions<sup>2</sup>. The demonstration early in 1964 of successive cyclical transmissions of *P. berghei* by the bite of experimentally infected *Anopheles quadrimaculatus* in mice, hamsters and tree rats<sup>3</sup> has brought new interest and a renewed attempt to solve the problem of the existence of a primary tissue phase of *P. berghei*. We report here some preliminary results of these investigations and our findings of pre-erythrocytic stages of development of this parasite.

The prepatent periods of 94 sporozoite-induced infections in laboratory-bred animals (white mice, golden hamsters, young albino rats and *Thamnomys*) were investigated. It was found that the incubation period varied greatly. The earliest appearance of parasites in the peripheral blood was observed 65 h after intracardial sporozoite inoculation. The majority of the animals showed a prepatency of 3–6 days. A longer prepatent period of 8–10 days was also observed on several occasions. The investigation brought to light the fact that the route of sporozoite inoculation and the animal-host species had but little effect on the duration of the prepatent period. It was the number of viable, invasive sporozoites introduced by bite or inoculation that was significant. Intracardial inoculation of 360 sporozoites resulted in a patency of 6 days. 37,000 sporozoites from the same course also produced a patency of 6 days. The bite of a single, very lightly infected mosquito which could introduce no more than 30–50 sporozoites produced a parasitaemia in a mouse within 4 days. Sub-inoculation of heparinized blood obtained by cardiopuncture from animals during their prepatent period into clean rodent hosts revealed the existence of a negative, non-infective phase of the blood which lasted from 2 h after sporozoite inoculation to 51 h. This finding is in contrast with the results obtained from blood-induced infections in which an absence of incubation

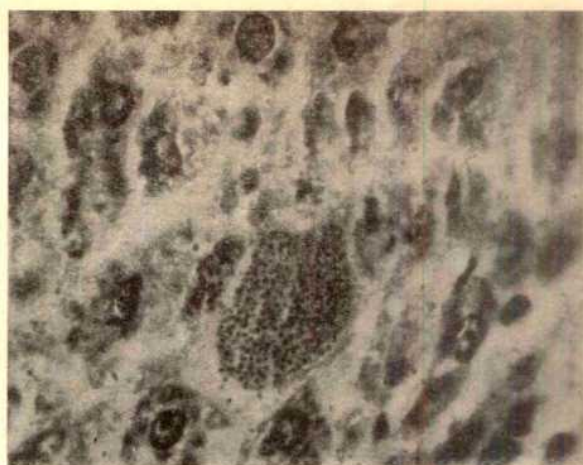


Fig. 2. Photomicrograph of section of liver (BH 2385) showing mature exo-erythrocytic schizont of *P. berghei* ('Polaroid' camera, oil immersion)

period after massive intraperitoneal trophozoite inoculation has been demonstrated by Schneider<sup>4</sup>.

Fifty-four experimental animals, which were exposed to bites of infected *A. quadrimaculatus* or inoculated with sporozoites, were killed during different stages of their prepatency. Large numbers of touch preparations from the liver, spleen, brain, kidney, bone marrow, lung, mesentery, muscles, heart muscle, adrenal, thymus, skin, intestine and lymph nodes were prepared from each animal. These were fixed in methanol, stained with Giemsa, and examined. Histological sections from the same organs and tissues were also prepared. Tissues were fixed in Carnoy and also in Zenker-formol. They were sectioned at 4  $\mu$  and stained in Giemsa colophonium and in Maximow stain. The present observations relate only to a single animal. The experimental procedure was as follows.

Ninety-one *A. quadrimaculatus* infected with KSP 11 strain of *P. berghei* were allowed to feed on a normal young hamster (BH 2385) on July 22–23, 1964. Fifty-five lightly- and medium-infected salivary glands and 62 heavily-infected crushed midguts were inoculated intramuscularly and intraperitoneally during the same period. The animal was killed at 3 p.m. on July 24, 51.5 h after the first infective bites and 23.5 h after the final sporozoite inoculation. Examination of stained blood smears of the hamster at the time of death did not show any parasites in the peripheral blood. However, two white mice which were inoculated with the heparinized blood obtained by cardiopuncture showed parasites in their blood five days later. A control hamster that was exposed to bites and sporozoite inoculation of the same batch of infected mosquitoes showed parasites in its blood after a five-day prepatency. Examination of touch preparations from the different tissues and organs of the experimental hamster did not reveal forms of parasitic nature. In sections of the liver stained in Giemsa colophonium a few forms were found which show all the morphological particulars of true mammalian plasmodial tissue forms as described in monkey and human malaria. What appeared as a mature schizont in a liver cell was seen through three successive 4  $\mu$  sections. It was 36.5  $\mu$   $\times$  35  $\mu$  and contained approximately 560 nuclei (Figs. 1 and 2). In its appearance the schizont greatly resembled *P. imai* tissue forms as described by Garnham<sup>5</sup>.

The quick development and growth of the pre-erythrocytic stages of *P. berghei* (51 h) are of great interest. It is as yet too early to theorize or to predict if only a single generation of tissue forms develop from the sporozoites or if some schizonts remain in the liver and cause relapses. An intensive search is at present being carried out to find in the material available and in new experimental infec-

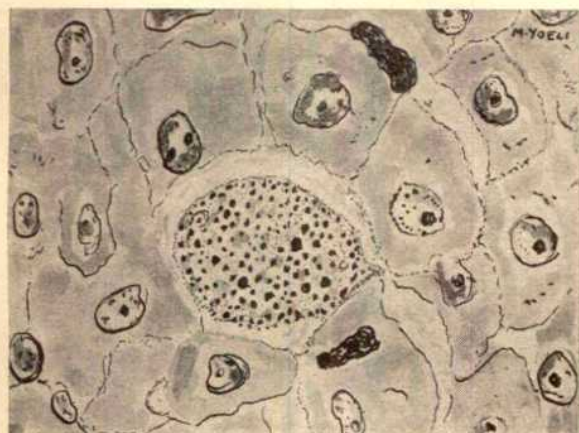


Fig. 1. Drawing from section of liver of baby hamster, BH 2385, showing pre-erythrocytic schizont 51 h after initial sporozoite inoculation (oil immersion  $\times$  c. 750)



tions all the different developmental stages in order to trace their true pattern.

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## IMMUNOLOGY

### Partial Immunological Restoration of Neonatally Thymectomized Rats with Thymus-containing Diffusion Chambers

SEVERAL investigators have demonstrated the important part of the thymus in the development of immunological competence<sup>1-3</sup>. A point of considerable significance is whether this thymic function is mediated by a cellular or a humoral mechanism. There is both direct and indirect evidence of a humoral factor in thymic activity in the mouse. Thus, genetic studies have established that the cellular constitution of thymus tissue grafted to mice thymectomized at birth is of host rather than donor origin<sup>4,5</sup>, and other work has established that partial immunological restoration of such thymectomized mice can be achieved by thymus-containing diffusion chambers which do not permit the passage of whole cells<sup>6,7</sup>. However, since the restorative effect of such chambers is incomplete, and since thymic extracts have not produced appreciable restoration, it remains unsettled whether a cellular mechanism is involved in addition to the humoral one and investigation in a second species appears to be warranted. The present note reports our investigations over the past 18 months of immunological restoration of neonatally thymectomized rats with thymus-containing diffusion chambers. The results are in general agreement with the mouse experiments, and show that partial immunological restoration is achieved but the restored animals are not immunologically normal.

New-born Sprague-Dawley rats were thymectomized within 24 h of birth by a technique previously described<sup>8</sup>. Diffusion chambers were constructed of TW (pore size 0.45 $\mu$ , thickness 100 $\mu$   $\pm$  10 $\mu$ , nylon reinforced) 'Millipore' filter disks (obtained from the Millipore Filter Corporation, Bedford, Massachusetts) cemented with Millipore No. 1 cement to lucite rings of 13 mm outer diameter, 3 mm height and 1 mm wall thickness<sup>9</sup>. Two different experimental procedures were followed. In the first (Tables 1-4), chambers either empty (thymectomy control) or containing a single whole thymus from 1-3-day-old rats were placed with strictly sterile technique into the abdominal cavity of 11-14-day-old thymectomized animals. Ten days later a second diffusion chamber either empty or containing a 3-7-day-old thymus was similarly placed. Approximately 10 days after the second chamber was inserted, total and differential blood counts on tail vein blood were performed (Table 2) using standard haematological technique. When the animals were 8 weeks old they were immunized<sup>10</sup> in a foot pad with 0.1 ml. of complete Freund's adjuvant containing 0.3 mg of tubercle bacilli and 0.5 mg of bovine serum albumin (BSA). Twenty days after immunization the animals were skin tested with 0.1 ml. of a 1-10 dilution of Old tuberculin

Table 1. MORTALITY \* IN NEONATALLY THYMECTOMIZED RATS

	No. animals	Deaths	Mortality	Excess mortality
Normal controls	24	5	21%	
Thymectomized controls (empty chamber)	44	29	66%	45%
Thymectomized (thymus in chamber)	42	20	47.5%	26.5%

\* In first 140 days of life.

Table 2. LYMPHOCYTE COUNTS IN THYMECTOMIZED RATS

	Lymphocytes (thousands per mm <sup>3</sup> )					
	2-4	5-6	7-8	9-10	11-12	>12
Normal controls	2	3	5	6	5	2
Thymectomized controls (empty chamber)	11	11	6	4	3	1
Thymectomized (thymus in chamber)	8	12	6	6	4	4

Table 3. DELAYED HYPERSENSITIVITY TO BOVINE SERUM ALBUMIN IN THYMECTOMIZED RATS

	Diameter of reaction		
	0-3 mm	4-7 mm	>7 mm
Normal controls	0	0	16
Thymectomized controls (empty chamber)	9	1	6
Thymectomized (thymus in chamber)	7	2	11

Table 4. DELAYED HYPERSENSITIVITY TO TUBERCULIN IN THYMECTOMIZED RATS

	Diameter of reaction		
	0-2 mm	3-5 mm	>5 mm
Normal control	5	2	6
Thymectomized control (empty chamber)	3	8	2
Thymectomized (thymus in chamber)	4	3	10

Table 5. SKIN HOMOGRAFT REJECTION IN THYMECTOMIZED RATS

	Day of rejection		
	<14	14-24	>24
Normal control	8	1	1
Thymectomized control (empty chamber)	1	2	9
Thymectomized (thymus in chamber)	8	2	4

(OT) and 0.1 ml. of BSA (30  $\mu$ g) injected intracutaneously into opposite shaved flanks, and the delayed reaction (average diameter of induration in mm) was measured after 24 h and is recorded in Tables 3 and 4. The skin-grafting experiment (Table 5) was performed differently: the first thymus-containing diffusion chambers were inserted when the animals were 4 weeks old, and the animal was grafted<sup>11</sup> with Sprague-Dawley skin of an unrelated animal of the same sex 3-4 days later. A second chamber was not inserted. The protecting plaster casts were removed 7 days after grafting, exposing conventional full-thickness skin grafts which were examined visually at that time and every 2-3 days thereafter.

While the part of secondary infection in the thymectomy wasting syndrome remains uncertain<sup>1,12,13</sup>, wasting and death are regular consequences of neonatal thymectomy in the rodent. In the present investigation thymus-containing diffusion chambers reduced the excess mortality of neonatally thymectomized rats to approximately half that of animals with empty chambers (Table 1). On post-mortem examination it was found that about half the animals that died had advanced suppurative disease of the lungs, pleura and pericardium, a disease which tends to spread to unthymectomized cage mates, thus accounting for the mortality among unoperated animals. It will be seen in Table 2 that thymus-containing diffusion chambers have only slight restorative value on the depressed lymphocyte counts of neonatally thymectomized rats.

Our results confirm the finding of Waksman *et al.*<sup>2,10</sup> that in the neonatally thymectomized rat delayed hypersensitivity is depressed, and that this depression varies from animal to animal. This variability may be caused by ectopic thymic tissue<sup>14</sup> or may simply be due to the individual variation of a non-inbred species. Nevertheless, in our experiments thymus-containing diffusion chambers do partially restore delayed hypersensitivity to both bovine serum albumin and tuberculin of rats thymectomized neonatally. Thus 13 or 20 restored animals displayed delayed reactions to BSA, whereas only 7 of 16 thymectomized controls with empty chambers reacted. Partial restoration of immunological responsiveness can also be seen in the skin-grafting experiment (Table 5). Only 1 of 12 thymectomized controls rejected skin grafts

in less than 14 days, but 8 of 14 thymectomized animals bearing thymus-containing diffusion chambers had shown rejection at this time. Similar results have been reported in a preliminary communication from another laboratory<sup>15</sup>.

Thus the neonatally thymectomized rat, like the mouse<sup>6,7</sup>, can be partially restored immunologically by thymus-containing diffusion chambers which do not allow the passage of whole cells. However, as in the mouse, this chamber has little influence on the depressed lymphocyte count, and the improvement in mortality, delayed hypersensitivity and homograft reaction is only partial. If anything, restoration is less complete than in the mouse, a fact which may be related to the disadvantage of the larger thymus of the larger animal in diffusion of metabolites and its consequently more limited viability<sup>16</sup>. However, it appears that a humoral mechanism is involved. It remains uncertain whether the incompleteness of restoration of immunological function reflects the inefficiency of the thymus-containing chambers, or implies a second (cellular) mechanism of thymic action.

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### Correlation between Complement-fixing Cell Antibody and Immunofluorescent Nuclear Antibody in Hamsters bearing 'SV40'-induced Tumours

THE fact that transformation of hamster cells by 'SV40' results in the synthesis of new viral-induced antigen(s) was first inferred by *in vivo* immunity tests<sup>1-4</sup>. This phenomenon was later detected *in vitro* by complement-fixation<sup>5</sup> and by immunofluorescence<sup>6,7</sup>. This latter approach has characterized the antigen as being particulate, intranuclear and present in 100 per cent of the transformed cells. Corresponding information regarding the intracellular site of the complement-fixing antigen and the proportion of cells in a culture synthesizing it has not yet been reported, and, indeed, such data are difficult to obtain by a direct approach.

Experiments were performed using both the complement-fixation and immunofluorescent techniques in order to correlate the two types of reactions. The sera tested were from tumour-bearing hamsters bled about three months after inoculation with 'SV40'-transformed cells.

Serum dilutions of 1:2 were used in the immunofluorescence assays and 1:10 in the complement-fixation tests. Control sera were obtained from hamsters with tumours induced by the hamster cell line 'BHK21' (ref. 8), which was shown to be free of 'SV40'-tumour cell antigen<sup>9</sup>. Two sources of complement-fixing antigen were used: extracts of 'H-50' and '2X-10' cells. The first is a stable virus-free cell line derived from an 'SV40'-induced hamster tumour<sup>10</sup> and has afterwards undergone 40 passages; the second is also a stable virus-free cell line but derived from hamster cells transformed *in vitro*<sup>11</sup>. Two full units of complement were used, in a semi-micro method to be described. For the immunofluorescence tests the same cell lines were used. They were grown as monolayers on round, 15-mm coverslips and stained with the sera under test as described<sup>11</sup>. Known positive and negative sera were included in each run.

Table 1. COMPARISON OF RESULTS OBTAINED BY COMPLEMENT-FIXATION AND IMMUNOFLUORESCENCE ON SERA FROM TUMOUR-BEARING HAMSTERS

Immunofluorescence	Complement fixation			Totals
	Positive	Questionable	Negative	
Positive	21	1	2	24
Questionable	1	1	2	4
Negative	2	1	28*	31
Totals	24	2	32	58

\* With few exceptions, the negative sera were from animals with small tumours (<10 × 10 mm).

Similar results were obtained in both complement-fixation and immunofluorescence tests with both hamster cell lines. The results of these paired experiments are summarized in Table 1. Of 58 different sera tested, 5 gave questionable reactions in one or the other test. Of the 53 sera yielding clear-cut results in both tests, 49 (93 per cent) gave either positive or negative results both in the complement-fixation and in the immunofluorescence tests. Of these, 21 were positive in both tests and 28 were negative in both tests. The 'BHK21' control sera were uniformly negative against the 'SV40' cell antigens.

From the high degree of correlation between the complement-fixation and immunofluorescence results, both antibodies must develop simultaneously in hamsters carrying the 'SV40' tumours, or the two different methods are detecting the same antibody. If this is true, then the complement-fixing antibody would be the result of the animal's immune response to the particulate, intranuclear, viral-induced antigen present in all virus-free transformed cells. Furthermore, if the antigens detectable by complement-fixation and immunofluorescence elicit the same antibody, then the intracellular site of synthesis of the complement-fixing antigen would also be the nucleus, and the antigen would be produced in every cell of a transformed culture.

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### Use of DEAE-'Sephadex' for the Fractionation of Allergic Serum

RECENTLY, Gelotte *et al.*<sup>1</sup> have reported that chromatography on DEAE-'Sephadex A-50' of a 7S fraction of normal human serum resulted in the isolation of IgG ( $\gamma_{ss}$ ) and IgA ( $\gamma_{1A}$ ) components which appeared to be free of other protein constituents. We have modified their procedure for the isolation of fractions containing skin-sensitizing activity from the sera of ragweed-sensitive individuals.

The sera from a treated (B.P.) and untreated (R.A.) allergic patient were used for these investigations and gave essentially identical results. The allergic serum (7 ml.) was dialysed overnight against 0.1 M *tris*-hydrochloric acid buffer, pH 8.0, and filtered on a column (2.5 cm  $\times$  100 cm) of 'Sephadex G-200' (Pharmacia). Elution with the *tris*-hydrochloric acid buffer resulted in 3 peaks corresponding to 19S, 7S and albumin components (Fig. 1). The eluates of appropriate cuts were pooled and the pools concentrated to the initial serum volume (7 ml.) by dialysis against polyethylene glycol M 20. The concentrated fractions were exhaustively dialysed against 0.15 M sodium chloride and analysed for skin-sensitizing activity by the passive transfer (*P-K*) test<sup>2</sup>. As shown in Fig. 1, reaginic activity was localized in 3 fractions (I, II and III) between the descending portion of the 19S peak and the ascending portion of the 7S peak (cf. refs. 3 and 4). A 2-ml. aliquot of 'Sephadex' fraction II, containing the bulk of the skin-sensitizing activity, was dialysed overnight against 0.1 M *tris*-hydrochloric acid buffer, pH 8.0, and chromatographed on a column (1.0 cm  $\times$  26 cm) of DEAE-'Sephadex A-50' (Pharmacia); the resin was previously equilibrated with the same buffer. Two protein fractions were eluted with 0.1 M *tris*-hydrochloric acid and additional fractions after a linear gradient was established from 0.1 to 0.5 M *tris*-

hydrochloric acid of the same pH (Fig. 2). A final fraction was obtained by passing 1.0 M *tris*-hydrochloric acid (pH 8.0) through the column. The cuts for the various fractions shown in Fig. 2 were made according to the shape of the optical density curves. The fractions were concentrated to the initial serum volume (2 ml.) and examined

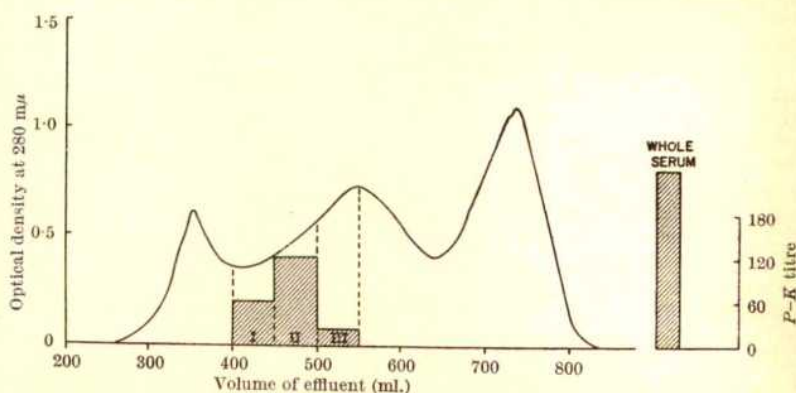


Fig. 1. Gel filtration of allergic serum (R.A.) on 'Sephadex G-200' in 0.1 M *tris*-hydrochloric acid buffer, pH 8.0, showing distribution of skin-sensitizing antibody. The *P-K* titre of fractions I, II and III are compared to that of the whole allergic serum

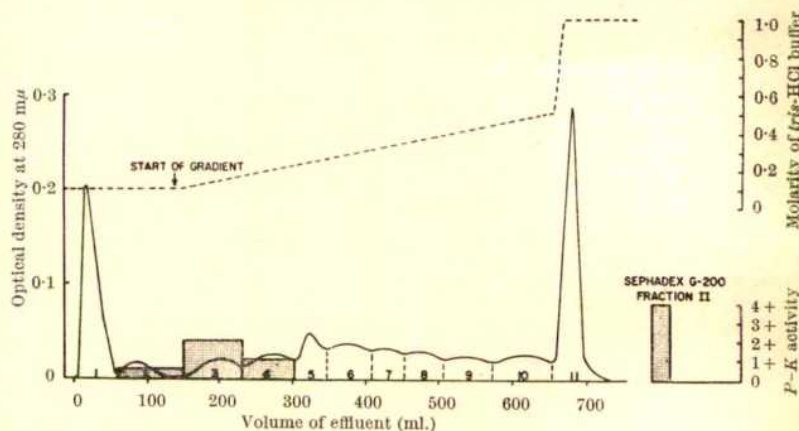


Fig. 2. Chromatography of fraction II of serum R.A. on a column of DEAE-'Sephadex A-50'. Elution was started with 0.1 M *tris*-hydrochloric acid, pH 8.0, followed, at the arrow, by a linear gradient of 0.1-0.5 M *tris*-hydrochloric acid, pH 8.0. A final fraction, 11, was obtained by applying 1 M *tris*-hydrochloric acid, pH 8.0, directly to the column. The *P-K* activity of fractions 2, 3 and 4 are compared with that of whole fraction II

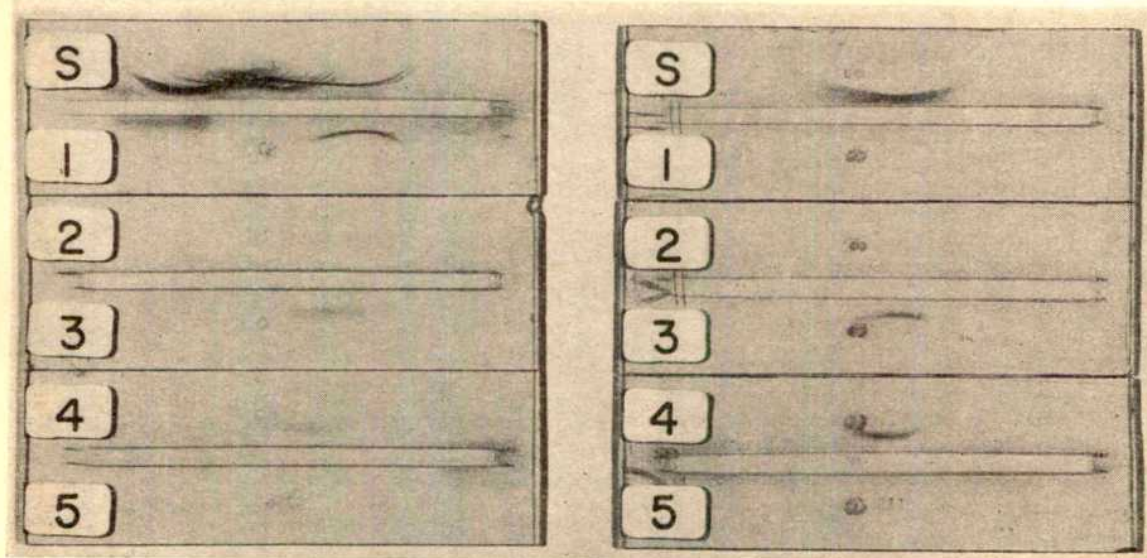


Fig. 3. Comparison of immunoelectrophoretic patterns of whole allergic serum (R.A.) and sub-fractions obtained by chromatography of fraction II on DEAE-'Sephadex A-50'. The precipitin arcs were developed with horse anti-serum to whole human serum (left) and with specific goat anti-serum to human IgA (right). S = allergic serum; 1-5 = the various sub-fractions. The precipitin arc obtained with fraction 2 and anti-whole serum appeared as a very faint band in the IgG region and was too weak for reproduction in the photograph



by *P-K* test and by immunoelectrophoresis<sup>5</sup> using specific antisera (Hyland) to human serum proteins (Fig. 3). Fractions 1 and 2 consisted of *IgG* components only: fraction 1 was consistently inactive whereas fraction 2 had slight but definite activity. Fractions 3 and 4 contained the bulk of the skin-sensitizing activity and consisted of *IgA* components. It is noteworthy that the *IgA* components of fractions 3 and 4 had slightly different electrophoretic mobilities.

The chromatographic procedures described appear efficacious for the isolation of discrete serum components with skin-sensitizing activity. Active *IgA* components have been isolated free of other immunoglobulins when tested by immunoelectrophoresis. These findings are in accord with recent investigations<sup>3,4,6</sup> which have indicated the association of skin-sensitizing antibodies with *IgA* components. On the other hand, we have also found an *IgA* component having slight but definite skin-sensitizing activity. Investigations are in progress to determine the presence of trace amounts of *IgA* in this fraction.

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## BIOLOGY

### Lowering of Immunity in Sheep following Injections of Chlorambucil

THE effects of amino-chlorambucil, an alkylating agent, on the establishment of homografts of haemopoietic tissue in rabbits have been described by Cree<sup>1</sup>. The preliminary experiments described here illustrate the effectiveness of a related compound, chlorambucil, in suppressing certain immunological responses in the sheep.

Preliminary investigations showed that a single intravenous dose of 4 mg/kg body-weight resulted in the death of one sheep from lung oedema in less than 18 h. Post-mortem examination revealed widespread degeneration of

walls of the blood vessels, haemorrhage and extensive necrosis of the kidneys and early necrosis of lymphoid tissue of the spleen, lymph nodes and Peyer's patches. An intravenous dose of 2 mg/kg to an adult Suffolk sheep (85 kg) was, however, well tolerated and produced within 5-6 days a rapid fall in the total circulating leucocyte count from values of about 10,000 to 1,000-3,000 mm<sup>3</sup>. In the absence of further doses of chlorambucil the circulating leucocyte counts rose slowly over a period of weeks to the pre-treatment level. A dose of 2 mg/kg was lethal in two young splenectomized rams.

Four adult Suffolk and one Clun sheep, all of which had been examined regularly over a period of a year by haemagglutination and complement-fixation tests for antibodies against gastro-intestinal nematodes were injected intravenously with chlorambucil (2.0 mg/kg body-weight). Total and differential leucocyte counts were carried out daily.

Intravenous doses of chlorambucil were continued in an attempt to keep the peripheral white cell count at values of approximately 1,000 mm<sup>3</sup>, and at this figure circulating lymphocytes were about 5 per cent of their original values. No ill effects were observed in the sheep. In one animal a single dose of 'Epodyl' (triethylene glycol diglycidyl ether) at the rate of 130 mg/kg body-weight and a single dose of the corticosteroid betamethasone at the rate of 0.24 mg/kg were injected.

Haemagglutination and complement-fixation tests, using an antigen prepared from third-stage infective larvae of *Haemonchus contortus*<sup>2</sup>, were performed at intervals of a few days. In all cases the complement-fixation and haemagglutination titres fell to values of one half or less within 3 weeks of the first injection. The results on two sheep injected with chlorambucil and one injected with chlorambucil and the other drugs are shown in Table 1. The remaining two sheep showed no increase in worm egg counts but antibody-levels fell from titres of 1,280 to 640 and 160 to 80 with the haemagglutination technique and 160 to 40 and 160 to 40 with the complement-fixation technique, respectively.

The post-mortem burden of parasites of sheep No. 66 was as follows:

<i>Ostertagia</i> spp.	1,500
<i>Trichostrongylus axei</i>	400
<i>Bunostomum trigonocephalum</i>	295

Several points of interest arise from these preliminary observations. First, when certain animals are used in homografting experiments it may be necessary to take measures against the parasite burden of the animal as well as taking the usual precautions against bacterial infection.

The rise in egg count which followed the administration of chlorambucil occurred at an interval which was too short to allow for re-infection and, moreover, the animals were housed under worm-free conditions calculated to prevent re-infection. Furthermore, the egg count of ewe No. 66 had been very low or negative for several weeks

Table 1. EFFECT OF CHLORAMBUCIL ON TOTAL WHITE CELL COUNT COMPLEMENT-FIXATION AND HAEMAGGLUTINATION TITRES

Sheep No.		1	2	3	4	5	6	7	9	13	18	21	28	32	39
76	Total WBC count	8,000	7,500	7,600	6,100	4,500	1,200	3,800	—	—	—	—	—	—	—
	C.F. test	160	—	—	80	—	—	40	—	—	—	—	—	—	—
	H.A. test	320	—	—	160	—	—	160	—	—	—	—	—	—	—
	Egg count	Neg.	—	—	Neg.	—	—	—	—	200	—	100	—	—	900
66	Total WBC count	15,000	—	11,300	—	7,500	—	4,000	2,500	6,000	1,000	6,300	—	—	—
	C.F. test	320	—	—	—	—	—	—	—	160	80	80	—	—	—
	H.A. test	1,280	—	—	—	—	—	—	—	320	320	160	—	—	—
	Egg count	Neg.	—	—	—	—	—	—	—	750	2,800	1,800	—	—	—
80	Total WBC count	9,500	—	3,800	1,400	1,000	2,000	3,400	3,000	3,200	2,200	1,200	900	1,000	1,700
	C.F. Test	160	—	—	—	—	—	—	—	80	80	80	40	40	20
	H.A. test	1,280	—	—	—	—	—	—	—	1,280	1,280	640	640	640	320
	Egg count	100	—	—	—	—	—	—	—	200	100	100	100	100	200

Sheep No. 76. Wt., 80 kg; 160 mg chlorambucil injected intravenously on day 1.

Sheep No. 66. Wt., 80 kg; 160 mg chlorambucil injected intravenously on day 1 and 320 mg in divided doses between days 5-20.

Sheep No. 80. Wt., 85 kg; 170 mg chlorambucil injected intravenously on day 1 and 290 mg in divided doses between days 6-15. Day 21. 'Epodyl' intravenous. 11G. 'Betamethasone' intramuscular 20 mg. Day 22. Bone marrow transplant from unrelated sheep.

before the injection of the compound and consequently the parasites must have been dormant or in an arrested stage of development, either as non-gravid adults or possibly as late fourth or young fifth larvae.

Though the phenomenon of arrested development is well known with the *Ostertagia* species, it is interesting to note that it may also occur with hookworms (*Bunostomum*). Other work has verified this. The absence of a substantial rise in egg counts in the other sheep may indicate a fortuitous result with regard to No. 66, but it is felt a more satisfactory explanation might be that they possessed only a small number of parasites. Comparable sheep usually possess low burdens at this time (November).

The haematological effects of chlorambucil in sheep are similar to those observed in the rat by Elson<sup>3</sup> and to those described as occurring in man when the drug is given for the treatment of Hodgkins's disease<sup>4</sup>. The effect of the drug on lymphoid tissue is of special interest since recent investigations have indicated that the lymphoid series along with circulating antibody probably play an important part in the immune response of the host to helminth infection<sup>5</sup>.

These investigations were conducted on sheep exposed to natural gastro-intestinal nematode infection and the burden could not be determined before the injections of chlorambucil were given. With defined experimental infections different types of cytotoxic drugs should be of value in assessing the relative role of the various cell types in immunity to helminths.

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## Homotransplantation of the Hamster Uterus

THE investigation of utero-ovarian relationships would be facilitated by the development of a successful technique for the transplantation of the uterus. Transplants of the uterus have been examined in several species: in the guinea-pig by Butcher *et al.*<sup>1</sup>, Hechter *et al.*<sup>2</sup>, and Loeb<sup>3</sup>; in the rabbit by Mishell and Motylloff<sup>4</sup>, Chu *et al.*<sup>5</sup>, and Sessums and Murphy<sup>6</sup>; in the mouse by Parfenoff<sup>7</sup> and Hall<sup>8</sup>; and in the dog by Cheval<sup>9</sup>. In these investigations intraperitoneal, intramuscular or subcutaneous sites were used for the transplantation of the entire uterus, uterine slices, endometrium, endometrial scrapings or suspensions of uterine tissue. Histological observations of the transplants either were not always presented or, when reported, usually exhibited degenerative changes. The development of a successful technique for the heterotransplantation of rabbit oviduct to the cheek pouch of the Syrian hamster as reported by McDaniel and Black<sup>10</sup> prompted a similar investigation using uterine transplants. The objective of this investigation was to evaluate the survival of uterine transplants in the cheek pouch of the hamster.

Twenty adult female hamsters (*Mesocricetus auratus*) weighing between 60 and 100 g were used as donor animals. Sub-total hysterectomies were performed on the donor animals anaesthetized with sterile 'Nembutal' (7 mg/100 g of body-weight). Each hysterectomized animal then became a host. The uterus was rinsed in sterile saline and placed in a prophylactic bath consisting of  $5 \times 10^6$  units of buffered penicillin G and 10 g of dihydrostreptomycin sulphate dissolved in 80 ml. of Locke's solution.

After removal of excess connective tissue, the uterus was divided into six segments (each approx. 6 mm long) with both ends of each segment closed with nylon thread ligatures. The segments were then cut from the uterus with a razor blade or fine scissors. The host was then anaesthetized and the cheek pouch everted and washed with 'Zephiran' chloride. Three small slits were cut in the cheek pouch epithelium, and a segment of the uterus was introduced into each one. To prevent loss of the transplants, the incisions were sutured. Afterwards, the transplants were examined at various times with the aid of transilluminated light to determine their condition.

Later (95–132 days after transplantation), the centre transplant from each pouch was removed from five animals. The excised transplants were fixed in 10 per cent neutral formalin, sectioned and stained with haematoxylin and eosin. Histological observations are recorded in Table 1.

Table 1. HISTOLOGICAL APPEARANCE OF THE SURFACE AND GLANDULAR EPITHELIUM OF UTERINE HOMOTRANSPLANTS IN THE HAMSTER

Transplant No.	Days after transplantation	Day of cycle	Surface epithelium	Glandular epithelium
D2 <sub>1</sub>	132	1	+ + + + *	+ +
D2 <sub>2</sub>	132	1	+ + +	+
D3 <sub>1</sub>	122	1	+ + +	+ + + +
D3 <sub>2</sub>	122	1	+ + +	+
D4 <sub>1</sub>	122	3	+ + + +	+ +
D4 <sub>2</sub>	122	3	+ + + +	+
D5 <sub>1</sub>	123	1	+ + + +	+ + + +
D5 <sub>2</sub>	123	1	+ + + +	+
D8 <sub>1</sub>	95	1	+ +	+ +
D8 <sub>2</sub>	95	1	+ + + +	+

\* Relative amounts of epithelial cells.

+ Sparse; + + + +, considerable.

Excised transplants were examined for the condition of endometrial tissue and general histological organization. A lumen of variable size was present in all ten transplants. In any one transplant, the height of the surface epithelium varied from squamous to tall cuboidal or columnar and was intact and healthy in all transplants. Glandular elements with lumina were relatively numerous in five transplants. In the remaining transplants, glands were either sparse or appeared without a lumen. All transplants showed a moderate although variable degree of fibrosis that disrupted the organization of the myometrium and endometrial stroma. Sites of lymphocyte infiltration were infrequent and small. Few plasma cells were seen.

The larger animals weighing 80–100 g were found to be more suitable hosts since their larger cheek pouches more readily accommodated the three transplants. The use of sutures to close the site of transplantation and the use of a prophylactic bath are modifications of the procedure described by McDaniel and Black<sup>10</sup>. These steps prevented the loss of transplants and increased their chance of survival by decreasing the incidence of infection. The most significant result of this investigation is the long-term survival of endometrial tissue in all transplants. The technique described here is at present being used in this laboratory to investigate utero-ovarian relationships in the Syrian hamster.

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## Effects of High-power Green Laser Radiation on Cells in Tissue Culture

THE availability of high-power, coherent radiation at new wave-lengths, by means of non-linear optics, has made possible new experiments in the field of radiation effects on cells in tissue culture. It has been shown<sup>1,2</sup> by exposure of melanin granules to ruby radiation at 6943 Å and its harmonic at 3471 Å, that injury sustained by cells in tissue culture is strongly dependent on wave-length. This confirmed the observations by Bessis *et al.*<sup>3</sup> that erythrocytes were destroyed by ruby laser radiation while unstained leucocytes were not affected. Because particular cellular constituents such as pigment granules, haemoglobin and reduced cytochromes *b* and *c* show absorption at 5300 Å, there was great interest in exploring the radiation effects from the second harmonic output at 5300 Å of neodymium (Nd) Q-switched lasers. In the work reported here, a variety of cell types were irradiated with power density at this wave-length in the range of 0.05–100 MW/cm<sup>2</sup> to observe their response.

The experimental arrangement used is shown schematically in Fig. 1. The unfocused 10-MW output from the neodymium laser was passed through a potassium dihydrogen phosphate crystal which was properly index-matched for maximum energy conversion from 1.06 μ to

5300 Å. A dispersive prism separated the 1.06 μ and the green 5300 Å radiation. The green radiation was reflected from a high-quality mirror and focused with an 8-in. lens on the specimen. The power was monitored by measuring the energy incident on a calibrated thermocouple and photomultiplier and by measuring the pulse width by means of an *EG* and *G* travelling wave oscilloscope. The measured pulse width at 5300 Å was 10 nsec and at 1.06 μ it was 15 nsec.

Pigmented retinal epithelium, originating from 12-day chick embryos, provided a suitable cell type containing melanin granules. Figs. 2A and 2B show the destructive effect of 100 MW/cm<sup>2</sup> of the focused green laser energy. Additional experiments indicated that pigmented cells were destroyed at the time of exposure by using power densities of as little as 1 MW/cm<sup>2</sup>.

Having observed previously that the ruby laser produced a slowing of rhythmic muscular contraction<sup>1</sup>, isolated pulsating cardiac muscle cells from a 5-day chick embryo were monitored for changes in their beating rates following exposure to 5300 Å. The response of individual cells showed a wide variation which might have been due, in part, to the location of the specimen within the target area. Two exposures of the cells to 100 MW/cm<sup>2</sup> each produced an average decrease in the beating rate of 45 per cent of the pre-irradiated rate. The morphology of the cells was unchanged.

Acting on the assumption that cytochromes *b* and *c* would absorb sufficient energy at 5300 Å to be modified, the mitochondria of isolated 9-day chick embryo kidney cells were irradiated with a focused laser beam. The morphology of the mitochondria within these tubular epithelial elements was not significantly altered with as many as 20 exposures of 50 MW/cm<sup>2</sup> each, although a transitory beading along the course of the filamentous organelle was observed in some of the cells.

A sheet of chick amnion tissue was mounted in a modified Rose multi-purpose culture chamber and the rate of oxygen consumption measured with a miniaturized Clark electrode<sup>4</sup>. The oxygen was replenished by a

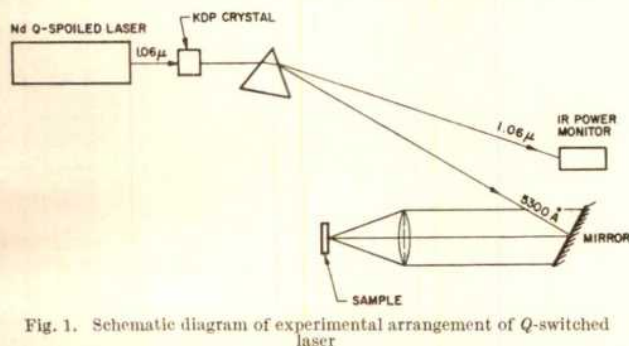


Fig. 1. Schematic diagram of experimental arrangement of Q-switched laser.

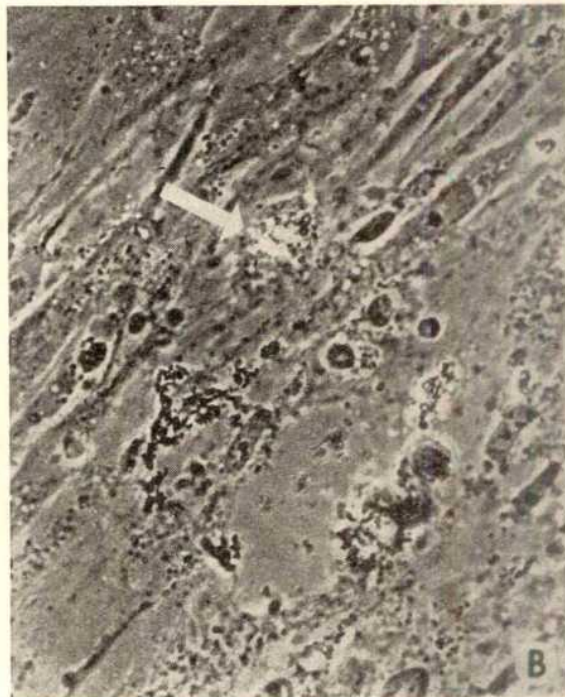
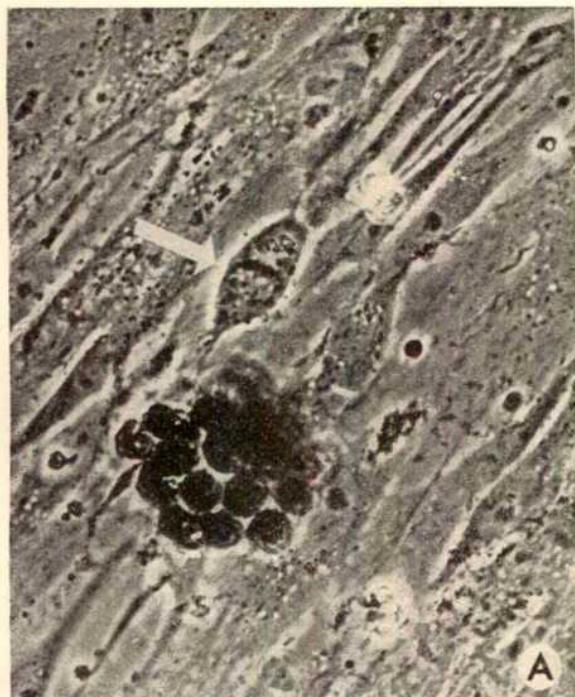


Fig. 2A. An image of pigmented retinal epithelial cells from a 12-day chick embryo before irradiation. The dark spheres constitute a cluster of pigment granules which were not spread on the coverslip. The arrow directs attention to a metaphase figure containing pigment granules. B, The same area as shown in A immediately after irradiation with 100 MW/cm<sup>2</sup> at a wave-length of 5300 Å. The cluster of pigment granules and the mitotic figure (arrow) were disrupted. Other elements in the background were injured or killed, depending on the density and number of pigment granules per cell.



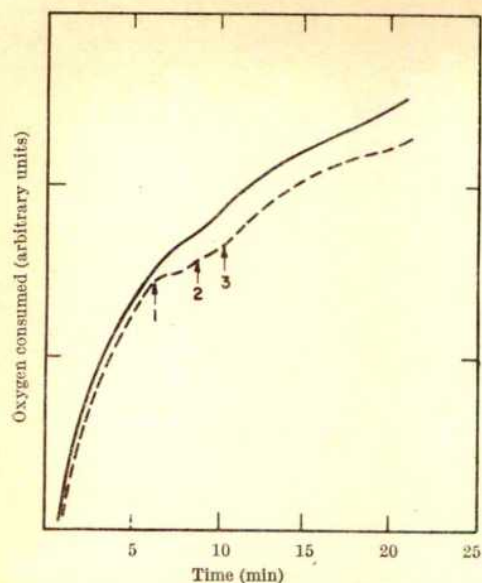


Fig. 3. Representative recorder tracings from a physiological gas analyser, depicting oxygen consumption rates by a sheet of chick amnion in tissue culture. Both lines were consecutive recordings from the same tissue. During the second tracing (dashed line), the tissue was exposed three times to MW of power at 5300 Å each (arrows)

medium exchange and a new record was prepared for the same tissue. The amnion was exposed to multiple flashes of an unfocused laser beam during the second record with a total incident power density of 3 MW/cm<sup>2</sup>. Comparing the control and irradiated rates of oxygen consumption, it was observed that respiratory activity was reversibly inhibited for approximately 2 min after the first exposure to the 5300 Å wave-length (Fig. 3).

It was concluded that cells *in vitro* containing pigment granules absorbed the 5300 Å to such an extent that they were destroyed by as little as 1 MW/cm<sup>2</sup>. Muscular contraction was slowed or stopped by an accumulated dose of 200 MW/cm<sup>2</sup> and oxygen consumption was temporarily inhibited following exposure to 3 MW/cm<sup>2</sup>, but the cells survived. Consistent with previous investigations<sup>1,2</sup>, cellular injury produced by the green laser light was considered to be a function of the amount of energy absorbed by specific cellular constituents.

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### Angiospermous Hemiparasites

LITTLE research has been conducted into the nutrition of hemiparasitic plants since the early part of this century. These plants, predominantly confined to the sub-family Rhinanthoideae of the Scrophulariaceae, become parasitic by establishing root connexions with the host plant through haustoria.

Koch<sup>1</sup>, Ewart<sup>2</sup>, Kostytschew<sup>3</sup> and Heinricher<sup>4</sup> maintained that the hemiparasites obtain only water and mineral nutrients from a host plant. Bonnier<sup>5</sup> showed

that the hemiparasites have only a weak photosynthetic activity and claimed that these plants are dependent on the host for a supply of assimilates. In 1962, Rogers and Nelson<sup>6</sup>, using radioactive tracer techniques, showed that carbon-containing compounds passed from *Zea mays* hosts to mature plants of the specialized hemiparasite *Striga asiatica*. In the present investigations, using a radioactive tracer technique, it is shown that the hemiparasite *Odontites verna* does receive assimilated carbon-containing compounds from the host plant.

*Striga* and *Odontites* lie at opposite ends of a scale of hemiparasitic specialization, *Odontites* being unspecialized

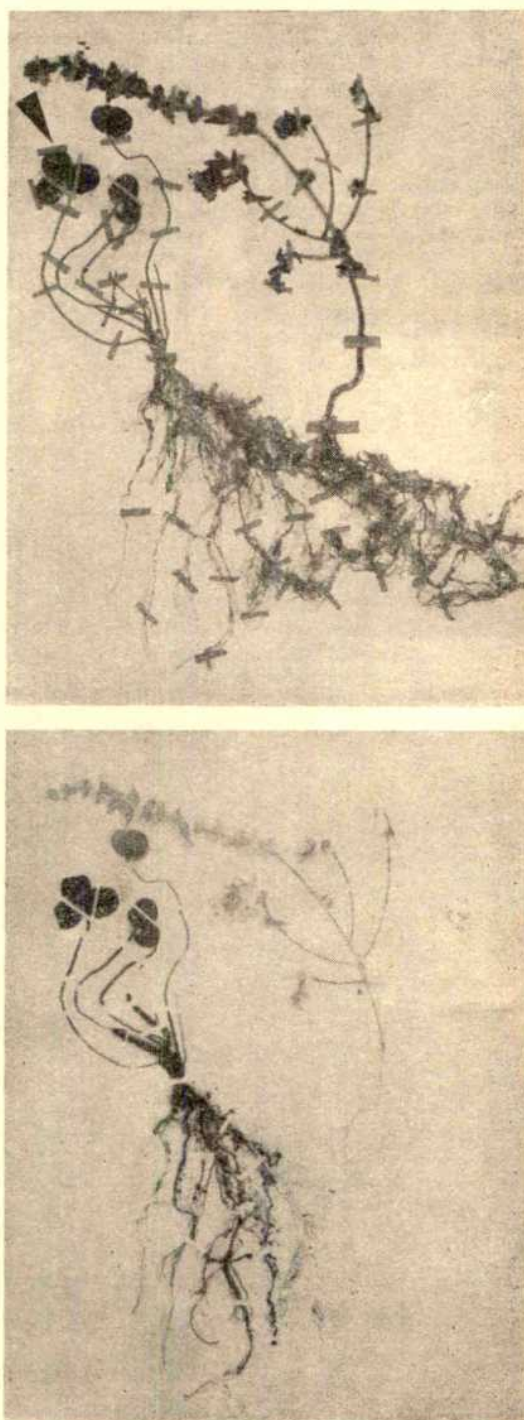


Fig. 1. Photographs of the treated plant (above) and the autoradiograph (below) showing the movement of labelled carbon from the *Trifolium repens* host to *Odontites*. The arrow indicates the fed leaf

in host requirements and capable of germinating, and completing its life-cycle, with the production of viable seeds, in the absence of a host.

Host plants (*Trifolium repens*) parasitized by individuals of *Odontites verna* were grown in pot culture. Individual leaves of the host were exposed to  $^{14}\text{CO}_2$ , in the light, for 24 h. The pairs of plants were then freeze-dried, mounted and exposed to X-ray plates. Fig. 1 shows one of the resulting autoradiographs demonstrating the presence, in the hemiparasite, of carbon-containing compounds from the host plant. Similar  $^{14}\text{C}$  transfer to the parasite has been observed from gramineous hosts. No transfer occurred when parasite and host were growing together but without haustorial union.

The demonstration that both *Odontites* and *Striga* receive assimilates from a host plant may indicate that this is a general characteristic of the Rhinanthoideae.

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### Long-distance Migration of Atlantic Salmon

In recent years a number of authors have reported the recapture in the Greenland area of salmon originally tagged as either smolts or spent fish in British, Canadian and Swedish waters<sup>1</sup>. During the month of October 1964 four salmon originally tagged in rivers in the west of Ireland in County Mayo as clean or spent fish have been reported as having been recaptured off the west coast of Greenland. The details are given in Table 1.

Release data		Table 1		Recapture data	
Date	Place	Date		Date	Place
5/9/63	Carrowmore Lake	6/10/64	Near Julianehaab (60° 40' N, 46° 15' W)		
12/9/63	Carrowmore Lake	21/10/64	Near Supperthoppen (65° 25' N, 53° 00' W)		
25/3/64	Burrishoole River	18/10/64	Off Kangamiut (65° 49' N, 53° 19' W)		
25/4/64	Burrishoole River	17/10/64	Off Kangamiut (65° 49' N, 53° 19' W)		

The first two fish were tagged by an official of the Fisheries Division of the Department of Lands, Dublin, and the second by officials of the Salmon Research Trust of Ireland, Inc., sponsored by Arthur Guinness, Son and Co., Ltd., and the Minister for Lands for Ireland.

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### MICROBIOLOGY

#### Effect of pH and Temperature on Structural Integrity of an L Form of *Streptococcus pyogenes*

L FORMS obtained from group A *Streptococci* are osmotically fragile. Suspensions of the L form, which lacks the rigid bacterial cell wall, in osmotically unprotective environments results in structural disintegration

and loss of viability<sup>1</sup>. A paucity prevails in the literature, however, concerning the effect of temperature and pH on the integrity of the L form in its protective growth medium. This communication deals with a comparison of the effect of such alterations of the growth environment on the structural stability of a group A  $\beta$ -haemolytic *Streptococcus* and a derived stable L form obtained from it with the aid of penicillin.

The *Streptococcus* and L form are the same as those used earlier<sup>2</sup>. The medium for each of these organisms was described<sup>3</sup>. Sterilization of all media by either heat or filtration afforded identical results. For these investigations, 50 ml. of media in 125 ml. Erlenmeyer flasks equipped with side-arm tubes were inoculated with 0.5 and 1.0 ml. of overnight cultures of the coccus and L form, respectively. All incubations ( $\pm 0.25^\circ\text{C}$ ) were performed in a thermostatically controlled water bath. Each organism was grown at  $37^\circ\text{C}$  until an optical density of between 0.250–0.300 (approximately mid-logarithmic growth) was attained before adjusting cultures to the experimental pH with small volumes of either concentrated sodium hydroxide or hydrochloric acid. Cultures were transferred to water baths adjusted to the desired temperature and lysis followed spectrophotometrically; growth and lysis being determined at frequent intervals with a Coleman model 14 spectrophotometer at 650 m $\mu$ . The temperatures and pH's examined are indicated in one of the figures. Un-inoculated media served as blanks for growth; pH-adjusted aliquots of the supernate of collected cultures served as blanks for the lysis investigation. Each value is an average of two determinations. Only logarithmic cocal and L form cultures, the growth rates of which (doublings/h) at  $37^\circ\text{C}$  were close to 1.1 and 0.7 respectively, were used. The rate of L-form lysis is expressed as the negative slope of a culture that had undergone at least 50 per cent lysis within 50 min.

Fig. 1 illustrates a typical example of the effect of an increase in pH on the structure of logarithmically growing cocal cells before and after cell wall removal (that is, stable L form). Fig. 2 summarizes the results of temperature and pH alterations of the growth medium on structural stability. As is apparent, L form structural disintegration was most pronounced at  $48^\circ\text{C}$ , with maximum destruction of cell structure occurring in combination with a pH of 9 (Fig. 2). The decrease in turbidity was correlated with L form structural disintegration by dark phase-

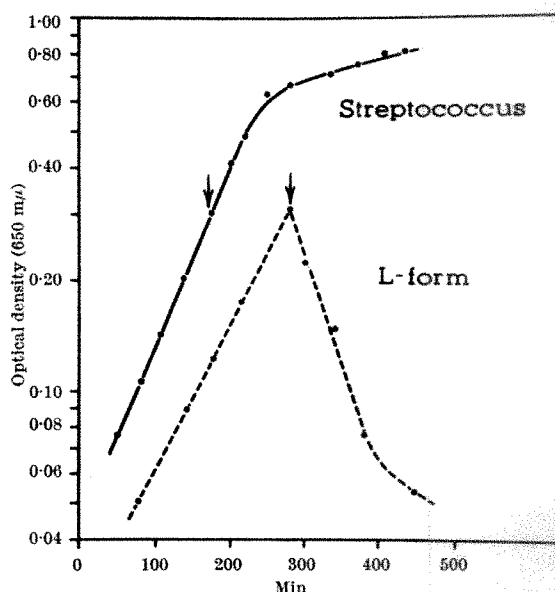


Fig. 1. Effect of pH increase on structural stability of the coccus and derived L form. Arrows indicate time each culture was adjusted to pH 9 and reincubated at the growth temperature ( $34^\circ\text{C}$ ). Growth and lysis rates determined from the straight line portion of curves after pH and temperature changes.



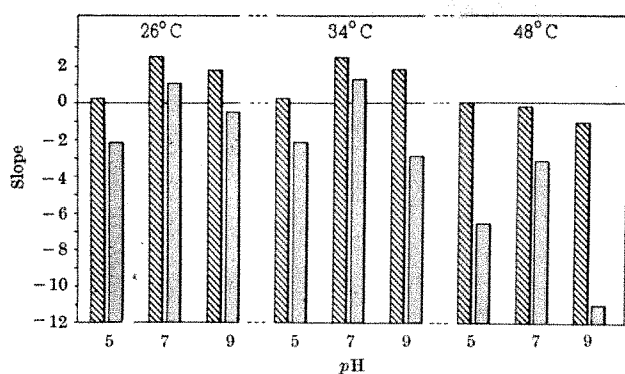


Fig. 2. Summary of results of temperature and pH changes of growth medium on structural stability. Hatched, *Streptococcus*; stippled, *L* form.

contrast microscopy. Microscopic examination of the coccus failed to reveal similar pronounced morphological alterations following identical treatment. Under these conditions of investigation, pH 7 was found to afford the greatest stability to *L* form structure within the wide limits of temperature and pH examined. The effect of alkalinity on *L* form structure increased with increasing temperature; pH 5 exerted its maximal detrimental effects only at 48° C. Following lysis of the *L* form at pH 5 at temperatures of 26° and 34° C, a slow but constant increase in turbidity over an additional 100 min re-occurred. This new growth proceeded at 0.11 and 0.25 doublings/h at 26° and 34° C, respectively. Growth was ascertained by phase microscopy which revealed the reappearance of the typical *L* form structure. Similar results were not noted at the other pH's and temperature examined.

These results suggest that the unprotected membrane of the *L* form is initially affected by drastic changes of pH and temperature on the growth medium. These alterations have provided an insight into the structural instability of the *L* form. Since the *L* form mimics the acidity production of the parent coccus, controlled pH and temperature of the growth medium appears necessary in order to prevent harmful effects on *L* form structure during growth. Coupled with the great diversity of individual cell sizes observed during *L* form growth<sup>4</sup>, these findings may also prove useful in further investigations of *L* form viability and physiology.

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## ENTOMOLOGY

### Sex Attraction in *Rhopaea* (Coleoptera : Scarabaeidae)

THE presence of sex pheromones in insects has been well documented<sup>1</sup>. Travis<sup>2</sup> and Jeannel<sup>3</sup> reported a natural sex lure in the scarab beetles *Lachnosterna lanceolata* Say and *Pachypus cornutus* Olivier respectively, and our investigations with *Rhopaea* spp. provide another such record for the Scarabaeidae.

The *Rhopaea* group of pasture cockchafers are believed to be of considerable economic importance in some areas in

Australia. It was therefore of great interest when a sex attractant was first indicated by large numbers of male *Rhopaea magnicornis* Blackburn trying to dig their way into a field cage containing a virgin female of the same species. Although several species of *Rhopaea* may exist together in mixed populations the sex attractants appear to be species-specific.

It was later found that the scent left in cups which had housed 4- to 15-day-old virgin *R. magnicornis* females was alone capable of eliciting the typical graded male response of fanning out the lamellae of the antennal club and excited walking, culminating in rapid fluttering of the wings and searching behaviour.

Marked *R. magnicornis* males were used in release and recapture trials to determine distances over which caged females could attract males. Field tests were conducted during the first 1-2 h after sundown when the beetles were very active. Recoveries of marked males were affected by wind speed. If breezes were unsteady or less than 3 m.p.h. the males flew at random in search of females; but in steady breezes of about 5-7 m.p.h. the males flew upwind to females. Thus, within 15 min marked males released downwind from the centre of a line of caged females placed at right angles to the wind direction were attracted to virgin females caged up to 30 yd. away. Presumably, competition of wild field females prevented recapture of marked males beyond 30 yd.

In the laboratory *R. magnicornis* females took from 3-8 days to reach the surface of the soil after emerging from their pupal cases and could produce an attractant by the third day of adult life.

Under favourable conditions a caged virgin *R. verreauxi* Blanchard female could attract as many as 50 males within a few minutes. Virgin females of *Rhopaea morbillosa* Blackburn and *R. verreauxi* were capable of emitting their respective attractants on at least three successive nights and, presumably, until a successful mating is achieved. However, in Nature, mating probably takes place soon after a female begins producing an attractant because the multiple-mating habits of males make available a large number of males.

Apparently, no attractant is produced after mating occurs. When caged virgin females were allowed to mate, males which had clustered outside the cage departed within a minute.

The attractant is emitted from the abdomen of the female. Five males were attracted to the abdomen of a dissected *R. magnicornis* female, after which one male was allowed to copulate with it. No males were attracted to the head or thorax.

*Rhopaea* females are capable of flight, unlike the short-winged females of *Lachnosterna lanceolata*<sup>4</sup> and the wingless females of *Pachypus cornutus*. However, females of some *Rhopaea* spp. are seldom seen in Nature, although those of *R. verreauxi* are known to make short dispersal flights after emerging from the ground at dusk.

*R. verreauxi* females were often observed resting or mating on the leaves of *Eucalyptus pauciflora* Sieber ex Spreng, a habit unknown for other species in the genus. Females of *R. heterodactyla soror* Blackburn and *R. magnicornis* have been observed only on tufts of grass or on the ground.

The adults of many scarab species in Australia are attracted to a common food source, such as *Eucalyptus* trees, where they feed and mate; such species have less need to rely on sex pheromones to bring the sexes together and are not known to produce them. On the other hand, adults of the North American scarab, *Lachnosterna lanceolata*, feed on a variety of plants, for example, alfalfa, clover, collards, wheat and oats<sup>5</sup>, and a sex pheromone is presumably of great selective advantage as a mechanism for uniting the sexes. The species of *Rhopaea* investigated are not known to feed as adults, and have only a rudimentary digestive tract. The females release



a potent chemical which attracts the males of the same species. *R. verreauxi*, the only species which has been found on trees, has the best-developed (but non-functional) digestive system; this leads us to suggest that the change in adult feeding habits in this species may have occurred more recently than in the others.

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## GENETICS

### Sex Ratio in Laboratory Populations of *Drosophila*

LABORATORY populations of *Drosophila* have been widely investigated in recent years, and extensive data are available for changes in the frequencies of alternative genes or chromosomal rearrangements. However, little attention has been given to changes in the sex ratio in such populations.

Twelve populations of *Drosophila melanogaster* were examined in this laboratory, using a modification of the cage designed by Thomson<sup>1</sup>. Each population was derived from an inbred line maintained by single sib matings for 14–19 generations.

From each cage a random sample of 300 adult flies was taken periodically to follow changes in the frequency of two segregating sex-linked allelic mutants (white and apricot eye, or white and blood eye), and the proportion of males was also determined.

In 80 of the 229 samples the proportion of males was significantly different from 0.5. There were more significant excesses of males than females, 58–22. For individual cages, the results varied from one significant sex ratio in 21 samples to 18 significant sex ratios in 22 samples. The results for two of the populations are illustrated in Fig. 1, where the proportions of males differing significantly from 0.5 are indicated by small circles.

Heterogeneity of the sex ratio was tested for each population using the Brandt and Snedecor  $\chi^2$  test. In all cases a significant  $\chi^2$  was obtained, the probability of the  $\chi^2$  being 1–2 per cent for two populations and  $P < 0.001$  for all other populations. There seemed to be no association between significant departures from a 1:1 sex ratio and either gene frequency or population size,

and in consequence it is suggested that this variability in sex ratio should not be limited to populations segregating for these sex-linked mutants.

Most of the published accounts of either large or small laboratory populations record changes in frequency of autosomally inherited characters, and do not present the results separately for the two sexes. Frydenberg<sup>2</sup> reported the sex ratio in a number of populations of *Drosophila melanogaster* of average size 550. 50 per cent of these showed significant departures from  $r = 0.5$ , but, as each population was only sampled once, no information is available on variability with time of these sex ratios. A number of investigations of populations with segregating sex-linked genes have reported variable sex ratios, but these have mainly been detected in small populations<sup>3</sup> where chance effects might be expected to contribute largely to the extreme sex ratios obtained. Thomson<sup>4</sup> reported variable sex ratios in large laboratory populations of *Drosophila melanogaster* segregating for sex-linked alleles at the white eye locus. He stated that the excess of one or other sex appeared to be an intrinsic character of each population, but did not indicate whether or not there was heterogeneity of the sex ratio with time.

Thomson's populations were similar to those examined in this laboratory in that each was homogeneous at all loci except perhaps for those closely linked to the white-eyed locus. It is possible that such populations are extremely sensitive to small unavoidable environmental fluctuations, leading to an instability of the sex ratio which would not be observed in a genetically less homogeneous population. This suggestion is now being examined by comparing the variability of the sex ratio in populations of *Drosophila melanogaster* derived directly from laboratory stock bottles with that in populations originating from the same stocks but made homogeneous at all loci. Both wild-type populations and populations homozygous for an autosomal or sex-linked mutant are being considered.

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### Difference in the Behaviour of Eu- and Hetero-chromatin: Crossing-over

THE recombination map-length (0.04 per cent) for the heterochromatin of the X chromosome of *Drosophila melanogaster* is far less than would be anticipated from its mitotic bulk (half the chromosome at metaphase) and considerably less than would be estimated from its polytene-length (17 bands of 1,024)<sup>1</sup>. An exchange frequency comparable to the euchromatic average for the 66 map units of the X chromosome should result in a heterochromatic map-length of at least 1.6 per cent and possibly as high as 33 per cent, depending on which cytological measurement more closely approximates the actual physical length of X heterochromatin. In order to decide whether this low frequency of crossing-over reflects a basic difference in the exchange behaviour of eu- and hetero-chromatin rather than an over-estimate of the physical length of X heterochromatin, use was made of the ability of autosomal inversions to increase crossing over on the X chromosome, the interchromosomal effect on recombination.

The region between suppressor of forked (*su-f*, 66.0) and non-yellow *Dp(scV1y+)* located on the short right arm of the X includes most of the X heterochromatin while the carnation (*car*, 62.5) to *su-f* region includes the euchromatic heterochromatic junction and adjacent euchromatin for comparison. Sisters of the genotype

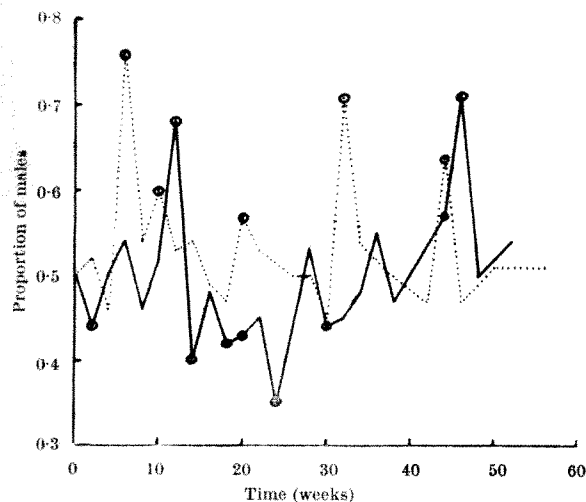


Fig. 1. The proportion of males plotted against time for two of the populations. Readings differing significantly from 0.5 are indicated by small circles.

*y f car su-f/y f. Dp(sc<sup>V1</sup>y<sup>+</sup>)* with or without the autosomal inversions, SMI and Ubx<sup>130</sup>, were crossed to *y f car su-f/Y* males and the *F*<sub>1</sub> scored for recombination.

It can be seen from Table 1 that the *car-su-f* region which includes euchromatin exhibits a pronounced increase in recombination in the presence of the autosomal inversions while the region which is heterochromatic is refractory to the interchromosomal effect. The interchromosomal effect on recombination which has hitherto been described as most pronounced in 'heterochromatic regions' is more accurately described as pronounced in the proximal euchromatin but absent in heterochromatin.

Table 1. RECOMBINATION IN EU- AND HETERO-CHROMATIN OF THE X CHROMOSOME

Autosomal inversions present	No. of flies scored	Per cent recombination Euchromatic <i>car-su-f</i>	Heterochromatic <i>su-f-y<sup>+</sup></i>
None	5,005	3.54	0.04
SMI; Ubx <sup>130</sup>	9,278	9.07	0.04

Crossing-over apparently occurs in the early growth stages of the oocyte<sup>3</sup>. In spite of the generally unfavourable nature of the cytological material, heterochromatin is demonstrable in the oocyte nucleus throughout these early growth stages. Moreover, the nucleolus organizer is in X heterochromatin, and when the nucleolus is first visible (stage 3) and in succeeding stages it is seen to be associated with heterochromatin<sup>3</sup>. It has recently been demonstrated that condensed, pycnotic chromatin is relatively inactive in the synthesis of RNA compared with the more diffuse chromatin<sup>4</sup>. The results recorded here make it possible to correlate another aspect of chromosome behaviour with a particular cytological state and indicate a fundamental difference in the exchange behaviour of eu- and hetero-chromatin owing, apparently, to the condensed state of heterochromatin at the time of exchange. It follows that the more diffuse chromatin condition is a prerequisite for the regular and frequent exchanges that occur during meiosis.

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### Pseudocholinesterase Variants in Japan

SINCE Kalow<sup>1</sup> in 1956 demonstrated a genetically controlled atypical (dibucaine resistant) variant of human pseudocholinesterase (acylcholine acylhydrolase [Enzyme Commission: 3.1.1.8]) much information has been gained on the polymorphism of this enzyme, an esterase of the serum of which the function is not known. Besides the most common gene (*Ch<sub>1</sub>U*) responsible for the synthesis of the usual pseudocholinesterase at least three other genes have been described on the same autosomal locus, namely, the alleles *Ch<sub>1</sub>D*, *Ch<sub>1</sub>F* and *Ch<sub>1</sub>S*, each producing variant enzymes different from the normal pseudocholinesterase. There is no difference in health between various genotypes of the usual gene *Ch<sub>1</sub>U* and those of the alleles *Ch<sub>1</sub>D*, *Ch<sub>1</sub>F* and *Ch<sub>1</sub>S*, unless either homozygotes or heterozygotes of each of the latter three genes are subjected to the application of the muscle relaxant succinylcholine (succinylidicholin) when an abnormally prolonged apnoea is observed. Heterozygotes with both a variant gene and the usual gene do not show this sensitivity.

Various methods have been developed to identify the different types of the enzyme in the homozygotes as well

as in the heterozygotes. Among the inhibition-tests the most common are those using dibucaine<sup>2</sup> or NaF<sup>3</sup> as inhibitors.

The incidence of the heterozygotes for the atypical allele *Ch<sub>1</sub>D* in Europe and Canada seems to be almost constant among various populations so far investigated: about 3-4 per cent<sup>4-7</sup>. Recent investigations on 433 Israelites suggests a higher frequency of the gene *Ch<sub>1</sub>D* (6.3 per cent) among them<sup>8</sup>. Australian Aborigines and Malaysians are said to show no significant difference from the European populations<sup>9,10</sup>. Consequently the atypical type (*Ch<sub>1</sub>D*) of pseudocholinesterase is believed to have a remarkable uniformity of incidence in widely different populations, suggesting that there are (or have been) differences between the selective values of pseudocholinesterase-variants of the various populations<sup>10-12</sup>. Investigations on the incidence of the gene *Ch<sub>1</sub>F* (fluoride resistant) suggests it has about the same frequency as that of the gene *Ch<sub>1</sub>D* (refs. 7, 12). Homozygotes for the 'silent gene' *Ch<sub>1</sub>S* are supposed to be found one in 100,000 (ref. 11). We have recently investigated 100 serum samples from Japan, randomly collected in Tokyo from healthy blood donors. The whole material was investigated at first by two screening tests, the diffusion-test<sup>13,14</sup> and the inhibition-test with RO2-0683 as inhibitor according to Kalow<sup>15</sup>. The dibucaine and fluoride numbers were measured afterwards on each of the 100 sera. Neither heterozygotes nor homozygotes for the gene *Ch<sub>1</sub>D* were found by the four methods described, while two sera were found to be heterozygous for the gene *Ch<sub>1</sub>F*.

The esterase level in our material was found to be lower than that of other populations. We do not believe that the depressed enzyme activity can be explained by a five days' transport from Japan to our laboratory at uncontrolled temperature, but further investigations will be necessary. Both the dibucaine and the fluoride numbers showed normal values.

The foregoing result was confirmed by the screening tests<sup>13-15</sup> on a further 150 Japanese sera, in which no *Ch<sub>1</sub>D* phenotype was found. The frequency of the heterozygotes for *Ch<sub>1</sub>F*, on the other hand, seemed to be about the same as found in the earlier investigation. This may suggest that the incidence of the gene *Ch<sub>1</sub>D* is not as evenly distributed as was assumed from earlier investigations.

This work was supported by the Alexander von Humboldt-Stiftung, the Deutsche Forschungsgemeinschaft and the Bundesministerium für Wissenschaftliche Forschung, Bad Godesberg.

We thank Dr. H. Sakura, Department of Legal Medicine, Medical Dental University, Tokyo, for sending us the sera.

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<sup>1</sup> Kalow, W., *Lancet*, **ii**, 576 (1956).

<sup>2</sup> Kalow, W., and Genest, K., *Canad. J. Biochem. Physiol.*, **35**, 339 (1957).

<sup>3</sup> Harris, H., and Whittaker, M., *Nature*, **191**, 496 (1961).

<sup>4</sup> Kalow, W., and Gunn, D. R., *Ann. Hum. Genet.*, **23**, 239 (1958).

<sup>5</sup> Kattamis, Chr., Zannos-Marjolea, L., Franco, A. P., Liddell, H., Lehmann, H., and Davies, D., *Nature*, **196**, 599 (1962).

<sup>6</sup> Goedde, H. W., and Altland, K., *Nature*, **198**, 1203 (1963).

<sup>7</sup> Goedde, H. W., Altland, K., and Bross, K., *Dtsch. med. Wschr.*, **88**, 2510 (1963).

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<sup>9</sup> Horsfall, W. R., Lehmann, H., and Davies, D., *Nature*, **199**, 115 (1963).

<sup>10</sup> Lehmann, H., and Liddell, J., in *Progress in Medical Genetics*, edit. by Steinberg, A. G., and Bearn, A. G., **3** (Grune and Stratton, New York and London, 1964).

<sup>11</sup> Motulsky, A. G., *Progress in Medical Genetics*, **49** (1964).

<sup>12</sup> Goedde, H. W., and Schoepf, E., *Deutsche Med. Klinik*, **59**, 1849 (1964).

<sup>13</sup> Harris, H., and Robson, E. B., *Lancet*, **ii**, 218 (1963).

<sup>14</sup> Goedde, H. W., and Fuss, W., *Klin. Wschr.*, **42** (6), 286 (1964).

<sup>15</sup> Kalow, W., and Davies, R. O., *Biochem. Pharmacol.*, **1**, 183 (1959).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 15

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 3 p.m.—Colloquium on "Design of Solid State Power Supplies".

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Mr. R. M. Young: "Mind, Brain, and Behaviour: Gall to Ferrier".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. A. J. Clarke: "Hatching Agents for Cyst-Forming Nematodes".

## Tuesday, February 16

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (joint meeting with the Agriculture Group, at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Meeting on "Potatoes".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. H. J. Ralston: "National Parks of the Western United States".\*

UNIVERSITY OF LONDON (at the London School of Economics, Houghton Street, London, W.C.2), at 5 p.m.—Dr. J. H. M. Beattie: "Ritual and Social Change".\*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Sir Alfred Sims: "Warship Design: Interdependence of Science and Engineering" (James Forrest Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Universities Under Fire" opened by Prof. W. E. J. Farvis.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. L. F. Boyd: "The Moon". (Afternoon lecture for Sixth Form Boys and Girls in Schools from London and the Home Counties. To be repeated on February 17, 23 and 24.)

UNIVERSITY OF LONDON (at the Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. E. R. Laithwaite: "Propulsion Without Wheels" (Inaugural Lecture).\*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. K. B. Roberts: "Peripheral Blood Lymphocytes". (Tenth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Achievement of Availability in Gas-Cooled Reactors".

PLASTICS INSTITUTE, LONDON SECTION ENGINEERING SUB-GROUP (at "The Coachmakers Arms", 88 Marylebone Lane, London, W.1), at 6.30 p.m.—Meeting on "Compression Moulding".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Kingston College of Technology Chemical Society, at Kingston College of Technology, Penrhyn Road, Kingston, Surrey), at 7 p.m.—Prof. S. F. Mason: "Circular Dichroism".

## Wednesday, February 17

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. A. A. Sharp: "Defibrination Syndrome".\*

ROYAL SOCIETY OF MEDICINE, COMPARATIVE MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5 p.m.—Meeting on "Electroencephalography in Animals".

INSTITUTION OF ELECTRICAL ENGINEERS (at the Central Hall, Westminster, London, S.W.1), at 5.30 p.m.—Mr. F. C. McLean, C.B.E.: "Colour Television" (Faraday Lecture).

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. F. R. Whatley: "Botany Revisited" (Inaugural Lecture).\*

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, RADAR GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Mr. R. Aste: "Transistorized Equipment Designed for Television Exploitation of Radar Information".

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Trends in Casting Versus Welding".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. Trevor Owen: "A Criticism of the Contemporary Approach to Environmental Engineering".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. G. L. Holbrow: "Dirt Pick-Up of Paints".

ROYAL AERONAUTICAL SOCIETY, GRADUATES' AND STUDENTS' SECTION (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Prof. A. R. Collar: "Research at Bristol University".

ASSOCIATION OF THE WILLIAM PENGELLY CAVE RESEARCH CENTRE, in conjunction with IMPERIAL COLLEGE CAVING CLUB (in the Physics Department, Imperial College, London, S.W.7), at 8 p.m.—Mr. G. de G. Sleveking: "Painted Caves of France and Northern Spain".\*

## Thursday, February 18

PARLIAMENTARY AND SCIENTIFIC COMMITTEE (in the River Room, Savoy Hotel, London, W.C.2), at 11.30 a.m.—Annual General Meeting followed by the Annual Luncheon.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Battersea College of Technology, London, S.W.11), at 2 p.m.—Symposium on "Colloidal Solutes".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, joint I.E.E./I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m. and 5.30 p.m.—Colloquium on "Automatic Aids to Machine Fault Finding".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Academican M. V. Keldysh (President of the Academy of Sciences of the U.S.S.R.): "Organization of Science in the U.S.S.R.".\*

UNIVERSITY OF LONDON (at King's College Hospital Medical School, Denmark Hill, London, S.E.5), at 4.30 p.m.—Sir Robert Platt, Bt.: "Hereditary and Environment in Hypertension—the Summing Up" (Wiltshire Memorial Lecture).\*

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. A. P. Prosser, Dr. A. J. Wright and Dr. J. D. Stephens: "Physical and Chemical Properties of Natural Copper Silicates which Resemble Chrysocolla"; Dr. A. J. Wright and Dr. A. P. Prosser: "Study of the Reactions and Flotation of Chrysocolla with Alkali-Metal Xanthates and Sulphides".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. G. E. H. Foxon, Mr. I. R. Bishop, Mr. E. C. Tatchell, Mr. D. Walliker and Dr. J. C. Garnham: "An Expedition to the Lower Amazon, 1964".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. R. F. Churchhouse: "The Use of Computers to Obtain Results in the Theory of Numbers".

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. Benno Hess (Heidelberg): "Metabolic Control Mechanisms". (Further lecture on February 22.)

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. R. F. Leggett: "Winter Construction Practices in Canada".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. O. L. Zangwill: "Experimental Psychology in Relation to Injury or Disease of the Brain". (Eleventh of sixteen lectures on "The Scientific Basis of Medicine", organized by the British Postgraduate Medical Federation).\*

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. H. H. Pearcey: "Swept Wing Aerodynamics".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (joint meeting with the Institution of Highway Engineers, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. P. D. Thompson: "The Use of Rubber in Bituminous Road Surfacing".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Discussion on "Six Months Experience with B.C.C.-2".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Médecin-Général Inspecteurs M. A. Vauzel and P. Riehet: "Le Service de Santé des Troupes de Marine et la Médecine Tropicale Française".

## Friday, February 19

BRITISH INSTITUTE OF RADIOLOGY (in the Reid-Knox Hall, Institute House, 32 Welbeck Street, London, W.1), at 2 p.m.—Radiobiology Meeting.

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 2.30 p.m. and 5 p.m.—Colloquium on "Electrostatic Precipitators".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. J. Vallance-Owen: "Modern Views of Diabetes".\*

QUEEN MARY COLLEGE (University of London) (in the New Lecture Theatre, Mile End Road, London, E.1), at 5.30 p.m.—Prof. M. W. Thring: "Mankind and Machines" (Inaugural Lecture).\*

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. R. C. Cookson: "Circular Dichroism".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.45 p.m.—Twenty-first Annual General Meeting, followed by Mr. D. W. Wilson: Retiring Chairman's Address.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. S. Tolansky, F.R.S.: "The Microtopographies of Surfaces".

## Saturday, February 20

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. A. Mourant: "Blood Groups, Race and Evolution".\*

## Monday, February 22

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. Emrys Jones: "The London Atlas".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. A. Saxton, Mr. J. A. Lane, Mr. R. W. Meadows and Mr. P. A. Matthews: "Layer Structure of the Troposphere".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. S. Kaplan (Stanford): "Unfolding Concepts of Mechanisms in Leukaemogenesis and Carcinogenesis".\*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Transport in the Year A.D. 2000".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. R. Calvert: "Curing Systems".



## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER Grade II (preferably with an interest in numerical analysis or operational research) IN THE COMPUTER UNIT, DEPARTMENT OF MATHEMATICS—The Registrar, University of Bristol, University Senate House, Bristol, 2 (February 19).

ASSISTANT LECTURER or LECTURER (preferably with an interest in microbiology, particularly in the fields of mycology and soil studies) IN THE DEPARTMENT OF BOTANY—The Registrar, The University, Liverpool, quoting Ref. CV/443 (February 26).

ASSISTANT LECTURER (preferably with experience, or an interest in space physics) IN PHYSICS—The Registrar, The University, Sheffield (February 27).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, BIOCHEMISTRY, PHYSICS, ENGINEERING, CHEMOTHERAPY and related subjects—The Deputy Secretary, Academic Council, Queen's University, Belfast, Northern Ireland (February 27).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS in various branches of CHEMISTRY, including INORGANIC CHEMISTRY, CHEMICAL ENGINEERING, ENGINEERING, TECHNOLOGY, PHYSICS and allied subjects—The Registrar, The University, Manchester, 13 (February 27).

LECTURER or ASSISTANT LECTURER (applied mathematician or statistician) IN THE DEPARTMENT OF THEORETICAL MECHANICS—The Registrar, The University, Nottingham (February 27).

LECTURER (preference may be given to candidates whose interests and experience centre on the biochemistry of cellular processes in plants) IN THE DEPARTMENT OF PLANT SCIENCE—The Registrar, The University, Newcastle upon Tyne, 2 (February 27).

PROGRAMMING ASSISTANT IN THE COMPUTING LABORATORY—The Secretary of the University Court, The University, Glasgow (February 27).

TURNER AND NEWALL RESEARCH FELLOWS IN ENGINEERING, INORGANIC CHEMISTRY, PHYSICS and allied subjects—The Registrar, The University, Manchester, 13 (February 27).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, PHYSICS, METALLURGY, ENGINEERING, GEOLOGY, AGRICULTURAL SCIENCE or related subjects—The Registrar of the University of Wales, University Registry, Cathays Park, Cardiff (March 1).

LECTURER IN ENGINEERING MATHEMATICS—The Secretary, Queen's University, Belfast, Northern Ireland (March 1).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS—The Secretary, The University, Edinburgh (March 1).

LECTURER IN THE DEPARTMENT OF ANATOMY—The Secretary, The University, Aberdeen (March 6).

LECTURER or ASSISTANT LECTURER IN ORGANIC CHEMISTRY—The Secretary, The University, Aberdeen (March 6).

SENIOR LECTURER IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leicester (March 6).

SENIOR LECTURER, LECTURER and an ASSISTANT LECTURER IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leicester (March 6).

LECTURER, SENIOR LECTURER or READER IN ANIMAL HUSBANDRY at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 10).

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF MEDICINE; and a JUNIOR LECTURER or JUNIOR RESEARCH FELLOW IN THE DEPARTMENT OF PHYSIOLOGICAL SCIENCES, University of Lagos Medical School—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 10).

SENIOR LECTURER IN PURE MATHEMATICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 05G (March 12).

READER IN ORGANIC CHEMISTRY at King's College—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 15).

SENIOR LECTURER or LECTURER (with a degree in chemistry or agricultural chemistry, and postgraduate experience, preferably in soil science or plant nutrition) IN AGRICULTURAL CHEMISTRY at Makerere University College, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 15).

LECTURER IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY, School of African Studies, University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (March 26).

LECTURER IN HYDRAULICS AND HYDROLOGY IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, The University, Newcastle upon Tyne, 2 (March 31).

POSTDOCTORAL RESEARCH FELLOW IN ORGANIC CHEMISTRY for work which will involve chemical studies of the pteridine co-factors in amino-acid metabolism—Dr. H. C. S. Wood, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, C.1 (March 31).

AGRONOMIST (with a degree in agriculture plus two years postgraduate training in agronomy followed by at least three years field experience in carrying out statistical experiments with field crops) in the West Cameroon, to conduct experiments on food crops and to train junior staff—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/33/05.

AGRONOMIST (with an honours degree in agriculture or botany, and preferably postgraduate training or experience in pasture agronomy for at least two years) in Uganda, to investigate the selection of grasses and legumes and their development in mixtures suitable for Uganda conditions and the effect of leys on soil fertility and the use of subsequent crops—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/06.

LECTURER (with a higher degree of a university in Britain and research and teaching experience) IN ORGANIC CHEMISTRY at the University of Medical Sciences, Bangkok, Thailand—The Recruitment Division, The British Council, 65 Davies Street, London, W.1, quoting Ref. 64/UNI/107.

MASTER to teach Mathematics at all levels—The Headmaster, School House, Rugby.

POST-DOCTORAL RESEARCH FELLOW (biochemist or virologist) to be a member of a small group working on the biochemistry of interferon production—Dr. D. C. Burke, Department of Biological Chemistry, The University, Aberdeen.

RESEARCH ASSISTANT (graduate with an honours degree in botany or agricultural botany) to assist with investigation of the factors which determine weed seed content of crop seeds; and a MYCOLOGIST (with a degree in agricultural botany or botany or equivalent qualifications) IN THE POTATO BRANCH to assist in the assessment of disease resistance in potatoes—The

Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

RESEARCH DEMONSTRATORS (with a good degree in botany or horticulture) IN THE DEPARTMENT OF HORTICULTURE to assist with practical classes in horticulture or plant physiology—Prof. O. V. S. Heath, F.R.S., University of Reading Horticultural Research Laboratories, Shinfield Grange, Reading, Berkshire.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

The National Central Library. 48th Annual Report of the Executive Committee for the year ending 31 March 1964. Pp. 24. (London: The National Central Library, 1964.) [2511]

Industrial Diamond Information Bureau. Non-Cutting Uses of Industrial Diamonds. Pp. 12. (London: Industrial Diamond Information Bureau, 1964.) [2511]

The Royal Institute of Chemistry, London Section. Annual Report of the Committee, 1964. Pp. 10. (London: The Royal Institute of Chemistry, 1964.) [2511]

Ministry of Housing and Local Government. North Lancashire Rivers: Hydrological Survey (Hydrometric Areas Nos. 70 to 73 and part 74.) Pp. v+117. (London: H.M. Stationery Office, 1964.) 20s. net. [2511]

New Reference Tools for Librarians. Compiled and edited by Hans Zell. Pp. v+232. Supplement No. 1. Pp. 62. Supplement No. 2. Pp. 56. (Oxford: Robert Maxwell and Co., Ltd.; Long Island City, N.Y.: Maxwell Scientific International, Inc., 1964.) Gratis. [2511]

Northern Ireland: Ministry of Agriculture. Leaflet No. 5: Dishorning Cattle. Pp. 4. (Belfast: Ministry of Agriculture, 1964.) [3011]

The Royal Society. Report of Council for the year ended 30 September 1964. Pp. 74. Scientific Research in Schools Committee—Report to Council, 1964. Pp. 16. (London: The Royal Society, 1964.) [3011]

### Other Countries

Comité International des Poids et Mesures. Comité Consultatif d'Electricité, 10<sup>e</sup> Session—1963 (2-3 Mai). Pp. 78. (Paris: Gauthier-Villars et Cie., 1964.) [3011]

Commonwealth of Australia: Department of National Development. Bureau of Mineral Resources, Geology and Geophysics. Report No. 60: Stratigraphic Drilling, Canning Basin, Western Australia. By S. D. Henderson, M. A. Condon and L. V. Bastian. Pp. 78+4 plates. Bulletin No. 69: A Survey of Phosphate Deposits in the South-West Pacific and Australian Waters. By W. C. White and O. N. Warin. Pp. 173+7 plates. Publication No. 38: Barlee Gravity Survey, Western Australia. By West Australian Petroleum Pty., Ltd. Pp. 10+7 plates. (Canberra City: Bureau of Mineral Resources, Geology and Geophysics, 1963 and 1964.) [3011]

India: Council of Scientific and Industrial Research. Investment in Scientific and Technological Research During the Fourth Five Year Plan. By Dr. S. Husain Zaheer, A. Rahman and N. Sen. (Working Paper.) Pp. 31. (New Delhi: Council of Scientific and Industrial Research, 1964.) [3011]

Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series, No. 10: General Report on Lands of the Bundaberg, Territory of Papua and New Guinea. Comprising papers by H. A. Haantjens, S. J. Paterson, B. W. Taylor, R. O. Slatyer, G. A. Stewart and P. Green. Compiled by H. A. Haantjens. Pp. 115+map. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1964.) [3011]

Chemistry of Cellulose and Cellulose Derivatives. (Symposium held on 14th January, 1964, under the joint auspices of the Royal Institute of Chemistry, Great Britain (Pakistan Section), and the Dacca University Chemical Society.) Pp. ii+17. (Dacca: Dacca University, 1964.) [3011]

Southern Rhodesia. Report of the Trustees and Directors of the National Museums of Southern Rhodesia for the year ended 31st December, 1963. Pp. 32. (Salisbury: National Museums of Southern Rhodesia, 1964.) [3011]

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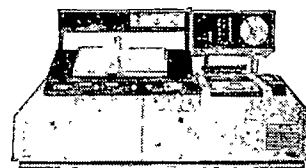
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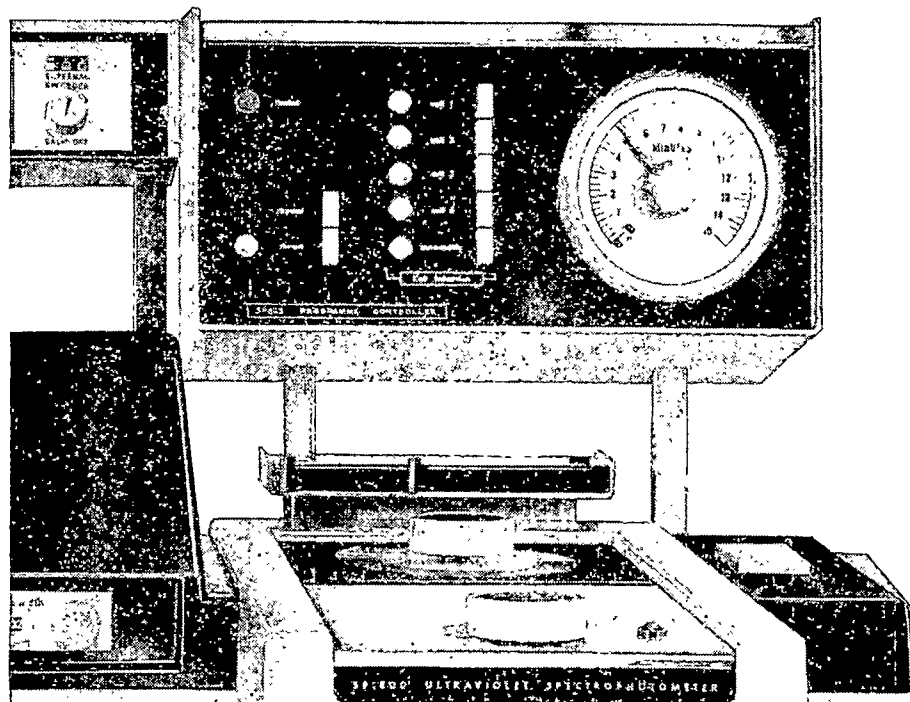
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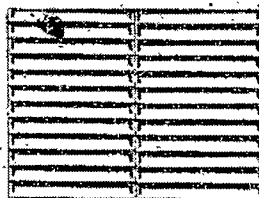


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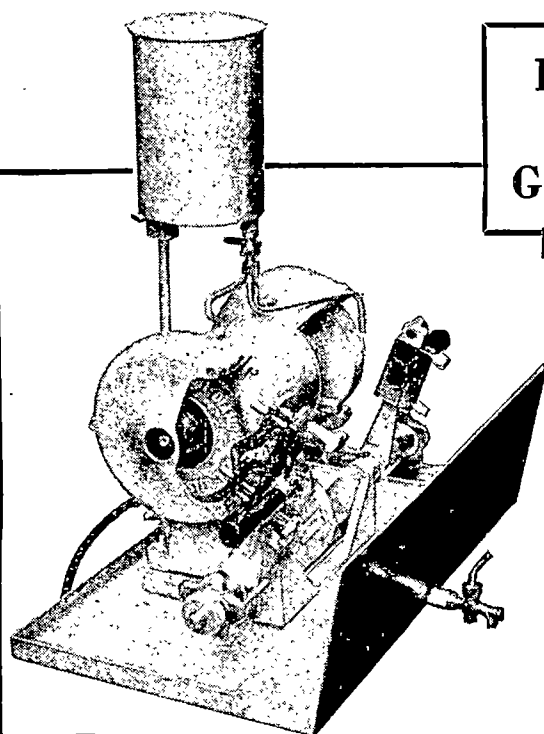
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## A POLICY FOR THE SOCIAL SCIENCES

ACCORDING to the Prime Minister's written answer to a question in the House of Commons on November 20, the Committee appointed, with Lord Heyworth as chairman, to review current research in social studies in Government departments, universities and other institutions, and to advise on any changes needed in the arrangements for supporting and co-ordinating this research, is expected to report in the spring. Meanwhile, the Council of the Tavistock Institute of Human Relations has issued a pamphlet which examines the whole question of social research in the wider framework of a national policy for research and development\*. Criticism of the present position is to be found, for example in the report on research in the humanities and the social sciences issued by the British Academy in 1959, in the memorandum by Prof. D. V. Glass and Prof. M. Gluckman on the social sciences in British universities which was submitted to the Robbins Committee by the British Association for the Advancement of Science (*Nature*, 196, 704 (1962)) and in the inaugural lectures by Prof. F. LaFitto at Birmingham in May 1962 and by Prof. R. K. Kelsall at Sheffield in November 1960. In this pamphlet these criticisms are discussed in the light of the findings of numerous subsequent enquiries such as those of the Robbins Committee, the Trend Committee, Lord Franks's Report on British business schools, and others from Prof. Buchanan, the Royal Institute of British Architects, the National Economic Development Council and the Organization for Economic Co-operation and Development.

The paper from the Tavistock Institute of Human Relations interprets the term 'social services' to cover the range of empirical disciplines concerned with social behaviour. It distinguishes broadly between the purely social disciplines, such as social and developmental psychology, and certain growing inter-disciplinary ones, such as social and psychological medicine, social and industrial administration, market research and management studies, operational research and systems analysis so far as they include social, psychological and academic variables. Neither the historical sciences, nor those branches of the behavioural sciences which are only indirectly concerned with social behaviour, are included.

In this description of the scope of the problem, the importance of social research in relation to the formulation of any national policy for science and technology, and even for academic growth, is unmistakable. The connexion is adumbrated by the main submission of the Council, that the priority task in supporting research in the social services in Great Britain is to foster a general development of the field as a whole. The present practice of piecemeal development is condemned as flatly as in the survey made by the British Academy. Furthermore, such a general development should be rapid rather than slow, and should be effected within the next 15 years. It must reach a certain magnitude before it can bring about the

progress made available by present knowledge, and for this purpose the Council suggests that annual expenditure should increase from £3.5 million in 1964-65 to £25 million by 1980, and that research workers should be increased from 2,000 to 11,000.

These estimates mean little except in relation to the scale and distribution of the national scientific effort as a whole. Taking the estimate of the Trend Committee for civil research expenditure through Government channels in 1962-63 of £151 million, adding £200 million for defence research, and the Treasury estimate of research expenditure by private industry in 1963 of £366 million, a figure is given of £700-£750 million for current national expenditure on research and development. The last report of the Advisory Council on Scientific Policy estimated Government expenditure in civil research in 1964-65 at £205 million, but gave no estimate for expenditure on defence research or by private industry. If, moreover, research and development expenditure in Britain reaches £1,500 million by 1980, or 3.0 per cent of the gross national product, in accordance with the target set by the National Economic Development Council, compared with 2.3 per cent in 1960, the proposed support will represent no more than 1.7 per cent of our scientific resources for social research in 1980. Even expenditure of this order falls short of the present-day expenditure of a single institution in the United States, the National Institute of Mental Health, where much of the research lies in social science. More than 3 per cent of research expenditure in the United States is now in the social services.

Unless social research in Britain is raised to such a level, serious results cannot be expected, but a change of the order envisaged depends on a widespread conviction that solutions to many of the important problems require contributions from the social sciences. This was urged by Profs. Glass and Gluckman in their memorandum to the Robbins Committee on the social services in British universities in 1962, and there have since been welcome signs of an increasing recognition of this situation and greater readiness to commit resources to it. Examples are the establishment of budgets for social research in the Home Office, the Ministry of Education, the Ministry of Health, the Board of Trade and the Central Office of Information, and the expansion of the Economic Section of the Treasury, while the subsequent changes introduced by the present Government as well as other pressures towards growth trend in the same direction. The demand for information capable of more effective statistical analysis is another factor. It is suggested in this paper that what is most needed in the United Kingdom in this field at the present time is some way of imparting a general stimulus simultaneously to the development of knowledge in the social services, for example in the universities and in specialized research organizations, and to its utilization by Government and other bodies. Accordingly, the Council of the Tavistock Institute suggests that the action to be taken first should be such as will later facili-

\* *Social Research and a National Policy for Science*. (A paper of the Council of the Tavistock Institute of Human Relations, Tavistock Pamphlet No. 7.) Pp. viii + 42. (London: Tavistock Publications (1959), Ltd., 1964.) 7s. net.



tate specific action at interdependent points. Much of the social research to be undertaken should thus grow out of attempts to help with practical problems. The line of advance is to a professional rather than a scientific discipline, as in fact has happened with medicine, engineering and architecture.

The paper then considers the problems of co-ordination and control that are presented by the heterogeneity of the social sciences and the variety and nature of the existing organizations carrying out social research. Here its analysis is sometimes rather deeper than that which marked the Trend Report on the organization of civil research generally. It finds strong reasons for keeping the new type of research organization closely linked with the executive departments directly concerned with their activities. First, an operating organization is unlikely to develop the scientific outlook imperative under conditions of increasing technological and environmental change unless it is made responsible for its own research and development. Secondly, so many Government departments are now operating on such an immense scale that the scale and heterogeneity of their tasks constitute an internal world, large and varied enough for a research and development organization to belong to it and still retain many degrees of freedom.

The paper does not underestimate the importance of scientific independence, and the Council's view is that autonomous research councils are still necessary, one being suggested for basic and another for applied research. It does not discuss the question of objectivity which has been urged as an important reason for keeping the Road Research Laboratory, for example, outside the Ministry of Transport, but it clearly does not regard the appointment of scientific advisers at a high level in a department as being, in itself, sufficient to ensure a scientific outlook. However, Lord Todd's point as to the imperative necessity of the scientist and technologist reporting at the highest level in the making of policy is duly taken. It should be noted, moreover, that it suggests that a new organization is more likely to develop effective research establishments of its own if it first collaborates with experienced outside organizations. The paper visualizes that by 1980 there may be perhaps 30 relatively small internal headquarters of about 6 senior people concerned chiefly with the identification and appreciation of problems, the head of which acts as chief social science advisor, and more than 150 field investigation groups, each with a director and a staff of about 15.

Considering next the university departments, after stressing the need for larger grants from the University Grants Committee for the development of teaching and research in the social services, the paper urges that in the short run the highest priority must be given to increasing and improving the supply of research workers in the social sciences. Here the paper assigns the first priority to graduate studies, including, however, intensive courses of one year for those with some undergraduate preparation, and of two years for those with none. Many of the best students come to hear about the social sciences only quite late, and it is neither possible nor desirable that more than a proportion of those who afterwards enter careers in social science shall have pursued, as undergraduates, the major disciplines of the social sciences. Moreover, the courses envisaged should include possibilities of acquiring practical experience, such as could be provided by attachment for periods of 3 months during the summer vacation to suitable centres in such organizations.

Because the advancement of knowledge and the extension of practice in the social sciences are interdependent, the universities must provide professional training as well as training in research, and such a development would change both the size and the character of the social science departments. Further extensive pre-doctoral programmes must be organized, so that those proceeding to a career in research undergo a thorough apprenticeship, if they are to be capable of entering into relations with those in other disciplines and of developing the broader outlook and competence essential for effective participation in inter-disciplinary programmes. For these purposes, graduate courses of two kinds, basic and professional, are envisaged, an undergraduate honours school, and other undergraduate courses given as part requirements in other departments. On the research side there would be Ph.D. candidates to supervise, various postdoctoral projects in basic research, and others of a contract character. By 1980 over the whole range of social science some 300 departments and 60 universities would be attempting these tasks, involving the equivalent of 3,000 full-time workers and an expenditure of £7 million per annum.

Discussing the special institutes for research, of which forty are envisaged by 1980, thirty (including most of the smaller ones) within the universities and ten outside, employing some 3,000 research workers and representing an expenditure of about £10 million per annum, the paper, while stressing the crucial importance of such institutes for later development, questions whether some would have more than a transitional purpose. To be effective, such an institute should have a recurrent annual expenditure of £100,000–£500,000 and a research staff of perhaps 35–60 or 70. Most of the special institutes in Britain are at present too small, comparing unfavourably with those in the United States or in Europe. Moreover, they require some basic support over and above ordinary research grants and contract fees.

It is in discussing the general system into which those various bodies would fit that the Tavistock Institute of Human Relations is most critical of the Trend Committee for its neglect of the social sciences—and of some other sciences—a neglect quite unjustified by the establishment of the Heyworth Committee while the Trend Committee was sitting. In the present paper the Council of the Institute attempts to redress that neglect by fitting its proposals for the social sciences into the framework of a general policy for the national science resources as a whole. It envisages a National Sciences Development Council to work with the Secretary of State for Education and Science, and it regards the new Science Research Council as a step in this direction, though it does not seem entirely compatible with the existence of a separate Council for Technology under the Minister for Technology. Its proposed council would recommend the allocation and utilization of the national scientific resources over the whole field of scientific endeavour. Neither a purely internal nor a purely external body is contemplated. The suggested Development Council is intended to bring Government and scientific interests together and reach common conclusions. Its logical position would appear to be, as Dr. S. Toulmin has suggested for the Advisory Council on Scientific Policy, in the Cabinet Office, directly under the Prime Minister.

The constitution suggested for this Council is interesting. Its members should include, as with the National Economic Development Council, the cabinet ministers most directly concerned, representatives of a wide range of new interests

including, besides scientific civil servants, employers' organizations, the Trades Union Congress and the University Grants Committee, together with scientific members from each of the main disciplinary groups (reflecting both pure and applied science) and learned and professional societies, including the social sciences. The Development Council would work largely through a number of commissions, each concerned with a main scientific grouping; these in turn would work with and through science research councils, applied research councils, the University Grants Committee, and other grant-giving bodies.

It is into this general pattern that the Development Commission for the Social Sciences would fit. This Commission would have as its tasks the determination of the nature and extent of available resources for social science, public and private, appraising their present-day use and adequacy, and making recommendations on the scope and direction of their future development. While responsible for recommending the total expenditure from Government resources on social research in each year or quinquennium, the Development Commission, like the other Development Commissions and the Development Council, would not itself award individual grants; its main findings would be made public for discussion and comment. The Commission would, however, strengthen the policy of according substantial social science budgets to individual Government departments, and it would also assist the series of applied research councils proposed—for example, for medicine, agriculture, natural resources, human resources, technology and industry, etc.—to foster the activities of the special research institutes.

The other main recommendation is the establishment, as one of some nine science research councils, of a Social Sciences Research Council. This Council would have as its main functions encouraging research expenditure or projects and programmes in neglected or new areas where it sees a need; fostering long-range programmes; giving institutional support through untied funds to special institutes; and provision of training fellowships, travel and sabbatical facilities, and funds enabling more social scientists from other countries to work in Britain. Whether such a Research Council should set up establishments of its own as well as award grants to other bodies is doubtful, particularly as its main function would be to ensure balanced support of the various types of research organizations.

The proposals from the Tavistock Institute are well argued and supported by analyses of the details of the proposed system which deserve the close attention of the Minister of Technology and the Secretary of State for Education and Science, even before the Heyworth Committee reports. It is not that it condemns the recommendation of the British Academy, made five years ago, and the subsequent findings of Profs. Glass and Gluckman, or shows how little has been done since the Chapman Committee reported almost two decades ago. It has constructive suggestions covering the whole field of civil science for its organization and the development of civil research, and the proposals merit attention before the recently planned changes in the structure of civil science are finally confirmed. No reader of this paper can mistake the magnitude of the contribution that social research could make to the economic as well as the social development of Britain, and no Government which fails to make effective provision for that attention can claim to have, in any real sense, a policy or a programme for economic, scientific or technical development.

## THE IDEA OF A UNIVERSITY IN TROPICAL AFRICA

African Universities and Western Tradition

By Sir Eric Ashby. (The Godkin Lectures at Harvard University, 1964.) Pp. vi+113. (London: Oxford University Press, 1964.) Boards 15s. net.; Limp 10s. 6d. net.

SOON after the Second World War, in implementing the 1945 Asquith Report on higher education in the colonies, Britain began to found universities in what were then her tropical African territories—at Khartoum in the Condominium of the Sudan, at Accra in Ghana, Ibadan in Nigeria, Makerere in Uganda and Salisbury in Rhodesia. The foundations of these new African institutions were all laid by British administrators and teachers, working in close association with the Inter-University Council for Higher Education in the Colonies and the not yet independent African Governments.

Sir Eric Ashby, Master of Clare College, Cambridge, has recently subjected the development of these African universities to a searching analysis in his 1964 Godkin Lectures in Harvard University. He seeks to trace the pattern of tension which has arisen in Africa between the English (and to some extent European) idea of a university on the one hand, and, on the other, the demands of African politicians and intellectuals that their universities shall be the product of their own soil, shall reflect their national aspirations, and fulfil the expectations African peoples have of them. His theme, he tells us, is one of universities and cultural nationalism, and he illustrates it by drawing his examples mainly from the universities at Accra in Ghana and Ibadan in Nigeria. Sir Eric's chief criticism is that we have exported wholesale to Africa our English idea of a university with little or no thought for the demands, whether in structure, function or curriculum, likely to be made on it in its new habitat. He approvingly quotes C. W. Eliot, president a century ago of Harvard University, who declared that "a university must grow from seed. It cannot be transplanted from England or Germany in full leaf and bearing . . . When the American university appears, it will not be a copy of foreign institutions . . . but the slow and natural outgrowth of American social and political habits".

So, too, must it be, argues Sir Eric, with the universities of Africa, but in fact we have hindered such a development by our cultural nationalism. He asserts that "in the past, one of the symptoms of British cultural nationalism has been an invincible confidence in the efficacy of British education, not only for home consumption but also for export" and this applies equally to our idea of a university. So we have insisted too rigidly on autonomous constitutions which debar these new universities from any functional relationship with, or any measure of control by, the State. The universities of a number of European countries, he points out, are State-controlled, but this has meant no loss of academic freedom. We have insisted, too, on very high academic standards and specialization in an attempt to adhere to what he calls the "academic gold standard", thus frustrating national demands for high-level manpower and technological expertise. As for the social function of a university, we have here exported our traditional notion that this is principally to educate an élite. The result has been that, although in the words of Dr. Nkrumah these African universities are "the heart of the nation" and essential to its progress, they and their graduates are isolated from the life of the common people in a way that has had no parallel in England since the Middle Ages. Too often to-day there is between European culture and African culture a no-man's-land, in which thousands of African graduates pass their lives, not assimilated to Europe yet strangers to their own folk.

How far are Sir Eric Ashby's criticisms justified? There is little doubt that his main point—that universities in

Africa have not adapted themselves to the needs and aspirations of African society—is substantially correct. But to infer that this was due to intolerance or cultural nationalism on the part of the British pioneers who launched the new universities of West Africa is to do them little justice and to exaggerate the situation considerably. Hindsight is always a gift of dubious value, but never more so than when we attempt to judge in near retrospect the transplantation of key institutions from one culture to another. A university, perhaps more than many other aspects of a European culture, is a focus of its value-system, and these pioneers could give of their best only in terms of the best they knew—the best of their own values. To have asked them to do otherwise would have been to expect them to do something for which they were neither qualified nor experienced. Had they agreed to do so they would have earned neither the gratitude nor the respect of their African colleagues. Adaptation is seldom the task of the innovating pioneers from the sending culture; it is the task of those from the receiving culture who take over from them, for it is based on criteria which the foreigner cannot appreciate. When President Eliot of Harvard spoke of American universities as the slow and natural outgrowth of American social and political habits, this is what he meant—and this is what is now happening in the universities of tropical Africa.

P. C. C. EVANS

## EXTRA-MURAL EDUCATION

University Extramural Education in England, 1945-62. A Study in Finance and Policy. By Prof. S. G. Raybould. (Michael Joseph Books on Live Issues.) Pp. 207. (London: Michael Joseph, Ltd., 1964.) 42s. net.

PROF. RAYBOULD is fast qualifying for the post of keeper of the universities' conscience in matters extra-mural. His first book on the subject was published in 1951 and dealt with the period 1924-49. Now he has given us in *University Extramural Education in England* a kind of supplement covering the years 1945-62. This volume, like the first, is mainly statistical, but from the statistics Prof. Raybould adduces three major criticisms of the present position. First, the university extra-mural departments are organizing too many short courses which do not call for serious student effort; secondly, they are employing too many non-university tutors; and thirdly, that since, in view of these facts, the financial link with the Ministry of Education has so signally failed to protect university standards, it is time this link was done away with and the extra-mural departments left to stand on their own feet with such funds as their universities can be persuaded to give them.

The trouble is, I think, that in support of these contentions Prof. Raybould relies too much on the raw statistics of extra-mural courses. These statistics certainly show, for example, that there has been a disproportionate increase in the number of courses of less than twenty meetings, but they do not show that many of these short courses are conducted at a very high academic level for students who bring to the work a considerable background of previous knowledge. The statistics also show that large numbers of non-university tutors are employed on extra-mural work in a part-time capacity, but they do not reveal to what extent these tutors are employed: in some universities, at least, they play only a very minor part.

If these qualifications are borne in mind, Prof. Raybould's criticism of the Ministry of Education loses much of its force. There is a good case to be made for abolishing the present system under which the extra-mural departments have received part of their funds from their universities and part from the Ministry, and substituting a system under which, like other university departments,

they receive their funds from the University Grants Committee. This case rests, however, not on statistical considerations such as Prof. Raybould has brought forward, but on the need for the universities to have full control and full responsibility for their extra-mural teaching.

None the less, it would be foolish to dismiss the criticisms that Prof. Raybould makes as entirely without substance. Extra-mural work has been passing since the Second World War through a period of pretty rapid expansion. My own impression is that the academic quality of the work, and its reputation inside the universities, has been steadily rising. However, the statistical record, though it may not mean as much as Prof. Raybould thinks it means, does point to certain real dangers against which the universities should be on their guard, and it is of great value to have this clear and cogently argued volume to direct attention to these dangers.

T. KELLY

## SIGN-POSTS IN SOCIAL AND CULTURAL ANTHROPOLOGY

Explorations in Cultural Anthropology

Edited by Ward H. Goodenough. (Essays in Honor of George Peter Murdock.) Pp. xiii + 653. (New York: McGraw-Hill Book Company; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1964.) 100s.; 12.50 dollars.

Other Cultures

Aims, Methods and Achievements in Social Anthropology. By John Beattie. Pp. xii + 283. (London: Cohen and West, Ltd., 1964.) 32s.

Social Anthropology

By Prof. Paul Bohannan. Pp. viii + 421. (New York and London: Holt, Rinehart and Winston, Inc., 1963.) 6 dollars.

IT is presumably a sign of maturity in a subject when not only volumes of the *Festschrift* order celebrate the work of leading scientists but also enough firmness has been given to the framework of theory that diverse text-books embodying it can be written.

The essays in honour of Murdock's sixty-fifth birthday rightly celebrate the work of a man who has done a very great deal to systematize and stimulate our thinking about some of the basic aspects of family and kinship structure. Even those who have disagreed at times either with his terminology or with his generalizations have been stirred to further enquiry as the result of problems he has posed. It is appropriate, therefore, that much of this book should be concerned with questions of kinship and with refinement of methods of social and cultural enquiry. Hockett's reconstructed account of the putative 'parent' of the Central Algonquian kinship system a millenium or more ago, and Lounsbury's formal model of Crow- and Omaha-type kinship terminologies, are for specialists alone. Goodenough's componential analysis of the terminology of Lapp kinship from Perhson's account is also technically difficult; as with Conklin's paper on ethnogenealogical method, the effort is interesting and rich in analytical suggestion rather than complete and convincing in final statement.

Of the many other stimulating essays in *Explorations in Cultural Anthropology* one can only indicate a handful. Rouse examines Julian Huxley's and Kroeber's ideas on evolution from an archaeological point of view, and Whiting considers how far climate has had an effect through a long chain of nursing and sleeping habits on the practice of circumcision of boys. Voget examines the way in which warfare was formerly a primary focus for integration of Crow Indian culture; whereas Pospisil, equally sociological but more tough-minded, represents the feud among an inland Eskimo group as being an



antithesis rather than a manifestation of law. La Barre gives a fascinating account of a snake-handling cult in the American South East, and Lebar a very sober statistical statement of the results of a household survey in Truk. Roberts examines in terms of the now fashionable communication and management theory the political systems of four North American Indian tribes. The regional coverage of the essays is wide, their theoretical contribution considerable. The introduction by Goodenough not only places them in a general setting but also emphasizes a modern trend of anthropology in distinguishing the observed phenomenal order of social events from the ideational order, the way in which the members of a community organize their experience.

The two text-books on social anthropology do not present analytical material with such intensity, but they are naturally much more systematic. Both are written mainly for academic use, but also provide a thoughtful introduction to the subject for those who do not wish to specialize in it. Their theoretical framework is closely parallel, which is not surprising since both authors were trained at the University of Oxford. But, whereas Beattie—with "cultures" in the title—keeps very closely to his brief for social anthropology, Bohannan—while labelling his book *Social Anthropology*—has sections on art, language and science, culture and personality as well. Both are works of intellectual calibre, but where Beattie's is sober in tone, Bohannan's is jaunty and its scholarship sometimes uneven. Both authors make some very interesting observations on the nature of their science. Bohannan, after general consideration of "the study of man", examines the task of the anthropologist in the field and the task of translation which he faces on his return. He stresses the need for analytical theory, and here argues that anthropology resembles science rather than history in its procedures. Beattie has a whole chapter on the need for theory and, like Bohannan, directs attention to the complexity of the notion that the anthropologist observes "what actually happens". Both books, in accord with present-day practice, treat of "models", and both seem quite clear on the fundamental point that the purpose of such models is to serve as aids in explanation of social reality, not as mirrors of it. In their discussion of methodology both authors, in general agreement, set out with elegance and clarity the major principles involved.

The content of the two books follows broadly the classical scope of kinship, economics, politics and religion; both too, while not denying the significance of the time dimension for all dynamic investigations of institutions, still rather conservatively have a section at the end specifically labelled "Social Change". Ethnographically, perhaps, the range is rather narrow; Africa has pride of place in the examples given, and though Bohannan has the wider coverage even his societies cited include none from Middle or South America.

Differences in their treatment are partly idiosyncratic and partly due to national tradition, exemplified by Bohannan's bow to the theory of cultures and personality and that of social evolution. Bohannan's book is curiously uneven in its handling of some topics. The section termed "The Image of the Unknown", less than fifty pages in all, is occupied by a chapter on the history of anthropological and sociological thinking about magic, science and religion (but no mention of Lynn Thorndike) and another of equal length on witchcraft, whereas "Tribal Religions", of which the Manus of the Admiralty Islands are the sole example examined in detail, are compressed into a dozen pages. "Sacrifice", which "lies at the heart of African religion", is dealt with in four lines. Some thoughtful observations on economic anthropology are marred by the view that it is only Karl Polanyi who has made it possible to investigate allocation cross-culturally "without disturbing the science of economics and without becoming its slave" (p. 213). The material on politics, government,

law and warfare is, as we might expect from Bohannan's earlier work, of high quality. An interesting section on "the network of agreements" begins with a useful summary of material on contracts and associations and another on rank and other systems of inequality. An examination of "the chimera of race" in this context, presumably with the idea that it fits the treatment of social inequality, is less successful. "Negroes and whites exist in the United States *in the same way* (my italics) that the Republicans and Democrats exist or Catholics, Protestants and Jews exist. They are social categories that influence action" is a statement that strikes oddly to the readers of, say, the recent work of James Baldwin. However accurate may be the notion that human races are social categories based on observable physical differences, it seems rather excessive to state that "race" is the modern Western idiom for pariah groups (pp. 205-6).

Modern social anthropology has a long tradition of interest in the ideas, beliefs and values of the peoples examined. But it is only during the past decade or so that these have received so much overt systematic and detailed attention. Beattie's book rightly stresses this concern, and shows how social anthropologists are interested in symbols and symbolic thinking at two levels of analysis, the level of meaning and the level of social action. This he exemplifies in many fields, including the political and the economic as well as the magical and religious. But this preoccupation has led even Beattie, well known for his solid and perceptive field research, to underplay the quantitative side of empirical field data. "No amount of statistics", he tells us, "will help us to understand a people's conception of spirit or other notion of substance or even what they understand by marriage or by their different categories of kinship". Of course statistics alone are useless; rightly Beattie points to the need for imaginative effort in such understanding. But imaginative effort alone is no substitute for systematic field work. It is precisely because of the lack of adequate statements of the frequency with which terms are used and actions performed and of their distribution in different sectors of the population that we are still vague about the meaning of even some of the best-known ethnographical formulations about a concept of spirit or a notion of substance. But Beattie does give systematic and thoughtful analysis to a range of beliefs and values in institutions such as ancestor worship, magic and sacrifice.

All three books are to be recommended to readers who wish to know more of social anthropology: Beattie's as a straightforward text-book of a thoughtful kind; Bohannan's as a sparkling if somewhat erratic commentary; the essays edited by Goodenough for the specialist who wishes to understand more in depth about some significant trends in modern anthropology.

RAYMOND FIRTH

## AN ENCYCLOPAEDIA OF ORNITHOLOGY

### A New Dictionary of Birds

Edited by Sir A. Landsborough Thomson. Pp. 928+48 plates. (London and Edinburgh: Thomas Nelson and Sons, Ltd., 1964.) 105s. net.

IN celebration of its centenary in 1959 the British Ornithological Union dispatched expeditions to the Comoro Islands and to Ascension Island and undertook a third enterprise of a different character in the launching of *A New Dictionary of Birds*. It was a very good idea and it has been brilliantly carried into effect under the editorship of Sir Landsborough Thomson. It may well come to rank as the best English bird book of the twentieth century.

The editor has himself contributed about forty articles of encyclopaedia length on major subjects of general interest and, in addition, has taken on his own shoulders the burden of defining the terms used in modern ornithology and of writing scores of short articles on a wide range of subjects. This comprehensive book is thus both a new dictionary and an encyclopaedia. But it is more than the indispensable work of reference to be consulted on occasion in an impersonal library. The longer articles designed for "deliberate reading" will make it the constant companion of all those seriously interested in birds as bird-watchers or scientific ornithologists or as biologists concerned in some specialized aspect.

Here they will all find the results of much exciting scientific investigation and a wealth of field observations.

The editor's treatment of names and technical nomenclature puts much disputatious (but not scientific) learning into perspective.

In his discussion of English names he may possibly go too far in saying that the expressions a "charm" of goldfinches or even a "fall" of woodcock have become entirely obsolete, at least in local speech, and can be taken, if used, as only half-serious. But one can unreservedly agree that "to take a minority view in a matter of convention is merely a nuisance".

In paying a tribute to the pioneering publication of Alfred Newton, this work justly claims to be new in scope and plan. It has taken full account and advantage of the great growth in specialized investigations since his day so as to embrace a vast accumulation of new knowledge, and the value of the interplay of disciplines clearly emerges. The 'schematic arrangement' of the major articles on general subjects, listed under that description, is a guide to the entries designed for deliberate reading. Together with the following list of those on main bird groups, it is the key to the basic plan of the book.

The editor adopts the classification of Peters's *Check-List of Birds of the World*, based on the system proposed by Wetmore, and, at the end of the volume, there is an alphabetical list of genera.

The general subjects include form and function, systematics and evolution, distribution and ecology, ethology and birds and man. Within these groups, every topic of importance to ornithologists is separately treated, fully under most headings and adequately under all. Most of the contributors are British, but for some widely spread groups of birds it has often been appropriate to invoke the aid of foreign experts. Similarly, subjects like vegetation and climatology, and some of the more anatomical and physiological essays, have gained in authority from being entrusted to distinguished scientists who are not themselves necessarily concerned primarily with birds. Whatever subject the reader may select, the useful and abundant cross-references carry him on from one enlightening article to another and assist him to pursue his enquiry when the encyclopaedia treatment has split the main topic among several authors. The *New Dictionary of Birds* differs from its predecessor in this fundamental respect of diverse authorship (nor would any other course have been practicable) and in giving far less space to historical matter. It reflects a real shift in the range and direction of ornithological investigation since the end of the past century. Newton himself emphasized the fact that ornithology meant much more than a "perfect taxonomy". But, on the other hand, Sir Landsborough reminds us that, writing in 1903, Ridgway still regarded anatomy and systematics as "scientific", life histories and habits as merely "popular". Nowadays, in taxonomy itself the *New Dictionary of Birds* shows that "the trend is not all towards the laboratory" and that the investigation of the living bird and its behaviour provides material which the taxonomist needs to consider.

The treatment of general subjects may be illustrated by almost any example. The series of articles on the senses

of birds, for example, include a covering introductory note by the editor. As he observes, experiment in this field is difficult and analogies with man may be misleading. In the separate examination of particular senses, it is concluded that taste may play little part in the selection of food, which appears to depend more on shape and tactile response. Smell also requires experiment with a wider range of stimuli before any definite conclusion can be reached. The oil bird (*Steatornis caripensis*) is credited with a very well-developed olfactory apparatus and is said "probably" to locate fruit-trees by their scent. It is an isolated member of the Caprimulgiformes, is gregarious, nocturnal, lives in caves and has a faculty of echo-location.

The nocturnal kiwi is another taxonomically abnormal species and has the olfactory lobe of the brain well-developed. But the evidence for its sense of smell is not accepted as convincing. The nasal organs of the turkey vulture again suggest a well-developed olfactory sense; yet it is unable to detect carrion if it is covered.

Other groups of birds have been thought to possess a sense of smell, including ducks and geese. This view was held by the Dutch keepers who insisted on approaching their decoys with burning sticks in their hands lest the birds should get scent of human beings. To some who have accompanied them it seemed that the ducks were alerted by sound rather than scent.

There is no doubt of a bird's keenness of hearing and acuity of vision. Diurnal, as distinct from nocturnal, birds also possess colour vision. Here the special contributor concludes that the range of colours visible to them is about the same as in man and that the ability to distinguish one hue from another is similar. Some special adaptations of vision in birds are dealt with.

In the remaining sense of touch it has yet to be shown whether rictal bristles have any sensory function. Migration, a different major subject and one on which the editor has long been an authority, provides another instance of interesting treatment. Though no longer "a mystery scarcely penetrated", as Newton described it, it is one far from being completely solved. Many old ideas have been shown to be ill-founded. Dismissed as equally ill-founded is the more recent notion that birds are sensitive to variations in magnetic forces. Magnetic sense is, in fact, defined, with a Johnsonian touch, as a faculty "sometimes hypothetically invoked, but not known to exist". Nor is there evidence of the existence of any organ by which such variations could be measured. The editor's own important article on migration discusses what is known as to the manner in which birds migrate, and the crucial problem of their ability to orientate themselves in the absence of previously known landmarks is examined by Dr. Geoffrey Mathews, who writes on navigation. Briefly, he regards a power of simple compass orientation as certainly established and a more complicated form of grid navigation as "plausible" but not proved, both capacities being based on celestial clues in the sun and the star pattern.

The articles on radar and time measurement are relevant, and it is interesting to find a separate article on Moon-watching as a means of examining migration. Newton records that, at the end of the nineteenth century, observations of the Moon through an astronomical telescope at Princetown showed large numbers of birds passing at a height of one or two miles. As a result of various means of visual observation, and through radar and ringing, a great deal is now known about the modes of migration and the routes which birds follow.

In other articles, the allied topic of irregular irruptions of certain species is interestingly discussed, as is the difficult and even controversial question of the factors regulating bird populations. The scientific basis of this regulation is not yet fully understood, and Dr. Lack, writing on population dynamics, though adhering to his view that animal numbers are regulated by density-

dependent factors, admits that the whole subject is "still in its infancy".

Finally, a series of articles relating to birds and man includes all the issues arising from modern conceptions of conservation and protection, and the dangers attendant on introductions of exotic species.

For many years and even decades this impressively authoritative addition to ornithological literature will hold the field. Long before the British Ornithological Union celebrates its second centenary and contemplates another new dictionary, many and perhaps most of the unresolved scientific questions so ably discussed and now left open will have found their answer. Whether the variety, range and abundance of the world's birds and of habitats suitable for most of them will still be as fascinating for ornithologists to study and bird-watchers to enjoy a century hence is a speculation on which it is not possible to embark without misgiving and even anxiety.

HURCOMB

## EARTH SCIENCES

### The Planet Earth

Edited by Prof. D. R. Bates. Second edition, revised and enlarged. Pp. vii+370. (London and New York: Pergamon Press, 1964.) 42s. net.

### The Earth Sciences

By Prof. Arthur N. Strahler. (Harper's Geoscience Series.) Pp. xii+671. (New York, Evanston, and London: Harper and Row, 1963.) 75s.

### Introduction to Satellite Geodesy

By Prof. Ivan I. Mueller. Pp. xxi+415. (New York: Frederick Ungar Publishing Co.; London: Constable and Co., Ltd., 1964.) 15 dollars; 120s.

### Gravitation and Relativity

Edited by Dr. Hong-Yee Chiu and Dr. William F. Hoffmann. (Physical Investigations of the Universe.) Pp. xxxv+353. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 15.75 dollars.

THESE four books are of considerable interest to workers in the Earth sciences.

*The Planet Earth*, edited by Prof. Bates, has been published in a second edition. It is a useful survey in 18 chapters of the separate aspects of the physics of the Earth and its environment. Although in the preface to this edition it is stated that there has been considerable revision of the chapters to bring them up to date, there has, in the interval of six years, been revolutionary advance in many of the topics under discussion and the revision has in many cases not been sufficiently thorough. However, it is a book which should be read by all students of geophysics.

*The Earth Sciences*, by Prof. Strahler, is of a much more elementary level and is intended as an introduction to be read by students in American university departments of geology. It is extraordinarily well illustrated with much useful graphical presentation and some remarkable photographs. What is perhaps important in relation to teaching in geology departments in Britain is that geophysical and astronomical topics are tackled in detail and I think the treatment is successful. The presentation of meteorological, astronomical and oceanographical principles to students of geology who often have not specialized in physics and mathematics is difficult, and Prof. Strahler is to be congratulated on his attempt to present physical explanations in simple terms by very excellent illustrations.

The other two books are of a more specialized nature. *The Introduction to Satellite Geodesy*, by Prof. Mueller, is of great importance. The accurate determination of the small divergences of the Earth from hydrostatic equilibrium

will probably provide the solution to many of the more important questions in geophysics during the next few years. The question whether or not convection currents are occurring in the mantle of the Earth, and if so what their distribution is, is likely to be solved in this way.

The book by Dr. Chiu and Dr. Hoffmann on *Gravitation and Relativity* may seem to have little interest for the geophysicist. However, in the past the investigation of the solar system has revealed fundamental laws of physics which could not easily have been discovered in the laboratory. The geophysicist should not always assume that he is simply concerned with the application of the known laws of physics to explain natural phenomena. The distinguished contributions in the book by Prof. Dicke and Prof. Wheeler concern fundamental difficulties in the theory of gravitation, to the discussion of which geophysical data on the evolution of the Earth-Moon system may still produce a notable contribution.

S. K. RUNCORN

## ASPECTS OF RELATIVITY

### The Theory of Space, Time and Gravitation

By V. Fock. Translated from the Russian by N. Kemmer. Second revised edition. Pp. xii+448. (Oxford, London, New York and Paris: Pergamon Press, 1964.) 100s.

THE first edition in English of *The Theory of Space, Time and Gravitation*, published in 1958, was greeted as an important and original contribution to the literature; in subsequent years Prof. Fock's book has continued to be held in high regard, proving itself worthy of its initial acclaim. The recent publication of the second revised edition of this masterly work is indeed to be welcomed.

Able translated from the Russian by Prof. Kemmer, this book includes, within its role as a text-book for the graduate student in theoretical physics, the author's own powerfully argued and highly distinctive views and interpretations of the subject of relativity. It is a thorough exposition of what are usually called the special and general theories, although Fock insists that these are misnamed. Using the word 'relativity' to denote an invariance in the description of physical processes in different frames of reference, Fock justifies instead the nomenclature 'the theory of relativity' and 'the theory of gravitation', by pointing out that the principle of relativity is directly related to uniformity and that Galilean space has maximal uniformity.

The first four of the seven chapters of the book are devoted to the development of the theory of (special) relativity, including its formulation in tensor form. The remaining chapters, rather more controversial in character, deal with Fock's own interpretation of the foundations and principles of Einstein's theory of gravitation.

In its non-cosmological form, this is based on the hypothesis that the geometrical properties of real, physical space and time correspond to Riemannian, rather than Euclidean, geometry, any deviation from the pseudo-Euclidean form appearing as a gravitational field. Throughout the work, Fock is concerned with ensuring the uniqueness of this result, and he deals with the problem of boundary conditions by combining with the above hypothesis the assumption that there is an insular distribution of masses, so that the geometry at points far removed from the masses is Euclidean. (When the cosmological problem is briefly discussed in Sections 94 and 95, this assumption is replaced by that of a uniform distribution, which leads to a Friedmann-Lobachevsky space.)

The field equations then follow from a generalization of Newton's laws, and the remainder of the book deals, in the main, with the derivation of the equations of motion and with gravitational potentials. Much of the complexity



of the resulting equations is reduced by Fock's use of a preferred system of co-ordinates, which he calls 'harmonic'. Fock accords a particular significance to harmonic co-ordinates (each of which satisfies a linear, generally covariant, equation), apparently because a harmonic system is uniquely determined to within a Lorentz transformation, under suitable supplementary conditions.

The changes effected for the new edition are summed up in the author's preface as follows: "The second edition differs from the first by some additions and reformulations. The question of the uniqueness of the mass tensor is treated in more detail (Section 31\*) and is illustrated by two examples (Appendices B and C). The notion of conformal space is introduced and used as a basis for the treatment of Einsteinian statics (Sections 56 and 57). Greatest care has been applied to the formulation of the basic ideas of the theory and to the elucidation of those points in which the author's views differ from the traditional (Einsteinian) ones. Thus, in order to discuss the general aspects of the relativity principle Section 49\* has been added".

Prof. Fock's unorthodox views, expounded with a forceful clarity, undoubtedly help to advance our understanding of the theory of gravitation, making this a work of major importance.

ELIZABETH HILTON

## STELLAR STATISTICS

### Statistical Astronomy

(Dover Publication No. 301.) By Robert J. Trumpler and Harold F. Weaver. Pp. xxi+644. (New York: Dover Publications Inc.; London: Constable and Co., Ltd., 1962.) 24s.; 3.00 dollars.

*STATISTICAL Astronomy* deals with statistical investigations of the system of stars, and concentrates on such problems as the space distribution of stars, the distribution of stars on the *H-R* diagram, the distribution of stellar velocities, and the phenomenon of galactic rotation. The statistical techniques involved are generally elementary in principle and tedious in application, and are fully developed in the first part of the book.

On reading such a detailed account and development of the subject as is presented here, one cannot help feeling that statistical astronomy has absorbed a great amount of the energy of astronomers and produced singularly few results. The authors should be fully aware that, because of the existence of interstellar dust, any star-counts can only lead to a 'local' picture of the star system, and a very distorted picture at that, if allowance is made only for a 'smooth' distribution of dust clouds; yet the book spends more than 120 pages doing just that. Shapley's classical investigation of the globular clusters which first led to a correct assessment of the star system is not mentioned, presumably because it involved too little statistics. Again, the construction of *H-R* diagrams for stars in the solar neighbourhood involves much statistical manipulation and takes up a correspondingly large space in the book, yet it is nothing like as useful as the *H-R* diagrams for star clusters, which involve little statistics and receive correspondingly brief treatment. In the field of stellar motions, the book gives a good description of Kapteyn's two-stream model and Schwarzschild's ellipsoidal model; it then develops the latter along Charlier's method of moments. The treatment is complete from an observational point of view, but the most intricate part of the mathematical problem (p. 66) is shirked by a facile reference to the two-dimensional case. It omits to mention Ambarzumian's method of determining the distribution of space motions without assuming a model. It seems that the best part of the book is the part dealing with the phenomenon of galactic rotation. Oort's original theory and Bottlinger diagram are set out very clearly, and are further developed in a sound manner. This subject is

mainly kinematical and dynamical in character, and is only marginally statistical.

There is an unfortunate idea among astronomers that statistical astronomy is a dull subject, that nothing of value has come out of it that cannot be improved on by non-statistical means. This is not true. There are exciting problems in this field, and these problems can be nothing but statistical, to witness, the distribution of interstellar matter and the clustering of galaxies. It is a pity that the only book in English that bears the title of 'statistical astronomy' fails to include these more exciting problems, and presents the subject in a rather unattractive manner by confining itself to problems in stellar astronomy. But, since there is also a dearth of text-books on stellar astronomy, this book is quite worth its Dover edition price from the point of view of the serious student.

T. KIANG

## A BIOLOGIST LOOKS AT MAN

### Genetics and Man

By Prof. C. D. Darlington. Revised edition of *The Facts of Life*. Pp. 382+8 plates. (London: George Allen and Unwin, Ltd., 1964.) 42s. net.

IN the preface of the first edition of his book *The Facts of Life*, published in 1953, Darlington defined his purpose, "to show the immense possibilities which await the application of the elementary principles of heredity to the great problems of society". In the preface of the revised edition, retitled *Genetics and Man*, Darlington explains that it has been necessary to abridge the old text in order to make room for new material. Certainly the new title is more apt, because the volume does not contain much information about what are popularly considered to be the facts of life.

The first comment to make about this book is that it is most entertaining. Geneticists, particularly those concerned with human genetics, can complain that some of the arguments are developed by an oversimplification of the facts. The biologists may be annoyed by occasional lapses into teleology, "The fly therefore has to reduce recombination" (p. 120). The stylists may appreciate the musical analogies, "mitosis is a symphony consisting of four movements" (p. 69), but find some of the constructions rather tortuous. The cynics will comment on a certain naïvety in the approach to problems involving human sexual relationships. There are one or two places where the reader is arrested by prejudice, for example, in a discussion of the education of normal women, "Girls are therefore prepared for a life which most of them will never follow, or wish to follow, and which it would be disastrous to the community if they did follow" (p. 317). This appalling state of affairs is attributed to the great influence that the "positively homosexual type of women" (p. 316) has had on the educational system!

In order to develop the theme of genetic determinism, the reader is taken smoothly through the advances in genetics from Moses via Mendel to molecules. There are interesting excursions into the history of forgeries that disturbed the scientific world. Having laid the foundation, Darlington philosophizes on the importance of the genetic background in all human situations. Advances in medicine have so reduced the untreatable causes of morbidity and mortality that medical practitioners have become increasingly aware of the importance of the family history of their patients—if not their genes. So Darlington possibly overstates the case when he writes, "And, when medicine wakes up to the existence of biology and rediscovers a philosophy, the genetic individual will be restored to his place" (p. 243).

These criticisms do not, however, seriously detract from the merits of a book that can be read both for pleasure and profit by laymen and scientists.

SYLVIA D. LAWLER

## College Chemistry

An Introductory Textbook of General Chemistry. By Prof. Linus Pauling. Third edition. Pp. xxiv+832. (San Francisco and London: W. H. Freeman and Company, 1964.) 50s.

THE third edition of *College Chemistry* brings in a wider range of topics, including a final chapter on the thirty-four fundamental particles. The theory is gradually and clearly introduced and includes some more advanced topics, such as Boltzmann's theorem from both the classical and quantum aspects. The sections on molecular structure, based on the valence bond and resonance theories, are clear and provide numerical data and graphs which add to their usefulness. The theory of metals and alloys is dealt with. Statistical mechanics rather than thermodynamics is mainly emphasized, but free energy and entropy are used in connexion with equilibrium; otherwise enthalpy changes are given prominence. The descriptive chemistry, including some organic chemistry, is adequately presented for a book of this character, and some attention is given to the history of chemistry. In discussing the elements and compounds, emphasis is laid on systematization, but sufficient detail is given to make the information worth while. The reaction for the preparation of chlorine dioxide given on p. 371 is incorrect. On p. 377 iodine dioxide is said to be paramagnetic and hence to have the formula  $\text{IO}_2$  and not  $\text{I}_2\text{O}_4$ ; it is in fact diamagnetic and the formula cannot be  $\text{IO}_2$ . There is a good description of the transuranium elements and related subjects. There is no doubt that this book, which has been carefully planned and written, will continue to enjoy the popularity which it fully deserves.

J. R. PARTINGTON

## Order-Disorder Phenomena

By H. S. Green and C. A. Hurst. (Monographs in Statistical Physics and Thermodynamics, Vol. 5.) Pp. x+363. (London and New York: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1964.) 105s.

*ORDER-Disorder Phenomena* is Volume 5 in a series of monographs edited by Prof. Progogine. So far, this series has produced one volume which is a very advanced text-book and four learned monographs. One may ask at this stage whether the series really provides scientists and engineers with "a comprehensive treatment of the basic methods of statistical mechanics" as is claimed. I seriously doubt whether this aim has been realized, and the relatively excessive price of the volumes in the series indicates also a lack of conviction on the part of the publishers.

If, however, one considers this series to be aimed purely at the specialists, there is a great deal to recommend it since the volumes in the series are well written by authorities in the fields covered. *Order-Disorder Phenomena* is no exception, as both authors have made significant contributions to the investigation of the subject.

The title of the book is slightly misleading, for it is essentially concerned only with a discussion of the so-called Ising model and even then mainly with the case of the two-dimensional lattices. Within this restriction the treatment is extensive and covers most recent developments. References to other work are, as seems to be an increasing tendency, patchy. The early paper by Lassetto and Howe in which the matrix method was proposed, independently of Kramers and Wannier, is, for example, not mentioned. Not very surprisingly, great emphasis is placed on the Pfaffian method developed by the authors. Unfortunately, recent developments and simplifications by Rumer and by Schultz, Mattis and Lieb occurred too late to be included.

The book starts with a general discussion of phase transitions and a statement of the Ising problem. The authors then show how far this model is applicable to the theory of ferromagnetism, antiferromagnetism and

ferrimagnetism, to the theory of binary alloys and to the discussion of lattice models of fluids. A discussion follows of the rectangular two-dimensional Ising lattice and the Pfaffian theory applied to this case. Then come a chapter on the general theory and a chapter dealing with other lattices, such as the triangular and hexagonal lattices, and also discussing correlations and spontaneous magnetization. In conclusion, other methods and a few outstanding problems are discussed and six mathematical appendixes complete the book.

The book is well written and can be recommended as an introduction to various approaches to the Ising model.

D. TER HAAR

## Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik und Technik

Von Landolt-Börnstein. Sechste Auflage. Zweiter Band: Eigenschaften der Materie in ihren Aggregatzuständen. 2 Teil: Gleichgewichte ausser Schmelzgleichgewichten. Bandteil c: Lösungsgleichgewichte II. Bearbeitet von J. D'Ans, D. Jänchen, E. Kaufmann und C. Kux. Herausgegeben von Klaus Schäfer und Ellen Lax. Pp. viii+731. (Berlin, Göttingen, Heidelberg: Springer-Verlag, 1964.) Moleskin 388 D.M.

SUCH is the credit rating of Landolt-Börnstein that it is not necessary to do more than to indicate the scope of this book. It opens with a continuation, from the earlier volume, of data on solubility equilibrium of solids and liquids, limited here to organic compounds. The survey of binary systems occupies rather more than half the pages; the arrangement is not difficult to follow, but to assist there is a very full index, based on molecular formulae. To say that this part is amplified by 321 diagrams does not give a true idea of the extent of the use to which these are put, for nearly all illustrate several systems, sometimes as many as a dozen; a few give the impression of being rather overcrowded. Information on about 300 ternary and higher systems is limited to literature references.

The remainder of the space is devoted to equilibria in systems having immiscible liquid phases. After a general survey, presented in tabular form, ternary and quaternary systems are dealt with in detail, each illustrated clearly with diagrams. The last part tabulates distribution coefficients in two phase systems; most of the solutes are organic but not all; water is one of the solvents in the majority of the systems recorded.

As is acknowledged, some information has been supplied from outside sources, and one cannot but admire the manner in which it has been collected from world-wide literature and then collated. It appears to have been overlooked that *NN*-diphenylacetamide and *N*-acetyldiphenylamine are identical; however, the arrangement brings the two entries next to one another on p. 359, so confusion is minimized.

B. A. ELLIS

## 1963 Transactions of the Tenth National Vacuum Symposium of the American Vacuum Society

October 16, 17, 18, Boston, Massachusetts, U.S.A. Edited by George H. Bancroft. Pp. xxi+510. (New York: The Macmillan Company; London: Collier-Macmillan, 1963.) 23 dollars; 172s.

THE extent of the enormous surge forward in vacuum technology and its applications in the United States during the past decade can be well appreciated by an examination of this handsomely produced volume containing 93 papers presented to 1,600 delegates at the tenth National Vacuum Symposium. The scale of activity is much greater than in Britain, and it is particularly significant that considerable research is in progress in American universities, whereas in British colleges work on vacuum, especially in its engineering aspects, is comparatively slight.

This American effort has been greatly stimulated by their space programme. Apart from the problems of the design of space simulators, much research is recorded here on materials from the points of view of lubrication, friction and wear *in vacuo*, effects of ion bombardment, solar and reactor radiation (the latter anticipating the possible development of nuclear-reactor powered rockets) and gas sorption and desorption. Freeze-drying, though a well-established technology, is being actively developed, particularly to reduce costs in application to food dehydration. The new techniques of sorption and cryopumping are well represented by nine papers and there are five papers on getter and ion pumps. The vapour diffusion pump, which has been a vital tool in vacuum technique for half a century, so far from being ousted by newer methods, is becoming widely accepted in ultra-high vacuum systems now that it is clearly realized that its ultimate pressure is theoretically vanishingly small, and is therefore the concern of nine research papers. There are twenty papers on vacuum measurement representing the growing body of knowledge about ionization gauges, gas analysis by mass spectrometer methods and the problems of calibration. Thin film technology is the concern of thirty-six papers, classified under sputtering, semi-conductor films, film preparation and film properties.

These *Transactions of the American Vacuum Society*, appearing annually, are the best reviews available of the most recent vacuum technology. The problem exists of the conference where so many papers are read, often with too little attention directed to presentation both at the lecture and in the final written account, that the audience and the reader tend to suffer severe mental indigestion. However, the American Vacuum Society is undoubtedly a progressive and imaginative body from which consideration of this problem can be expected. J. YARWOOD

### Packing and Covering

By Prof. C. A. Rogers. (Cambridge Tracts in Mathematics and Mathematical Physics, No. 54.) Pp. viii+111. (Cambridge: at the University Press, 1964.) 30s. net; 5.50 dollars.

SUPPOSE that a large box is filled with spheres of equal size. The proportion of the volume of the box which is occupied depends, of course, on the closeness of the packing; it is possible to pack the spheres in such a way that this proportion is as high as  $\pi/3\sqrt{2}=0.74$ , but it is not known whether this is the closest possible packing. This is a very typical packing problem, and the fact that, despite its simple formulation, it continues to defy the efforts of mathematicians to solve it shows that the theory of packing is a non-trivial, and indeed a challenging, branch of pure mathematics. It is also important because of its connexions with the 'geometry of numbers' and hence with number theory.

The same problem can be posed in  $n$  dimensions. When  $n=2$  the answer is known; the closest possible packing of circles fills a proportion  $\pi/2\sqrt{3}=0.91$  of the area of the plane. When  $n\geq 4$  the best packing is not known, but certain packings thought to be quite good have been found. For example, in 12 dimensions Coxeter and Todd have a packing which fills a proportion  $\pi^6/19,440=0.05$  of the space.

Prof. C. A. Rogers gives a fascinating account of these problems in *Packing and Covering* and of the known results, particularly of those which give (incomplete) information when the dimension  $n$  is large. He is concerned not only with spheres, but also with more general bodies and with certain restricted types of packing.

A very similar problem is that of covering. Suppose, for example, that one tries to cover a region of the plane of large area  $A$  with circles of unit area. The minimum number required will be approximately  $1.21 A$ , where the number  $1.21$ , or more exactly  $2\pi/3\sqrt{3}$ , is a measure of the necessary overlap. The analogous problem in 3

dimensions is unsolved, but the number which must replace  $1.21$  is known to lie between  $1.43$  and  $1.47$ . Here again the problem can be generalized, and there are a few results where the dimension  $n$  is large.

It is, however, true that most of the interesting problems are unsolved, and one may hope that Prof. Rogers's well-written survey of the field will stimulate mathematicians to a renewed attack, while leaving them under no illusions about the difficulty of their task. J. F. C. KINGMAN

### An Introduction to the Solidification of Metals

By Dr. W. C. Winegard. (Institute of Metals Monograph Series, No. 29.) Pp. x+98+10 plates. (London: The Institute of Metals, 1964.) 42s.

THIS short monograph contains an elementary and concise survey of present-day knowledge on the solidification of metals. The book has been written for undergraduate students and those metallurgists and scientists who have no previous knowledge of the subject. Dr. W. C. Winegard has succeeded admirably in condensing much information which was only previously available in original papers and research reports.

The book attempts to relate the structure of the freezing solid with the conditions that prevail during the process of solidification. The opening chapters cover the relevant properties of solids and liquids, the thermodynamics of nucleation and the subsequent process of crystal growth in pure metals. An important chapter is devoted to the consideration of alloy systems and phase diagrams, where emphasis is placed on the concentrations that exist at the solid-liquid interface. These ideas are then used by Dr. Winegard in a discussion of the growth structures that occur when alloys solidify, the concept of constitutional undercooling being introduced, and there follows a review of growth structures in solid-solution alloys. Later chapters deal with segregation, eutectic growth, zone-refining and techniques available for growing single metal crystals from the melt.

The presentation of the material has been well thought out and the book provides a valuable introduction to more advanced reading on the subject.

D. T. TURNBULL

### The World of Birds

A Comprehensive Guide to General Ornithology. By James Fisher and Roger Tory Peterson. Pp. 288. (London: Macdonald and Co. (Publishers), Ltd., 1964.) 105s. net.

SCIENTIFICALLY, this book is light-weight, since it is mainly a work of interpretation and, although it has a bibliography, the entries are not specifically tied to the text. But, as a work of interpretation and as an aesthetic production, it will find a wide and appreciative audience. Birds lend themselves to artistic portrayal, and ornithologists will find a great wealth of material, on birds from all parts of the world, lavishly spread before them in the inimitable style of Roger Tory Peterson. The text by James Fisher deals with all aspects of bird biology under subject headings, and the illustrations are deployed to give the richest possible commentary to the text. The long series of maps depicting the world distribution of many families of birds, including those known from fossil material, will be a mine of comparative information to those ornithologists who have specialized in their own regional avifaunas. Finally, the sections dealing with species which have become extinct and those which are threatened with the same fate are particularly valuable. In addition there are useful chapters on technical aids and on the co-operative approach to the investigation of such subjects as migration, nest-recording and census work.

The standard of reproduction and presentation is first-class and, despite the high selling price, this is a book which will appeal specially to the talented amateur.

H. N. SOUTHERN



## MOLECULAR BIOLOGY IN CHINA

By PROF. M. ERRERA

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NOT having been a regular reader of *Scientia Sinica* or of other Chinese scientific journals, my first visit (together with five other Belgian scientists) to Chinese institutions was an experience as stimulating as visiting a museum full of recently discovered works of art or walking in a park built where there were slums half a dozen years ago. The Chinese people are advancing in all fields simultaneously, and the field of science is not remaining behind any other. Of course, the challenge of educating the people is tremendous and the problems with which Chinese scientists are involved are extremely complex: both technical and scientific education badly need to be developed with the greatest possible speed. This the National Science and Technological Commission, headed directly by one of the vice-premiers, and both the Academia Sinica and the Chinese Academy of Medical Sciences, have clearly understood: they have taken the necessary steps to make rapid progress. The leading scientists of the country have been re-grouped in the various institutes of the Academia Sinica or the Academy of Medical Sciences, which have started work mainly in Peking and in Shanghai, but also in the other big cities. The main responsibility of these groups is to develop research and graduate teaching, but some of them are also responsible for writing up-to-date Chinese text-books to be used in the universities. Members of the Biochemical Institute in Shanghai provide an example.

## Protein Chemistry

*Enzyme structure and function.* The approach is both theoretical and experimental: Tsou Chen-lu (Institute of Biochemistry, Academia Sinica, Shanghai) has examined data from the literature relating the modifications of functional groups of a variety of enzymes to their biological activity and has established equations from which the number and types of essential groups involved can be calculated. This defines the best experimental conditions for obtaining further results. This investigation covers many hydrolytic enzymes, some of which are now being examined experimentally, and it has led to the suggestion that, in many cases, two enzyme substrate intermediates are involved, one of the Michaelis type and the other an acyl enzyme intermediate in the case of proteinases or esterases. This type of mechanism has been generalized to other systems and shown to be compatible with data from present-day literature<sup>1,2</sup>.

A certain number of proteolytic enzymes have been approached experimentally: the action of sulphite on the S-S bridges of trypsin in the presence of *p*-chloromercuribenzoate has shown that for many artificial as well as natural substrates (benzoyl-L-arginine-amide, *p*-toluenesulphonyl-L-arginine methylester, Hb), three out of the six S-S bridges are essential for enzymatic action<sup>3</sup>. The study of *p*-nitrophenacyl bromide as an inhibitor of insulin, ribonuclease, papain and  $\alpha$ -chymotrypsin led to the suggestion that in the conditions used the imidazole group of histidine (as was known to be the case with RNase) is involved.

Further investigations on papain (photo-oxidation of histidine and of tryptophan residues as well as the oxidation of the latter by *N*-bromosuccinimide at pH 5.0) have established that one of each of these residues was essential for papain. These investigations also led to establishing that a polymer of leucine could be obtained by incubating leucineamide with papain at pH 7 as well as with a spleen preparation apparently different from

known cathepsins. With papain, a mixture of leucineamide and phenylalanine methylester formed a mixed peptide of leucine and phenylalanine. The active site of papain involved in peptidase activity is apparently different from that which is active in splitting benzoyl-arginineamide as deduced from the amount of ammonia evolved in the presence of one or of both of these substrates and of the differences in the kinetics of inhibition of papain with *N*-bromosuccinimide with both substrates<sup>4,5</sup>. These various investigations have also led Du Yu-cang and Tsou Chen-lu<sup>6</sup> to propose an original spectrophotometric method for determining protein SH groups by the absorption at 420  $\mu$ m of the complex formed between these groups and 1,4-naphthoquinone.

*Organic synthesis of insulin.* The research in protein structure and function led to an efficient method (> 50 per cent recovery using a biological test) of regenerating active insulin from its A and B chains obtained by the quantitative splitting by Na<sub>2</sub>SO<sub>3</sub> of the three disulphide linkages in the absence of urea<sup>7-9</sup>. Molecular properties of the B chain were examined and it was shown by optical rotatory and viscosimetric measurements that there was a low helical content<sup>10</sup>. This property apparently favours linear polymerization of the molecule when its S-sulphonate derivative is left at room temperature and at pH 6-9. The polymerization was inhibited in solutions containing 30 per cent dioxane chloroethanol or formamide, as well as in 5 M urea. These results suggested that hydrophobic bonds were the main forces holding the monomers together<sup>11</sup>. Both polymer and monomer were examined with the electron microscope. These investigations showed the polymer to be of indefinite length and 50-60 Å in diameter (which is slightly smaller than fibrous insulin); it seems to be formed by the assembly of units 200 Å long which are visible during the 'induction' period of polymerization<sup>12,13</sup>.

Then, in collaboration with the Institute of Organic Chemistry and the University of Peking which started the synthesis of the A chain, the workers of the Institute of Biochemistry began the synthesis of various peptides of the B chain<sup>14</sup>. At present complete A and B chains have been obtained<sup>15</sup>, and because of the excellent yields obtained for the re-synthesis of both natural chains of insulin both chains combined to their natural counterpart gave active insulin molecules.

*Comparative chemical and physico-chemical study of several strains of tobacco mosaic virus.* Four strains of TMV have been compared by serological methods, electron microscopy, fingerprinting of the proteins, etc. These observations suggest a close genetic parentship between the various strains (identical terminal threonine) but a closer similarity between the YMV<sub>15</sub> and DDV strains. During these investigations, a method using chloroethanol to precipitate the RNA was suggested for obtaining pure virus proteins. The proteins and nucleic acids can be recombined to form active virus. The amino-acid composition and the C-terminal pentapeptide structure of YMV<sub>15</sub> suggest that it is a new strain of TMV<sup>16,17</sup>.

*Muscle proteins and muscle functions.* Work on the molecular properties of muscle proteins has been commenced by the Institute of Biochemistry of the Academia Sinica in Shanghai; examinations of muscle are also being carried out in the Institute of Physiology. The first group is investigating with the electron microscope a number of para-crystalline forms of tropomyosin and paramyosin from a variety of biological species: both proteins, on the

basis of electron microscopic periodicities, are quite different; however, when present in the same muscle, as in some molluscs, some genetic relationship between them may exist on account of the intraperiod fine spacings of 140 Å found for *Anodonta* tropomyosin (recalling the characteristic fine spacing of the paramyosin from the same origin, in contrast to the usual 200 Å found for tropomyosin)<sup>18</sup>. Tropomyosins of various origins were also examined immunologically and by fingerprinting, and some interesting evolutionary aspects of shark and ray proteins were obtained<sup>19,20</sup>.

*Effects of ionizing radiations on some physical chemical properties of bovine plasma albumin.* It was shown that the binding of <sup>35</sup>S-methionine by bovine plasma albumin was increased after radon irradiation, but that the viscosity of the solution as well as its specific rotation did not change at neutral pH; however, at pH < 5 the specific rotation was lowered—but it was increased at pH > 7. The antigenicity of the protein was also reduced<sup>21,22</sup>.

### The Respiratory System

Earlier work by Wang Tsing-ying *et al.*<sup>23</sup> led to the partial purification of succinic dehydrogenase and the establishment, independently of other workers, that succinic dehydrogenase was a metalloflavo-protein containing non-haematin iron. Wu Chin-yung and Tsou Chen-lu<sup>24</sup> demonstrated that succinic dehydrogenase was linked to the cytochrome *c* part of the chain through a common velocity-limiting factor with NADH dehydrogenase. These investigations led Tsou Chen-lu and Li Wen-chieh<sup>25</sup> to an examination of cytochrome *c* from mammalian heart muscle and from yeast (see also Li Wen-chieh and Tsou Chen-lu<sup>26</sup>). The flavine prosthetic group of purified succinic dehydrogenase was separated after trypsin and chymotrypsin digestion from the proteins, and fractionated by electrophoresis into four flavine derivatives containing various amounts of attached amino-acids. These are inactive in the D-amino-acid oxidase test and are therefore distinct from FAD<sup>27</sup>. These flavin-adenine peptides do not show changes of absorption maxima in alkaline or acid solutions like FMN and FAD; this is probably due to the peptide linked to the isoalloxazine ring. The nature of the peptide and type of bond to the isoalloxazine ring awaits further characterization<sup>28</sup>. Continuing this work on the respiratory chains, Wu Chin-yung compared the action of various inhibitors on choline and L- $\alpha$ -glycerophosphate oxidase, and he concluded that both systems contain an antimycin A or BAL sensitive step, whereas cytochrome *b* is concerned only with choline oxidase<sup>29</sup>.

Further work on the relationship between the succinic and NADH oxidase systems by Liu Lin, Du Yu-cang and Tsou Chen-lu gave no experimental support for the suggestion that endogenous cytochrome *c* was directly linked to the succinic oxidase-NADH oxidase chain through a phospholipid<sup>30</sup>.

In 1964 Ao Shih-jou and Chang Yu-tuan<sup>31</sup> examined the oxidation of succinate in function of the concentrations of 2,4-dinitrophenol. At low concentrations of DNP respiration of succinate is increased, while at higher concentrations, after a short stimulation, there is an inhibition which can be prevented by ATP, which also prevents the inhibition of respiration that is obtained with amytal or arsenate. This led the authors to postulate that some activation energy is required for succinate oxidation.

Results leading to similar conclusions have been obtained by Wu Chin-yung *et al.*<sup>32</sup>. Ling Chi-shui and Wu Chin-yung further showed that no inhibition of respiration by DNP is observed when succinate is oxidized with 2,6-dichlorophenolindophenol—showing that DNP does not inhibit at the dehydrogenase level. In addition to ATP, restoration of respiratory activity could also be obtained in the presence of substrates of the NADH-linked

dehydrogenases; it was suggested that this high energy intermediate might be formed by the reversed reaction of the succinate linked endogenic reduction of NAD<sup>+</sup> in the presence of DNP. This reaction was demonstrated by Wu Chin-yung and Ling Chi-shui using sub-mitochondrial preparations in the presence of ATP; this reduction was inhibited by amital, dicoumarol and DNP and was completely dependent of magnesium, manganese and probably another metal which can also be removed by EDTA<sup>33</sup>.

Comparing the competitive inhibition by aged haematin on various flavoprotein enzymes<sup>34</sup>, it was shown that the inhibitory effect of this compound depended on the acceptor system, and the similarity of response on succinate and NADH oxidation (heart muscle mitochondria) with that of choline (rat liver mitochondria) and of  $\alpha$ -L-glycerophosphate (rabbit skeletal mitochondria) led to the suggestion that the two last-mentioned were also flavoprotein enzymes.

Whole-body irradiation was found to induce mitochondrial swelling within 30 min–3 h; this effect was greater in the presence of Ca<sup>++</sup> or thyroxine, and it is concluded that structural damage to the mitochondria had been induced. However, respiration and oxidative phosphorylation, which had been somewhat inhibited, became normal again after 4 h.

*Microsomal oxidation of NADP.* The oxidation of NADP in rat liver microsomes and supernatant was examined, and the use of various inhibitors showed that cytochrome oxidase is not involved, but that elements of both cell fractions seemed to be necessary for full activity<sup>35</sup>.

*Photosynthesis.* In addition to electron spin resonance investigations on chloroplasts in the Biophysics Institute, Peking, Tsou Chen-lu, of the Institute of Biochemistry, Shanghai, has devised kinetic equations for photosynthesis based on the assumption of two limiting reactions when flashing light is used—one photochemical and one biochemical. The relation between flash yield and flash period predicted by this equation agrees with experimental results recorded in the literature. The equation can be simplified under certain conditions and permits the calculation of the rate constant for both the limiting reactions as well as the 'concentration' of the 'photosynthetic unit'<sup>36</sup>.

### Nucleic Acids

Interest has concentrated on amino-acid transfer RNA (sRNA)—chiefly in the Institute of the Chinese Academy of Medical Sciences, Peking, as well as in the Institute of Biochemistry of the Academia Sinica in Shanghai.

*Preparation.* A method of preparation of sRNA from phenol bacterial extracts and fractionation with ammonium sulphate has been devised by Wu Kuan-yun *et al.*<sup>37</sup> and the isolation of an sRNA preparation with high methionine accepting activity homogeneous by sedimentation and electrophoresis tests has been obtained.

*Structure.* The same group devised a spectrophotometric method for the base analysis of RNA applicable to 100- $\mu$ g quantities<sup>38</sup>. An examination of 1-fluoro-2,4-dinitrobenzene derivatives of sRNA has enabled Hu Ping-cheng *et al.* to show that the reagent was fixed on 5'-phosphoguanosine; this finding should facilitate the analysis of the pG end of the sRNA chain<sup>39</sup>.

A paper electrophoretic and chromatographic method has also been described to obtain 'fingerprints' of sRNA digests with pancreatic RNase<sup>40</sup>. Differences between yeast and silk gland sRNAs have been described by this method<sup>41</sup>.

*RNase degradation.* Liu Wang-i and Wang Tch-pao<sup>42</sup> have shown the requirement of the Mg<sup>2+</sup> (or Ca or Na) for the activity of an RNase from *E. coli* on sRNA, whereas Ni<sup>2+</sup> is inhibitory. sRNA is much less susceptible to the enzyme than 'heavy' RNA—probably on account of the greater secondary structure of the former.

**Deamination.** The same authors<sup>43</sup> showed that the secondary structure of sRNA was altered after deamination with hydroxylamine in spite of the fact that this treatment did not alter the extinction coefficient at 260 mμ or the ultracentrifuge sedimentation pattern. However, the deaminated sRNA became more susceptible to RNase because of the alteration of its secondary structure.

**Effects of ionizing radiations.** Irradiation of DNA *in vitro* showed a drop in viscosity and rise in optical density at 260 mμ, which increased on heating the solution, indicating that hidden damage involving only one strand had occurred and that the double helix became less stable at temperatures nearing the normal melting-point<sup>44</sup>. After irradiation *in vivo* (1,000 r.) the DNA extracted from the spleen and thymus showed a reduced hyperchromic effect at alkaline pH indicating that the secondary structure might also be affected *in vivo*<sup>45,46</sup>.

**Protein synthesis.** Work on protein synthesis is only starting, but the stimulation of glycine incorporation into *E. coli* sphaeroplast proteins by RNA and various nucleotides (especially uridylic acid) has been shown by Tung Lin and Mann<sup>47</sup>.

In a combined cytochemical and biochemical investigation, K. H. Lu and P. Y. Wang have shown that glycine incorporation into silk fibroin appears to be a cytoplasmic process. In this investigation, RNA activity is also examined with phosphorus-32 (ref. 48).

A simple method for organ cultures on filter paper strips as a support for flowing culture medium has been described by Chen Jui-ming and Chuh Teh-ho<sup>49</sup>. By this procedure organ rudiments can proceed with differentiation and remain cytologically normal for 10–16 days, and the procedure appears very suitable for the examination of organ interactions, hormone action and many other aspects concerning the regulation of protein metabolism.

Antibody synthesis in rabbit popliteal lymph node cells cultured *in vitro* after antigen injection into the foot pad has been obtained in suspension cultures. In these preliminary investigations, a 20–30 per cent increase of antibody was recently observed—but in some experiments there was as much as 400 per cent increase. This was not seen if 2,4-dinitrophenol was added to the culture medium, but there was no modification of activity by cortisone acetate or by the addition to the immunized cells obtained from non-immunized ganglia<sup>50</sup>.

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## EVALUATION OF CHEMICAL DIETS AS NUTRITION FOR MAN-IN-SPACE

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INVESTIGATIONS at the U.S. National Institutes of Health have led to the development of water-soluble, chemical diets which were successfully used in quantitative nutritional investigations in which the more conventional semi-synthetic protein diets had hitherto proved neither suitable nor applicable<sup>1-6</sup>.

\* Work performed while employed at the California Medical Facility, Vacaville, California.

Such investigations were designed to exploit the high degree of flexibility possessed by completely chemical diets by virtue of their liquid nature and the fact that they are composed solely of highly purified and discrete chemical constituents, each of which may be added to, omitted from, or varied in the diet at will. Diets of this type are unique in that: (a) their essential and non-essential nitrogen is provided in the form of highly pure



Table 1. BASE DIET COMPOSITION

Amino-acids			
L-Lysine · HCl	3.58 g	Sodium L-aspartate	6.40 g
L-Leucine	3.83 g	L-Threonine	2.42 g
L-Isoleucine	2.42 g	L-Proline	10.33 g
L-Valine	2.67 g	Glycine	1.67 g
L-Phenylalanine	1.75 g	L-Serine	5.33 g
L-Arginine · HCl	2.58 g	L-Tyrosine ethyl ester · HCl	6.83 g
L-Histidine · HCl · H <sub>2</sub> O	1.58 g	L-Tryptophan	0.75 g
L-Methionine	1.75 g	L-Glutamine	9.07 g
L-Alanine	2.58 g	L-Cysteine ethyl ester · HCl	0.92 g
Water-soluble vitamins			
Thiamine · HCl	1.00 mg	α-Biotin	0.83 mg
Riboflavin	1.50 mg	Folic acid	1.67 mg
Pyridoxine · HCl	1.67 mg	Ascorbic acid	62.50 mg
Niacinamide	10.00 mg	Cyanocobalamin	1.67 µg
Inositol	0.83 mg	p-Aminobenzoic acid	416.56 mg
α-Calcium pantothenate	8.33 mg	Choline bitartrate	231.25 mg
Salts			
Potassium iodide	0.25 mg	Potassium hydroxide	3.97 g
Manganous acetate · 4H <sub>2</sub> O	18.30 mg	Magnesium oxide	0.38 g
Zinc benzoate	2.82 mg	Sodium chloride	4.77 g
Cupric acetate · H <sub>2</sub> O	2.50 mg	Ferrous gluconate	0.83 g
Cobaltous acetate · 4H <sub>2</sub> O	1.67 mg	Calcium chloride · 2H <sub>2</sub> O	2.44 g
Sodium glycerophosphate	5.23 g	Sodium benzoate	1.00 g
Ammonium molybdate · 4H <sub>2</sub> O	0.42 mg		
Carbohydrates			
Glucose	555.0 g	Glucono-δ-lactone	17.2 g
Fats and fat-soluble vitamins*			
Ethyl linoleate	2.0 g	α-Tocopherol acetate	57.29 mg
Vitamin A acetate	3.64 mg	Menadione	4.58 mg
Vitamin D	0.057 mg		

\* Provided daily in separate supplement.

L-amino-acids; (b) they are administered as single, crystal-clear solutions which are nutritionally complete in themselves.

In essence, the diets are composed of balanced (but varying) proportions of L-amino-acids, the required water-soluble and fat-soluble vitamins, the pertinent salts, glucose, and ethyl linoleate as the source of essential fat. As each of the separate components is purified prior to its incorporation into the diet, the exact chemical composition of the mixture is known. The dry ingredients are dissolved in distilled water to yield a solution of 50–75 per cent solids which is substantially sterile, has a nutritive value of 2–3 cal/ml. and possesses excellent storage stability. Use of these diets in long-term nutrition investigations, including those bearing on longevity, reproduction and lactation over several generations, and on pathological conditions of various kinds, demonstrated that they will support normal growth, life-span, and

reproduction in experimental rats. Further, clinical investigations with hospitalized patients revealed that complete chemical diets maintained body-weight, rapidly replenished protein stores lost during periods of nitrogen depletion, and produced no abnormalities of liver and kidney functions<sup>7</sup>; the absence of adverse complications suggested that suitably formulated chemical diets are practical, safe, and biologically effective.

In order to evaluate the adequacy of chemically defined diets as potential long-term sustenance for man-in-space, clinical investigation was carried out at the California Medical Facility, Vacaville, California. The experimental subjects were adult male inmates of the Facility. Selection of 24 healthy subjects, from a total of 160 volunteers, was made on the basis of past medical history and comprehensive physical, neurological, and psychiatric examinations. The subjects were 24–39 years of age, exhibited a range in weight of 135–208 lb., and represented a wide variety of body types. The experiments were made in a locked ward where the subjects were kept under constant medical surveillance. Scheduled daily physical examinations and twice-weekly psychological examinations were conducted with each subject, who was also expected to follow a daily activity schedule that included moderate physical exercise, and co-ordination and strength tests. Chemical diets in aqueous solution (Table 1) were provided at 4 intervals daily to each subject, who was permitted to ingest whatever amount of diet satisfied his subjectively determined need; ingestion of anything else, with the exception of distilled water which was provided freely, was prohibited. Dietary intakes of the individual subjects ranged from 2,100 to 3,700 calories daily. Of the original 24 subjects, 9 dropped out during the course of the investigation, all for other than medical reasons.

Results obtained over a 19-week experimental period revealed that chemical diets sufficed to maintain the subjects with no untoward physiological or psychological responses, and free of complications or effects of toxicity. Indeed, physical and psychological examinations revealed marked improvements in the physical status and psychological outlook of each of the subjects. Fasting blood, drawn prior to initiation of the investigation, weekly during the experimental period, and bi-weekly for a 3-month period after return of the subjects to institutional fare, revealed values within the normal range for all parameters tested (Table 2) and further attested to the

Table 2. ANALYTICAL DATA OF SUBJECTS ON CHEMICALLY DEFINED DIET STUDY

Test parameter (normal range)	Pre-experiment value		Chemical diet period*		Normal foodstuff period†	
	Av.‡	S.D.§	Av.‡	S.D.§	Av.‡	S.D.§
CO <sub>2</sub> -comb. power (24–32 m.equiv.)	—¶	—¶	29.77	1.17	29.35	0.96
Chlorides (95–105 m.equiv.)	101.7	2.66	103.0	1.51	105.1	2.44
Sodium (135–145 m.equiv.)	146.1	3.63	144.9	1.13	143.3	2.41
Potassium (3.5–5 m.equiv.)	4.75	0.41	4.65	0.24	4.74	0.32
Calcium (9–11 mg%)	—¶	—¶	10.34	0.55	9.96	0.25
Phosphorus (3.0–4.5 mg%)	—¶	—¶	3.57	0.26	3.67	0.45
Protein (6–8 %)	7.56	0.35	7.36	0.26	7.33	0.30
Albumin (55–63% of total)	—¶	—¶	62.03	2.98	64.29	2.84
α <sub>1</sub> -Globulin (2–4% of total)	—¶	—¶	3.83	0.31	3.23	0.40
α <sub>2</sub> -Globulin (8–12% of total)	—¶	—¶	8.03	0.67	9.97	0.97
β-Globulin (9–13% of total)	—¶	—¶	11.38	1.28	11.20	1.21
γ-Globulin (14–20% of total)	—¶	—¶	13.91	2.43	14.42	2.74
Creatinine (0.8–1.5 mg%)	—¶	—¶	1.17	0.12	1.26	0.12
Uric acid (2.5–6.0 mg%)	4.79	0.63	4.47	0.67	5.01	0.63
Fasting blood sugar (60–100%)	93.0	10.70	76.8	2.11	90.2	7.62
Blood urea nitrogen (10–20 mg%)	11.25	1.93	11.38	1.19	10.66	2.51
Cholesterol (150–260 mg%)	233.5	51.32	176.2	40.68	229.2	42.83
Cholesterol esters (90–200 mg%)	—¶	—¶	122.2	30.24	168.5	27.75
Phospholipids (150–350 mg%)	—¶	—¶	204.8	27.48	227.5	31.89
Fatty acid esters (250–500 mg%)	—¶	—¶	301.7	31.28	318.0	28.85
Total nitrogen (9.6–11.2 g%)	—¶	—¶	10.35	0.32	10.56	0.36
Red blood count (4.80–6.20 MM/mm <sup>3</sup> )	4.58	0.66	4.72	0.25	4.69	0.35
White blood count (5–10 MM/mm <sup>3</sup> )	7.23	1.65	8.29	1.18	8.30	2.43
Platelets (204–395 M/mm <sup>3</sup> )	270.0	34.05	258.7	10.19	275.1	24.77
Haemoglobin (12–17 g)	13.91	1.86	14.30	1.10	14.56	1.08
Haematocrit (40–54%)	43.7	5.31	45.3	2.37	46.0	2.69
Sedimentation rate	15.8	4.53	9.2	6.88	12.9	8.57
Corrected sed. rate (0–10 mm/h)	11.5	9.84	5.7	5.70	9.9	6.46

\* Values obtained weekly over 19-week period.

† Values obtained weekly over 12-week period.

‡ Values given are averages of the weekly mean average values for the 15 subjects who participated for the complete programme.

§ Standard deviation of averages.

¶ No pre-experiment values taken.

physical well-being of the subjects. It is of especial interest to note that faecal elimination in all individuals was strikingly reduced, as contrasted with that induced by natural diets; several of the subjects experienced smaller than normal bowel movements at regular intervals of 5-6 days. Another observation of interest was the progressive decrease of the total serum cholesterol-levels of all subjects from an average baseline value of 226 mg per cent to an average value of 151 mg per cent after 19 weeks on the chemical diet. This progressive decrease was interrupted only during a 3-week period (fifth-seventh week) when replacement of 25 weight per cent of the glucose component of the diet with an equal weight of sucrose was accompanied by a sharp rise in the serum cholesterol-levels of all the subjects; this latter observation is described elsewhere<sup>8</sup>.

A number of characteristics of chemical diets can serve to overcome some of the limitations inherent in alternative space-food sources now being evaluated. Among these characteristics are: (a) high nutritive efficacy in ultra-compact form—1 ft.<sup>3</sup> of the diet, as a 75 per cent solution in water, will provide a 154-lb. astronaut with all his required essential and non-essential nitrogen, salts, vitamins, and fats, in addition to his estimated requirement<sup>9</sup> of 2,830 calories per day, for a period of a month; (b) complete water solubility—provides advantages in the administration of the diets in liquid form under conditions which will not permit the use of solids; (c) low bulk—induces low faecal residues and mitigates the critical problem of disposal of solid wastes; (d) complete nutritive flexibility—allows alteration at will of the amino-acid ratios, carbohydrate content, and levels of all other components, thereby making it possible to tailor formulations to the specific dietary needs of the individual

astronaut; (e) complete digestibility—provides dietary components in most elemental form in the event of disturbances of the digestive system; (f) good storage stability either in the solid state or as aqueous solution.

The promising clinical results already obtained, together with the high degree of flexibility and nutritional adequacy which complete chemical diets afford, indicate that consideration should be given to them as potential man-in-space diets.

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## VOLCANIC ERUPTION ON RAOUL ISLAND, NOVEMBER 1964

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**R**AOUL, or Sunday, Island lies about 700 miles north-east of Auckland, New Zealand. It is about 5 miles across and is the largest island of the Kermadec group (Fig. 1). The only settlement now on the Island is a meteorological station serviced by the New Zealand Department of Civil Aviation.

Since the Island was discovered by d'Entrecasteaux in 1793, several volcanic eruptions are known to have occurred. The first recorded eruption<sup>1</sup> took place on March 9, 1814, when Captain Barnes, of the ship *Stella*, reported the presence of dark clouds, flame and suffocating vapour issuing from the Island. Two months later he returned and found in Denham Bay an island of three miles circumference covered with coarse grit. This was in a place where two months previously he had made soundings of 40-45 fathoms. The water in the bay was hot near the island.

In 1847 and 1853 settlers in Denham Bay were temporarily frightened away from the Island by earthquakes, and in 1872 an eruption occurred<sup>2</sup> from Green Lake within the main crater. The lake boiled vigorously and sent up a column of 'fire', killing trees immediately around the crater. At the same time, an island was again thrown up in Denham Bay, the earlier one having in the meantime disappeared, either by subsidence or erosion. By 1877 all that remained of this island was a shoal, and in 1887 Wolverine Rock and a shoal were charted. Another

eruption appears to have taken place between 1872 and 1876, when Dr. Stockwell<sup>3</sup> reported bare mud and rocks covering a belt extending from Green Lake to near the site of the present Meteorological Station on the north coast of the Island. Debris extended eastwards in the crater to Blue Lake, killing vegetation.

Since 1957 the New Zealand Seismological Observatory has operated a Willmore seismograph near the Meteorological Station, and several swarms of earthquakes originating in the Kermadec area have been recorded. On November 10, 1964, a series of extremely local earthquakes with *S-P* intervals of 1-2 sec began about 4 p.m. (local time, 12 h ahead of U.T.). Several of these shocks were felt, and by 8 p.m. the frequency of recorded shocks had risen to more than 80 an hour. The level of background tremor increased until by November 12 individual shocks were hard to distinguish. Thereafter, both the level of background tremor and the number of recorded shocks decreased. The largest earthquake (magnitude 5.7) occurred at 9.58 a.m. on November 14. The frequency of recorded earthquakes during the disturbance is shown in Fig. 2.

The water in Denham Bay was discoloured for a mile from the shore on November 12. One of us (E. F. L.) reached Raoul on November 19 in H.M.N.Z.S. *Lachlan* and found discoloured water, bubbles of gas and fragments of pumice reaching the surface in Denham



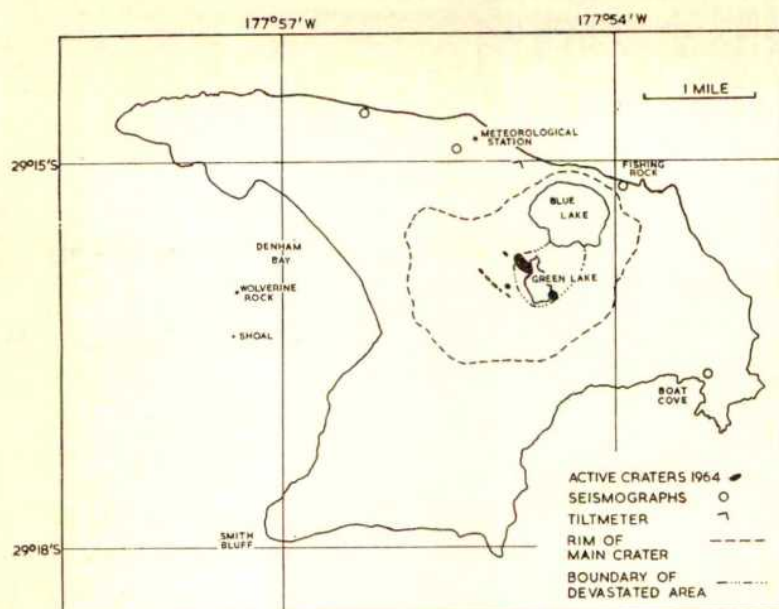


Fig. 1. Map of Raoul Island

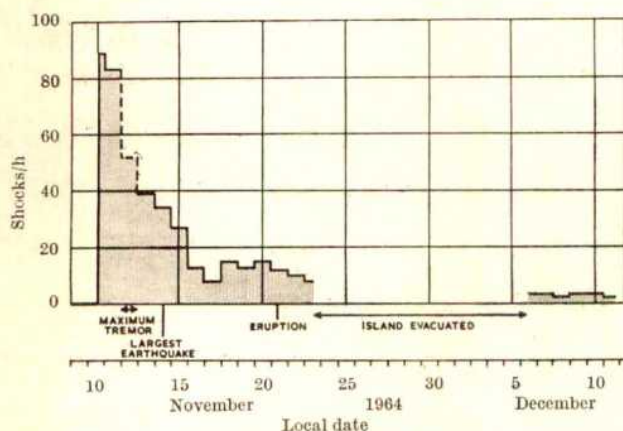


Fig. 2. Frequency of local earthquakes recorded at main seismograph station

Bay. In the main crater, Green Lake was found to be discoloured, and whereas it had formerly been cold the temperature had reached an average of 30° C with local temperatures of 52° C. It was estimated that the lake had risen 20 ft. and was still rising half an inch per hour. A number of new hot springs had formed at the south-west end of the lake and the ground was observed to be heating up at a few places. *Lachlan* left on November 20.

At 5.56 a.m. on November 21 an eruption occurred in the main crater. Steam, mud and rocks were thrown to a height of 2,500 ft. for 3-4 min, then to lesser heights for a further half hour (Fig. 3). During the eruption the seismograph recorded a continual vibration 30-40 times the amplitude of the normal background level. On hearing of the eruption, *Lachlan* returned to the Island, arriving the same day, and a further inspection of the crater was made. An aerial inspection of the Island was made late on the afternoon of November 21 from an R.N.Z.A.F. aircraft and revealed that an actively steaming crater has been blasted out at the north-western end of Green Lake (Figs. 4 and 5). Steam was also rising from points at the south-eastern and south-western ends of the Lake. Low cloud obscured most of the main crater. From a vantage point on the Island the same afternoon it was noted that Green Lake had risen approximately 50 ft. and a stream of hot water was flowing north to Blue Lake. The vegetation along the northern and

western side of Green Lake had been destroyed, and a broad area of vegetation extending eastwards to Blue Lake was completely flattened and covered with grey mud and stones. Sporadic eruptions from these craters and other smaller ones to the west followed during the next two days. Blue Lake was found to be rising and becoming slightly warmer, and steam was rising from a number of points along the eastern shore.

On November 23 the Island was evacuated as a precaution.

A further aerial reconnaissance on November 30 (Fig. 5) showed that the volcanic activity had declined. It was also noticed that a thin covering of grey dust extended to Smith Bluff at the southern tip of the Island. A patch of discoloured water was again seen in Denham Bay. On December 6 *Lachlan* returned to Raoul carrying the meteorological party and a party of ten from the Department of Scientific and Industrial Research, including ourselves. Other members of the party were R. R. Dibble, G. E. K. Thompson and R. H. Orr (Geophysics Division), Dr. B. G. Weissberg and J. V.

Sarbutt (Chemistry Division) and E. J. Thornley (Information Service).

A more detailed examination showed small explosion craters aligned north-west-south-east along the western side of the main crater. Craters at the south-eastern and north-western ends of Green Lake and another small fissure crater farther to the north-west were similarly aligned. Between these lines is another small crater. The discharged material consisted entirely of country rock, and the explosive eruption is considered to have been phreatic in type. A number of samples were collected so that some of the previous eruptions on Raoul Island could be dated by the carbon-14 method.

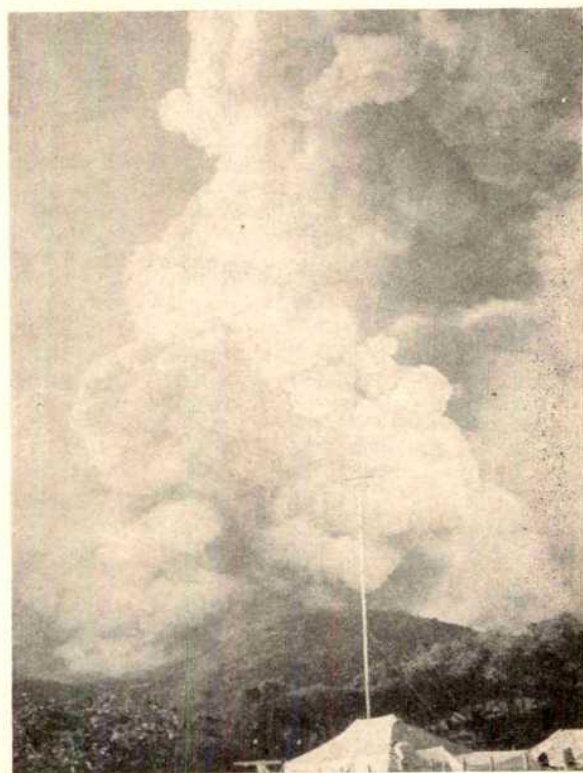


Fig. 3. View of main eruption from Meteorological Station. (E. J. Thornley photo)





Fig. 4. Outflow from new crater on edge of Green Lake. (E. J. Thornley photo)



Fig. 5. Air view of Raoul Island crater looking south-west on November 30, 1964. Blue Lake at bottom right, Green Lake at centre with new crater pit on far shore, and other new crater pits at top left. Part of the devastated area is shown in foreground. (E. J. Thornley photo)

shocks were in the vicinity of Denham Bay probably at a depth of less than 6 km. The number of recorded shocks had decreased to between two and four per hour and remained at this level while the party was on the Island.

A two-component tiltmeter was installed on a level area near the north coast, at a point one mile north of the main eruption centre at the north-western end of Green Lake (Fig. 1). Recording commenced at about 4 p.m. on December 9, and will be continued by the station staff for a period of 2-3 months. The record obtained up to December 12 showed nearly continual irregular tilt movements with peak-to-peak amplitudes of about 1 sec of arc, and periods of 10-20 min. Movements were often of similar amplitude on both components, indicating tilting about a north-west-south-east axis, but one component (azimuth  $258\frac{1}{2}^\circ$ ) showed noticeably greater irregularity of movement over the whole period. A few sharp transient disturbances, amounting to several seconds of arc, and corresponding to rises in the direction of Green Lake, were recorded. Longer-term tilt movements were also observed amounting to several seconds of arc, but these appeared to be due to diurnal (possibly tidal) effects. No evidence was found of any progressive tilt movement in the period covered.

Further soundings were made in Denham Bay on December 12 from the motor launch *Acheron* (Lieutenant-Commander J. L. Harrison, R.N.Z.N.R.), and conditions were found to be rather similar to those observed in 1887 by Captain Fairchild. Bubbles and a few small pieces of pumice were seen rising to the surface over an area near the middle of the bay. Wolverine Rock could not be located at the position marked on the chart, but rock was found within four fathoms of the surface near where the previous shoal had been charted, and two holes of 30 and 17 fathoms were found where the depth was generally 13-14 fathoms.

On December 12, the scientific party left the Island in H.M.N.Z.S. *Lachlan*. The meteorological party remained and will continue the geological and geophysical observations.

The hot waters involved in the activity were strongly saline. The chloride concentration in the water issuing from the orifice of the main crater (flow rate 1-4 ft.<sup>3</sup>/sec at 95.5° C) was 8,280 p.p.m. on December 7, and water samples obtained from the large geyser hole on December 8 (temperature 87° C) contained 6,840 p.p.m. chloride.

Three additional seismographs were set up on the Island and ran for several days while the party was ashore. These showed that the centres of the larger

We wish to acknowledge the generous assistance of Commander W. J. Doole, R.N.Z.N., and the ship's company of H.M.N.Z.S. *Lachlan*; of the Department of Civil Aviation; and of Mr. C. Phillips and the members of his meteorological party, without whom the visit to the Island and the stay there would not have been possible.

<sup>1</sup> Smith, S. P., *Trans. N.Z. Inst.*, **28**, 47 (1895).

<sup>2</sup> Smith, S. P., *Trans. N.Z. Inst.*, **20**, 333 (1887).

## DEPARTMENT OF ZOOLOGY, UNIVERSITY OF GLASGOW

### INCREASED FACILITIES

MAJOR alterations within the Department of Zoology of the University of Glasgow together with the erection of buildings associated with it, namely, the new University Field Station on Loch Lomond and the Wellcome Laboratory for Experimental Parasitology, represent the extension and great improvement of its facilities for teaching and research. Alterations include provision of

a unit for electron microscopy completed last session together with the recent completion of new accommodation for investigations on animal behaviour, of an electrophysiological unit and of an experimental aquarium together with an insect hot-room for use in Agricultural Zoology.

The electron microscope, an E.M.6 instrument made by Associated Electrical Industries, was purchased out





Fig. 1. University Field Station, Loch Lomond. View from the south-west, showing main laboratory on the left, and dormitory block on upper storey on the right, with research laboratories below

of a grant of £14,608 from the Department of Scientific and Industrial Research and is housed in the basement in rooms constructed out of a former store-room. A preparation room (11 ft.  $\times$  22 ft.), which communicates with the corridor, contains a fume cupboard, coating unit, facilities for embedding procedures, and a small section set aside for the filing of negatives and prints. Provision is also made for writing and studying. An L.K.B. 'Knife-maker' is available for preparing glass knives. A microtome room (11 ft.  $\times$  9 ft.) opens off the preparation room and contains L.K.B., Porter-Blum and Huxley microtomes; there are also facilities for phase-contrast microscopy. An interesting feature of this room is the low bench height of 27 in. Normal sitting bench height is 29 in.-30 in., but, when sectioning, the worker is generally operating controls some 3 in.-4 in. above the bench top.

The entrance to the microscope room (11 ft.  $\times$  12 ft.) is guarded by a light-trap which, in order to save space, is constructed from a concertina-type partition. Apart from the microscope, the room contains only a steel storage cupboard and a small working bench.

The photographic rooms occupy the remainder of the space. The printing room (11 ft.  $\times$  14 ft.) has its own entrance to the corridor and is equipped with a 5 in.  $\times$  4 in. 'Omega' enlarger, a rotary print drier and a large stainless-steel sink unit. The small dark-room (11 ft.  $\times$  8 ft.), used for loading and developing plates, opens both into the printing room and microscope room. It is here that the power unit of the microscope is housed.

The entire unit, originally planned by Prof. G. Owen and Dr. H. F. Steedman, and for which Mr. J. B. Cowey is now responsible, is extremely compact and an attempt has been made to utilize efficiently all available space. Wherever possible, storage is provided by underbench units and glass-fronted wall cupboards. Doors are of the sliding type and, at the entrance to the printing room, the thickness of the original structural wall (in this case 2 ft.) has been used to provide a light-trap, a sliding door being placed on each side of the wall.

Also within former storage space in the basement a small laboratory has been constructed and equipped for research on mammalian behaviour by S. A. Barnett and his colleagues. A new experimental situation has been designed for investigation of interacting components of behaviour, such as eating and exploration. The connexion between choice of competing activities and learning ability is to be investigated. For this purpose, contrasting species, and also different genotypes within species, are to

be compared; and the effects of heterozygosis and of a 'stressful' (cold) environment are to be examined. New equipment, which allows detailed recording of an animal's movements over long periods, is to be used.

Establishment of the new Wellcome Laboratory for Experimental Parasitology having freed the one-storey animal house at the back of the Department, this has been completely reconstructed internally to provide two laboratories, two research rooms, a preparation room, insect hot-room and other facilities. These now constitute a Unit for research and teaching in comparative aspects of electrophysiology. When the Unit is fully operative (summer 1965), a co-ordinated research programme, involving investigations of synaptic transmission in insects, will be undertaken. The full complement of research staff will consist of up to five members, a lecturer, Dr. P. N. R. Usherwood, a research assistant, two graduate students and a technician.

The budget should be sufficient to provide each research worker with a complete set of apparatus for stimulation and for recording of bio-electric phenomena. The research assistant will devote some time to histological and electron-microscopical examinations of excitable tissue. The full complement of research apparatus for each research worker will consist of the following: (1) Oscilloscope, with plug-in units for recording versatility. (2) A pre-amplifier with neutralizing capacitance control for use with high-impedance microelectrodes. (3) An oscillograph camera, with or without pulsed frame charge. (4) Stimulator giving a programmed pulse output. (5) Stimulus isolation unit. (6) Micromanipulators for recording and stimulating electrode assemblies. (7) Palmer rackwork and stands. (8) 'Zoom' stereomicroscope for microdissection. (9) Facilities for simultaneous visual and photographic recordings of oscillograph traces, either by use of slave oscilloscope assembly or by use of a bio-electric reflexor unit. One of the Tektronix oscilloscopes will be modified by Dion Electronics Co., U.S.A., for use with a slave oscilloscope. This company will also be responsible for modifying some of the plug-in units of the 561 A oscilloscope to give 90° rotation of display. They will also be responsible for converting four Tektronix 'Type 161' pulse generators to give input selection for positive and negative pulse, positive and negative saw-tooth output, extended pulse duration output and d.c. output.

Teaching in the Unit will almost exclusively concern the fourth-year honours class, although a short elementary course designed as an introduction to electrophysiological



techniques will be given to the second-year class. It will be possible to provide facilities for as many as ten honours students. One main object of the teaching course will be to familiarize the students with basic research techniques in electrophysiology and also to give them opportunities for advanced studies on some of the excitable tissues or preparations which they will have previously studied.

Meanwhile a new University Field Station has been erected within the Queen Elizabeth Forest Park three miles south of Rowardennan on the east shores of Loch Lomond (Fig. 1). Constructed with the help of a substantial interest-free loan from the Nuffield Foundation, this replaces the two large wooden huts which formerly served this purpose on the opposite side of the loch. Planned by Messrs. Thomson, McRae and Sanders and built by the Weir Housing Corporation, the Station consists of three units: a main building, a caretaker's house and a boat-house. These are handsome buildings of unpainted red cedar vertical timbering on foundations of sanded brick and roofed with grey-green tiles, the whole blending well with the surrounding woodland. Laboratories are on a lower level and living quarters on an upper, each with its own entrance but with an intercommunicating stairway. Since it is highly desirable that the amenities of the area shall not be disturbed, the woodland has only been cleared to a distance of 40 ft. from the buildings and, especially when the trees are in leaf, they remain screened from view on all sides.

The Station is designed to house classes of up to eighteen students for periods of one or two weeks, but for most of the time it will be occupied by a limited number of research workers. On the upper floor the three dormitories descend in size to allow economical distribution of varying numbers of students of both sexes. The two smaller ones are fitted with benches so that either or both may have alternative use as laboratories. On this floor also are three single bedrooms, lavatories and bathrooms, drying rooms for field clothing, store-rooms, a well-appointed kitchen, and a common room which also serves as library and dining room.

On the lower floor there are two research laboratories and a large laboratory for both teaching and research. Again with flexibility in mind, fixed benches run only around the walls, leaving a clear floor space where chairs may be placed for lectures or folding tables set up for demonstrations and practical work.

The technician-caretaker's house stands to the west of the main building and the boat-house on the shore below it. The latter is of sufficient size to contain several rowing boats, all the ancillary equipment for the launch moored out in the bay before it, as well as seine nets and other collecting gear.

The Station depends on electricity for lighting, heating and power. Water is drawn from Loch Lomond and drainage discharged into the adjacent Dhu Lochan after treatment in a large septic tank followed by a percolating filter to safeguard this relatively small loch from pollution.

The work of the Field Station covers both fresh water and the land, animals and plants being investigated in their natural environment. Dr. H. D. Slack, who continues in general charge, directs aquatic research, which at present is particularly concerned with the distribution of animals on the profundal bed and with plankton populations. Terrestrial ecology is under the supervision of Dr. A. F. G. Dixon, and the present research involves three post-graduate students particularly concerned with population dynamics of sycamore aphids as a preliminary to an analysis of energy flow in woodland communities.

The situation of the Station, on the largest lake in Great Britain and one as yet little affected by human activities, gives scope for work which may have economic value. Its services have been offered for participation in the fresh water section of the International Biological Programme initiated by the International Council of Scientific Unions in 1961 with the view of exploring man's rapidly increasing demands on his natural environment.

The Wellcome Laboratories for Experimental Parasitology, erected following a gift of money by the Wellcome Trustees in 1963, are situated in the grounds of the Veterinary School at Garscube. They form a two-storey building containing some 9,000 sq. ft. of floor space, the ground floor being occupied by the Unit of Experimental Parasitology formerly housed in the Department of Zoology and of which it still forms an integral part. The new accommodation comprises three research rooms equipped respectively for isotopic, analytical and microscopic work; a sterile unit consisting of two transfer rooms, two incubation rooms, medium preparation laboratory and sterilizing room; an animal unit and aquarium; and five staff rooms. Under the direction of Dr. C. A. Hopkins, reader in experimental parasitology, there is an academic and research staff of six which it is hoped will increase to ten over the coming two years.

The Unit's main work is concerned with the development of pseudophyllidean tapeworms such as *Diphylobothrium* spp. and *Schistocephalus* spp. A primary objective is the development of techniques for the maintenance of these tapeworms throughout their life-cycle in the laboratory, a problem which involves *inter alia* the maintenance of copepod, piscine and avian hosts. Meanwhile, investigations using material obtained in the field are proceeding. These include the cultivation of larval *Schistocephalus solidus* in an attempt to reproduce *in vitro* under known conditions development that normally occurs in the perivisceral cavity of sticklebacks; an investigation of the changes that occur when a larval worm passes from the aerobic habitat of the fish coelom to the anaerobic habitat of the caecum of a duck; the cause of the close host specificity shown by the larva which is confined to a few, possibly a single, species of fish; an electron microscopic examination of the cestode cuticle with particular reference to site and method of food uptake; and an investigation of the factors which influence the longevity and death of the adult worm.

## OBITUARIES

### Prof. Josef Tomcsik

JOSEF TOMCSIK, who died in Basle on December 30, 1964, at the age of sixty-seven, was born of a Protestant family in Transylvania, when that country was part of the Austro-Hungarian Empire. Although he had adopted Swiss nationality for many years, he always regarded himself as Hungarian and suffered greatly at the distress of his country. Shortly before he died he visited Hungary, at the invitation of the present Government, for the first time since the War. This was unquestionably a great physical and emotional strain, coming, as it did, not long

after the death of his wife, Olga, who had acted always as his companion and confidential secretary.

As a young man, Prof. Tomcsik served in the First World War, in the Imperial Army, and saw action as a sniper. After the return of comparative peace to Hungary he took a degree in medicine and became an active research worker in bacteriology and immunology. His interests were wide, but he is especially well known for his work on the antigenic structure of the anthrax bacillus. He travelled extensively in Europe, America and the Far East, but continued to do his main work in Budapest, where he was elected professor when still in his twenties.



He was an exceedingly likable personality and made friends everywhere, but he especially enjoyed his stay of several years in Peking, where he acquired a taste for Chinese food and Chinese furnishings that was reflected in his town house in Basle in later years. He frequently spoke of his travels, and told with relish of his discomfort when unable to match the skill of his Chinese assistants in picking up glass beads with sterile glass rods, chopstick fashion. He was a remarkable linguist, speaking German, English and French almost perfectly. When reading a paper, in English, at the Institut Pasteur, he was asked a question in French, to which he replied in French. He then continued to deliver the paper in French until, after several minutes, he recalled himself and continued again in English. The notes from which he was speaking were probably in German, with annotations in Hungarian.

During the last War, Prof. Tomesik was invited to become director of the Institute of Hygiene at Basle. He foresaw the coming tragedy, and with much emotional conflict he accepted. Thereafter, for many years, return to his native country was barred to him.

The Institute is a fine, half-timbered house in the Petersplatz, and it was promptly christened Uncle Tom's Cabin by the medical students of the University of Basle. Here, for the second half of his active career, Prof. Tomesik settled down to teaching and research. He adopted Swiss nationality and built a country cottage of great charm, at Weggis on Lake Lucerne, backed by a steep chestnut forest of which he gradually acquired a considerable area, and of which he was exceedingly proud. Tragically, his imminent intention of retiring there was never fulfilled.

Returning to his study of the polysaccharides of *Bacillus anthracis*, Prof. Tomesik determined to employ the new technique of phase-contrast microscopy to the elucidation of antigen-antibody reactions, and thus half-accidentally discovered his method of visualization of antigenic components in the bacterial capsule, which renewed his fame at an age when many scientists find themselves slipping away from the bench to the desk. This was one of the big advances in cytological method that have been made in this century. The observation that an apparently formless capsule conceals an elaborate lamination of polysaccharide and polypeptide layers is in itself an important and remarkable one. The full potentialities of the technique, applicable as it is to any biological material, has

not so far been realized, for the lack, perhaps, of others with his skill and patience to prepare antisera from antigenic fractions and apply them to the microscopic field. Perhaps, in the memory of a good and kind man, and a fine scientist, one of the institutions of the world will establish a grant to enable his work to be employed and extended as it deserves to be. He lived for his science and died in harness; he would ask no better memorial.

K. A. BISSET

### Prof. H. den Hartog

HENDRIK DEN HARTOG, professor of biophysics in the University of Amsterdam, died on July 15, 1964, at the age of forty-eight.

Den Hartog, whose early scientific research was concerned with cosmic ray physics, developed a lively interest in the ultimate performance of Geiger-Müller counters, which grew to encompass the fundamental limitations of measuring instruments in general. One of his many contributions to this field, in collaboration with F. A. Müller, is the now classic treatment of "Optimum Instrument Response for Discrimination Against Spontaneous Fluctuations", published in 1947. His originality and skill in the application of the principles of physical measurements are seen in a wide spectrum of instruments for use in fields as diverse as biochemistry and non-destructive materials testing.

In later years den Hartog directed his efforts toward problems of biology, especially sensory phenomena. Although his accomplishments in this field were many, he was not to witness the fruition of his more recent ideas on the investigation of biophysical phenomena. Appointed to the newly created chair in biophysics in the University of Amsterdam in the autumn of 1962, he became ill just as the toils of organizing a department and moving into a new laboratory were ending.

Den Hartog gave generously of his time to help others. His advice was as well considered as his own actions, and often unmasked hidden facets of brilliance. In this age of specialization when most persons are impatient for success, and change is often mistaken for progress, den Hartog was a rare man. He pursued a broad range of intellectual activities and considered no endeavour to be complete until the product was as close to perfection as human skills allow.

A. S. BRILL  
B. CHANCE

## NEWS and VIEWS

### Chairman of Natural Environment Research Council

SIR GRAHAM SUTTON has been appointed chairman of the new Natural Environment Research Council which will be set up by Royal Charter, in accordance with the Science and Technology Bill now before Parliament. The major part of Sir Graham Sutton's time will continue to be devoted to his responsibilities as director general of the Meteorological Office. The Natural Environment Research Council's functions, defined in broad terms in the Science and Technology Bill, are to support and carry out research in the Earth sciences and ecology (in particular, geology, meteorology, seismology, geomagnetism, hydrology, oceanography, forestry, nature conservation, fisheries, and marine and freshwater biology); to disseminate knowledge in these subjects; and to establish, maintain and manage nature reserves. The Research Council will take over the Nature Conservancy, the Geological Survey and Museum, the Hydrology Research Unit, the National Institute of Oceanography and the responsibilities hitherto undertaken by the Development Commission in relation to marine and freshwater biology and fishery research.

### Sir Graham Sutton, C.B.E., F.R.S.

SIR GRAHAM SUTTON has been director-general of the Meteorological Office since 1953, and a Fellow of the Royal Society since 1949. Born in 1903, Sir Graham Sutton was educated at Pontywarn Grammar School, and as a Scholar, at the University College of Wales in Aberystwyth and at Jesus College, Oxford. He was a lecturer at the former College from 1926 until 1928, and was made an Honorary Fellow there in 1958. From 1928 until 1941 he served in the Meteorological Office. During the War, Sir Graham Sutton acted as superintendent of research in the Chemical Defence Experimental Establishment at Porton, and as superintendent of Tank Armament Research. From 1945 until 1947 he was chief superintendent at the Royal Radar Research and Development Establishment at Malvern. In 1947 he was appointed Bashforth professor of mathematical physics at the Royal Military College of Science, and later dean at the same College. From 1950 until 1955 he was chairman of the Atmospheric Pollution Research Committee, and in 1951 he became scientific adviser to the Army Council. In the past he has served as president of the Royal Meteor-

ological Society and the Institution of Professional Civil Servants. Sir Graham Sutton was made a Commander of the Order of the British Empire in 1950 and a Knight Bachelor in 1955. Since then, Sir Graham has been a member of the Nature Conservancy, of the Executive Committee of the World Meteorological Organization and of the Council of the University College of Wales, Aberystwyth.

**Deputy Director of the General Post Office Research Station:** Dr. J. R. Tillman

DR. J. R. TILLMAN has been appointed deputy director of research of the Post Office Research Station, Dollis Hill, London, N.W.2. Born at Gillingham, Kent, in 1912, Dr. Tillman was educated at Gillingham Grammar School. While at the Imperial College of Science and Technology, he obtained an honours B.Sc. degree and a Ph.D. for research in electron diffraction. He was a Beit Research Fellow during 1934-36, and with P. B. Moon discovered the thermal neutron and the selective absorption of slow neutrons. His other academic qualifications include D.Sc. He is also a Fellow of the Physical Society, an Associate of the Royal College of Science, and an Associate Member of the Institution of Electrical Engineers. He joined the Post Office Research Station in 1936, becoming a principal scientific officer ten years later and a senior principal scientific officer in 1952. His work at the Station includes the design of receivers for the first international dialling installations and for the national system, and he carried out a detailed study of high-speed time division multiplex switches for electronic exchanges. During the 1950's he became an acknowledged expert on transistors, and his Division at the Station specialized in their long-term reliability for telecommunications applications, particularly submerged repeaters for under-sea telephone cables. He is joint author of a book *Theory and Practice of the Transistor*. Another of his activities is concerned with organizing the recruitment of graduates for work at the Station.

**Honorary Fellowship of the International Institute for Conservation of Historic and Artistic Works:**

F. I. G. Rawlins, C.B.E.

At a luncheon party in the Athenaeum Club on February 9, 1965, Mr. F. I. G. Rawlins was presented with a special issue of *Studies in Conservation*, marking his recent election to an Honorary Fellowship of the International Institute for Conservation of Historic and Artistic Works (IIC). IIC, which now includes in its membership a majority of the professional conservators throughout the world, owes a great deal to Mr. Rawlins. His contribution has two distinct aspects, represented, respectively, by his work as first secretary-general of the Institute (1950-58) and by his honorary editorship of the Institute's journal, *Studies in Conservation*, from 1952 until 1958. During this period, Mr. Rawlins was scientific adviser to the Trustees of the National Gallery, London. As secretary-general he gained and held the interest and support of those most prominent in the profession in Europe and America as well as in countries as far away as India and Japan, and provided a firm foundation for the later development of the Institute. As editor of *Studies in Conservation* he set an exacting standard of scholarship and scientific precision. Among those contributing to the special issue of the journal are the following present and past officials of the Institute: Dr. Arthur van Schendel, director of the Rijksmuseum, Amsterdam; Dr. Harold Plenderleith, director of the International Centre for the Study of the Preservation and the Restoration of Cultural Property, Rome; Mr. George Stout, who represents pioneering efforts in the United States; Dr. A. E. Werner, keeper of the British Museum Research Laboratory; Mr. N. S. Brommelle, who succeeded Mr. Rawlins as secretary-general and is

keeper of the Conservation Department at the Victoria and Albert Museum; and Mr. Garry Thomson, the present editor of *Studies in Conservation* and scientific adviser to the Trustees of the National Gallery, London.

**Director of An Foras Taluntais**

THE Council of An Foras Taluntais has, in accordance with the terms of the Agriculture (An Foras Taluntais) Act, 1958, appointed Dr. T. Walsh to the post of director of An Foras Taluntais (The Agricultural Institute) on a permanent basis, as from August 1, 1965. On the establishment of An Foras Taluntais in 1958 the Government appointed Dr. Walsh as the first director for a period of seven years as from August of that year (see *Nature*, 184, 28; 1959). This term of office expires on July 31 next.

**The U.S. National Science Foundation**

DR. L. J. HAWORTH, director of the National Science Foundation, has announced a major reorganization of the activities of the Foundation in support of science education. Three divisions have been established under the associate director for education, Dr. H. W. Riesen. These divisions, which correspond to educational levels, are: the Division of Pre-College Education in Science, headed by Dr. K. Kelson, formerly deputy director of the Division of Scientific Personnel and Education, which has now been abolished; the Division of Undergraduate Education in Science, headed by Dr. L. Shanor, formerly head of the undergraduate education in the Sciences Section; and the Division of Graduate Education in Science, headed by Dr. R. D. Fontaine, formerly in charge of the Fellowships Section.

**The Royal Society of London**

THE annual report of the Council of the Royal Society for the year ended September 30, 1964, confirming the earlier announcement that Nos. 6-9 Carlton House Terrace are to be converted to provide adequate and dignified accommodation for the Society under a 99-year lease now being negotiated with the Crown Estate Commissioners, expresses the hope that reconstruction will commence early in 1965 (Pp. 74. London: The Royal Society, 1964). The second expedition to North Borneo spent four months in the Pinosuk Plateau region of Mount Kinabalu, Sabah, Malaysia, in January-May 1964. Planning continues on the expedition which is to be sent to the Solomon Isles in 1965. A small expedition investigated the volcanic areas near the coast of the Aden Protectorate, and another small expedition carried out volcanological and petrological investigation on Ascension Island in June 1964. A committee appointed in October 1963 has become a joint committee with increased representation from the Department of Scientific and Industrial Research and authority to deal with both technical and financial implications of the proposal for a large telescope in the southern hemisphere. At the conference of scientific editors held in April 1964, a further conference was arranged for November, and the Council has also arranged a further meeting of officers of British scientific societies to discuss such matters as office and meeting accommodation and the need for an international conference centre in London. A Developing Countries Sub-committee of the International Relations Committee was set up in June 1964 to advise the latter Committee on action which the Society might take to assist scientific and technological research in developing countries, and on matters concerning the developing countries in the activities of the International Council of Scientific Unions. One hundred research projects are now being administered by the Scientific Research in Schools Committee, the report (pp. 16) to the Council of which is included with the Council's annual report. There is some reference to United Kingdom co-operation in international space research. Parliamentary grants administered by the

Royal Society for the fiscal year 1964-65 totalled £251,000 compared with £220,000 in 1963-64, of which £100,000 were for scientific investigations, £45,600 for research professorships, and £95,400 for international research and scientific congresses. Brief report on researches are appended, including lists of publications, from the research professors and Research Fellows.

### European Launcher Development Organization

IN a written answer in the House of Commons on February 3, the Minister of Aviation, Mr. R. Jenkins, stated that a conference, attended by all the Member States of the European Launcher Development Organization, met in Paris during January 19-21, to review the activities of the organization and to examine proposals for its future work. The cost of completing the first programmed launcher, based on conventional techniques and with a limited operating capacity, would be higher than the original estimate, and a working group had been set up to consider the feasibility of using the technical results already achieved and the experience acquired by the Organization to develop an advanced launching system making use of the most modern techniques. The system would continue to use *Blue Streak* as the first stage, and the Conference would be convened again later this year to review progress. Britain's part in the European Space Research Organization was the responsibility of the Secretary of State for Education and Science.

### Science Research Council

IN a written reply to a question in the House of Commons on February 4, Mr. A. Crosland, the Secretary of State for Education and Science, announced that a Science Research Council would be constituted after the enactment of the Science and Technology Bill which is now before Parliament. The Council, under Sir Harry Melville's chairmanship, would consist of: Lord Halsbury; Dr. A. Caress, director of research, Imperial Chemical Industries, Ltd.; Dr. D. G. Christopherson, vice-chancellor and warden of the University of Durham; Dr. S. C. Curran, vice-chancellor of the University of Strathclyde, Glasgow; Prof. G. C. Drew, professor of psychology in University College, London; Prof. M. R. Gavin, professor of electronic engineering in University College of North Wales, Bangor; Sir Ewart Jones, Waynflete professor of chemistry in the University of Oxford; Sir Bernard Lovell, professor of radio astronomy in the University of Manchester and director of Jodrell Bank Experimental Station; Prof. K. Mather, professor of genetics in the University of Birmingham and vice-chancellor-elect of the University of Southampton; Prof. C. F. Powell, Henry Overton Wills professor of physics in the University of Bristol; Prof. J. E. Smith, professor of zoology in Queen Mary College, University of London, and director-elect of Marine Laboratory, Plymouth.

### Committee on Manpower Resources for Science and Technology

IN reply to a question by Mr. J. Tinn in the House of Commons on February 4 concerning the arrangements for the continuation of the work formerly performed by the Committee on Scientific Manpower, Mr. A. Crosland stated that the Committee would be known as the Committee on Manpower Resources for Science and Technology. The Committee had now been re-appointed and the members were: Sir Willis Jackson, professor of electrical engineering, Imperial College of Science and Technology (chairman); Sir Leon Bagrit, chairman and managing director, Elliot-Automation, Ltd.; Prof. J. G. Ball, professor of physical metallurgy, Imperial College of Science and Technology; Mr. G. S. Bosworth, director of Group Personnel Services, the English Electric Company, Ltd.; Mr. S. L. Bragg, chief scientist in the Aero Engine Division of Rolls Royce, Ltd.; Dr. E. F. Brookman, managing director of the Paints Division, Imperial

Chemical Industries, Ltd.; Lord Brown of Machrihamish, chairman of the Glacier Metal Co., Ltd.; Dr. S. G. Hooker, technical director (Aero) of Bristol Siddeley Engines, Ltd.; Dr. F. E. Jones, managing director of Mullard, Ltd.; Prof. C. A. Moser, professor of social statistics, School of Economics and Political Science in the University of London; Prof. L. Rosenhead, professor of applied mathematics in the University of Liverpool; Prof. J. R. N. Stone, Leak professor of finance and accounting in the University of Cambridge; Sir Peter Venables, principal of the College of Advanced Technology, Birmingham; Mr. L. T. Wright, general secretary of the Amalgamated Weavers Association. Members from Government Departments: Mr. H. E. Bishop, Central Statistical Office; Mr. G. B. Blaker, Department of Education and Science; Mr. R. H. W. Bullock, Ministry of Technology; Mr. R. C. Griffiths, University Grants Committee; Mr. F. Jones, Ministry of Economic Affairs; Mr. F. Pickford, Ministry of Labour; Mr. W. G. Pottinger, Scottish Office; Mr. J. K. Thompson, Ministry of Overseas Development. The Committee would report its findings to the Secretary of State for Education and Science, and the Minister of Technology.

### Grants to Universities and Colleges in Britain

IN a written answer in the House of Commons on February 1, Mr. A. Crosland stated that since the settlement of the grants in aid of the recurrent expenditure of universities and colleges in Britain for the last three years of the quinquennium ending on July 31, 1967, was adjusted in February 1964 to take account of the acceptance of the Robbins Committee's proposals for immediate expansion, supplementary grants had been approved for the increases in academic salaries approved on the recommendation of the National Incomes Commission and for a further increase in the intake of medical students. The present level of grants had been reviewed following advice from the University Grants Committee, and, to assist the universities to meet the objectives of the Robbins Report, in view of increased costs since 1963, the grants for these three academic years would be increased by £5.8 million, bringing the total estimated recurrent provision, excluding rates, to £86.3 million in 1964-65; £99.3 million in 1965-66; and £109.8 million in 1966-67, or about £295 million in all.

### The University of Aston in Birmingham

THE College of Advanced Technology, Birmingham, which is shortly to submit a petition to the Queen in Council for the grant of a Charter as a new technological University, has agreed to request that the title of the new University be "The University of Aston in Birmingham". The matter of an appropriate title has been under discussion for some time, and the title now suggested has been agreed on by the Governing Body of the College after considering many views, including the results of questionnaires to members of staff. The College grew up in the City of Birmingham, and was run by the Corporation for many years until 1962, when an independent Governing Body was set up. The name now suggested will carry with it something of the history of the institution, and also reflects the fact that it is the intention of those responsible for the new University that it should remain on its present site near the centre of the City. It has already been announced that the Chancellor-designate of the new University is Lord Nelson of Stafford. The first Pro-Chancellor will be Mr. J. A. Hunt, chairman of the Board of Governors of the College, who for many years has been connected with further education, and education for commerce and industry. Mr. Hunt is director and general manager of the Hymatic Engineering Co., Ltd., in Redditch, and a director of other companies, as well as a member of many regional and national bodies connected with education, commerce and manage-



ment, including the Central Training Council, the National Advisory Council on Education for Commerce and Industry and the Redditch New Town Development Corporation. The first Vice-Chancellor will be Sir Peter Venables, at present the Principal of the College. Sir Peter has been Principal since 1956. The draft for the Charter of the new University has been submitted to the University Grants Committee for comment; it is anticipated that the petition for the Royal Charter incorporating the new University will shortly be submitted to the Privy Council, and it is hoped that the Charter may be granted before the beginning of the next academic session in October 1965.

#### Economics and Statistics Committee formed by the Scientific Instrument Manufacturers' Association

THE Scientific Instrument Manufacturers' Association has set up an Economics and Statistics Committee to meet the needs of the Association and its members. This Committee will co-operate closely with other bodies doing similar work. The importance of statistical and economic information has become increasingly recognized over the past few years. Government departments and the National Economic Development Council require fuller information in their task of formulating plans, and the demand from companies for data to assist in market research and to assess their own position has greatly increased. Basically two types of information are sought: accurate and detailed data extending back over a reasonable period of time on sales, exports, imports; and secondly, an assessment of trends within the industry and forecasts of future trends. The Economics and Statistics Committee will act as an advisory body to the Council of the Association for the provision of economic and statistical information. It will determine the information likely to be of value to the industry, advise on its collection, analysis and dissemination, and consider methods of improving it. Another aspect of the work of the Committee will be the formulation of terms and definitions for use in the statistics work of the Association. The Committee is composed of members representing each of the sections of the instrument industry and is under the chairmanship of Mr. H. C. Pritchard (Cambridge Instrument Co., Ltd.).

#### The Confederation of British Industry

THE Integration Committee appointed to supervise the establishment of a national industrial organization by the amalgamation of the National Association of British Manufacturers, the Federation of British Industries and the British Employers' Confederation has announced that a revised Charter (on the lines of the outline plan already circulated to members) is in the course of preparation, and it is expected that meetings of members of the three bodies concerned will be held on March 24 at which the integration agreement will be presented for approval.

On this basis the Integration Committee proposes that, to take effect as soon after the meetings as practicable (the anticipated effective date being early in August): (a) the name of the new organization shall be the Confederation of British Industry (C.B.I.); (b) the first president of the C.B.I. shall be Mr. M. Laing; (c) the first vice-presidents of the C.B.I. shall be Sir Peter Runge and Mr. L. Jenkins; (d) the office of deputy president shall be created, but no immediate appointment to this office is proposed; (e) the first director-general of the C.B.I. shall be Mr. J. Davies, at present vice-chairman and a managing director of Shell Mex and B.P., Ltd., who will take up his post on the effective date; (f) Mr. P. F. D. Tennant, at present deputy director-general of the Federation of British Industries, shall be relieved of general duties during the formative period of the C.B.I. in order to give special assistance to the director-general;

(g) appointments to the posts of deputy director-general of the C.B.I. shall be made by the director-general in consultation with the president in due course; (h) Mr. H. J. Gray will continue as director of the National Association of British Manufacturers until the effective date, when he shall be appointed director of regions in the C.B.I.; (i) Mr. D. Taylor, at present deputy director of the British Employers' Confederation, shall be appointed director of labour and social affairs in the C.B.I.; (j) Sir Norman Kipping will continue as director-general of the Federation of British Industries until the effective date and thereafter will be available to the C.B.I. on a part-time basis for consultation until his normal retirement date on May 11, 1966; (k) Sir George Pollock will resign his post as director of the British Employers' Confederation with effect from June 30. Mr. D. Taylor, the present deputy director, will assume the position of acting director of the body from July 1 until the effective date. Sir George will be available to the C.B.I. on a part-time basis for consultation and will also represent the C.B.I. at the International Labour Organization.

If the proposals for a revised Charter are accepted, a formal petition will be submitted to Her Majesty in Council, requesting the grant of a Supplemental Charter.

#### Laboratory Animals

A DIRECTORY of sources of laboratory animals, and of suppliers of equipment and materials connected with their maintenance, is prepared by the Institute of Laboratory Animal Resources. The fifth edition is now available (*Laboratory Animals. II, Animals for Research*. Publication 1199. Pp. 89. Washington, D.C.: National Academy of Sciences-National Research Council, 1964. 2 dollars). The directory comprises five sections: Section I lists the common mammalian and avian laboratory species, giving the strain designation and the breeding system; Section II lists the species of animals "obtained from Nature" and available through commercial suppliers; and Section III lists feedstuffs, cages and other equipment. Each item in these sections is referred to by a code number, and the fourth section of the booklet provides a guide to the manufacturer and suppliers of the animals and equipment coded in the first three sections. In the final section the animals are indexed by taxonomic and vernacular names.

#### Soils and Land-use in Buckinghamshire

A RECENTLY published memoir of the Soil Survey of Great Britain by B. W. Avery describes the soils and features of the region mapped on Sheet 238 (*The Soils and Land Use of the District around Aylesbury and Hemel Hempstead*. Pp. vii+216+11 plates. London: H.M.S.O., 1964. 35s. net). The total area amounts to 216 square miles, of which about two-thirds comprise part of the Chiltern plateau of Chalk upland, which falls in a south-easterly direction down to 400 ft. from an indented scarp north-east-south-west and rising to 800 ft. The 'Icknield Belt' lies between the escarpment and the Vale of Aylesbury to the north-west, which is mainly on Gault and Kimmeridge clays. This memoir is the first to give a detailed account of soils overlying Cretaceous rocks and associated superficial deposits. The first chapter concerns the geology, physiography and climate of the area and discusses local fluctuations from the average daily maximum and minimum temperatures of 55° and 42° F and the reasons for an average summer soil moisture deficit of about 4 in. More than half the volume is devoted to a discussion of soil formation processes and survey methods—there is a useful appendix defining the terms used in profile description—and to the classification of calcareous, brown earth, podzol and gley groups of soils, with details of representatives of various series. There is a chapter on land-use capability and requirements for which much valuable information has been derived from

the long-term experiments on Rothamsted soils that are typical of much of the area. There is also a chapter by J. M. B. Brown on the recent history of the woodlands, describing their exploitation for fuel and wood-working industries with changes in management according to economic and social demands. Beech is still the principal species, especially on well-drained soils of high base status, but in some places the conditions favour oak. There is a list of references to appropriate publications and a subject index.

### Standards of Radioactivity

THE National Physical Laboratory has added sodium-22, chromium-51, manganese-54, cobalt-57 and sulphur-35 to the list of standards of radioactivity issued by the Laboratory. During 1965 standards will be made available as follows:

Potassium-42	February	Phosphorus-32	June
Sodium-22	March	Cobalt-57	September
Manganese-54	April	Iron-59	October
Chromium-51	May	Sulphur-35	November

Standards are available from stock of strontium-90 in equilibrium with yttrium-90. Application forms for all these standards can be obtained from the Applied Physics Division of the National Physical Laboratory, Teddington, Middlesex. Intercomparisons, held during the past two years, of standards of cobalt-60 and sodium-24 issued both by the National Physical Laboratory and by the Radiochemical Centre, Amersham, have been in satisfactory agreement. Therefore, in line with the general policy of the National Physical Laboratory to transfer routine standardization to commercial organizations, cobalt-60 and sodium-24 will now be available only from the Radiochemical Centre, in a similar manner to gold-198 and iodine-131. Ultimate responsibility for primary standardization is with the National Physical Laboratory, and issues of nuclides which have been transferred to the Radiochemical Centre as outlined here will also be measured by the Laboratory.

### Organization for Economic Co-operation and Development Fellowships

THE Organization for Economic Co-operation and Development is offering a limited number of fellowship grants in the field of human resource development planning. Award winners will spend a year, from approximately July 1, in training, research and planning, within the framework of national planning teams engaged on specific projects sponsored by the Organization and member countries. Applicants should be qualified university graduates under thirty-five, with degrees in economics or other appropriate disciplines. Professional or postgraduate experience is a prerequisite, and candidates must be able to work in either English or French. Applications should be made before March 1. Further information can be obtained from the Directorate for Scientific Affairs (Fellowships), Organization for Economic Co-operation and Development, 2 rue André-Pascal, Paris 16.

### The Night Sky in March

NEW Moon occurs on Mar. 3d 10h U.T. and full Moon on Mar. 17d 11h. The following conjunctions with the Moon occur: Mar. 8d 15h, Jupiter 2° N.; Mar. 16d 17h, Mars 1° S. Mercury sets at 18h 00m, 19h 50m, 19h 50m on Mar. 1, 16 and 31 respectively. Venus is too near the Sun for observation. Mars makes its nearest approach to the Earth on Mar. 12d 01h; Mars is above the horizon all night during the month; its stellar magnitude is -0.9; its distance from the Earth on Mar. 12 is 62 million miles and during the month retrogrades through Virgo towards Leo. Jupiter sets at 0h 10m, 23h 25m, 22h 40m at the beginning, middle and end of the month respectively;

its stellar magnitude is -1.8; its distance from the Earth on Mar. 15 is 510 million miles and during the month advances into Taurus. Saturn is unfavourably placed for observation during the month. There is one occultation of a star brighter than magnitude 6 observation being made at Greenwich: Mar. 14d 01h 14.9m,  $\gamma$ Cnc (D). D refers to disappearance. The spring equinox occurs on Mar. 20d 20h.

### Announcements

DR. CASIMIR FUNK has been awarded the Carl Neuberg Medal for 1964, in recognition of his work on vitamin research in the United States.

DR. I. GIAEVER of the General Electric Research Laboratory, in Schenectady, New York, has been awarded the 1965 Oliver E. Buckley Solid State Physics Prize of the American Physical Society, for his discovery that electrons can tunnel through a simple insulating layer only 10-100 atoms thick between two thin films.

DR. P. LIMBERG, formerly lecturer at the Institute for Plant Breeding and Plant Genetics of the University of Giessen, has been appointed director of the Institute for Plant Breeding and Seed Research of the Agricultural Research Centre, Braunschweig-Völkenrode, in succession to Prof. O. Fischnich, who is now assistant director-general of the Food and Agriculture Organization in Rome.

THE British Federation of University Women, Ltd., offers a prize of £15 to women undergraduates for an essay on one of the following subjects: the responsibility of the artist in the modern world; the responsibility of the scientist in the modern world; the student abroad. The closing date for the competition is March 16. Further information can be obtained from the Secretary, British Federation of University Women, Ltd., Crosby Hall, Cheyne Walk, London, S.W.3.

A SYMPOSIUM on "Comparative Phytochemistry", organized by the Phytochemical Group, will be held in Cambridge during March 30-April 1. Further information can be obtained from A. H. Williams, Research Station, Long Ashton, Bristol.

A SPRING meeting on "Adsorption of Silver Halide Surfaces", arranged by the Scientific and Technical Group of the Royal Photographic Society, will be held in the University of Bristol during March 31-April 1. Further information can be obtained from Mr. R. Cox, Ilford, Ltd., Ilford, Essex.

THE Laboratory Apparatus and Materials Exhibition, sponsored by the Scientific Instrument Manufacturers' Association of Great Britain, will be held at Earls Court, London, during March 29-April 2. Further information can be obtained from the Press Officer, U.T.P. Exhibitions, Ltd., Racquet Court, Fleet Street, London, E.C.4.

A CONFERENCE for headmasters and headmistresses on "The Challenge of Modern Engineering", arranged by the Royal Society and the Engineering Institutions Joint Council, will be held in Churchill College, Cambridge, on March 30. Further information can be obtained from R. W. J. Keay, The Royal Society, Burlington House, London, W.1.

A SYMPOSIUM entitled "From Mendel's Factors to the Genetic Code", sponsored jointly by the Royal Society and the Genetical Society of Great Britain, will be held in University College, London, during March 10-13. The programme will include sessions on: the gene, its structure, function and control; chromosomes and genes; genetics of man; quantitative and population genetics. Further information can be obtained from Prof. D. Lewis, Department of Botany, University College, Gower Street, London, W.C.1.

## THE BRITISH COUNCIL

AN article describing the opportunities which the British Council provides for a career overseas accompanies the customary review of the year in the annual report for 1963-64\*. It is of particular interest to the scientist. The staff of the Council considered in this article now numbers about 500; most of them are overseas for about four-fifths of their service, and some 80 per cent of them work in developing countries and are directly or indirectly concerned in education. The total staff of the Council numbers 3,700, of whom 1,600 serve in London and some twenty other universities. Of the remaining 2,100 serving overseas, 400 were appointed in London. Although men and women with scientific training are particularly welcome as candidates for general service entry (at present about 20 a year), there are not many scientists in Council service at present, but the Council hopes to increase the number of full-time science liaison and information posts. It also requires more men and women with scientific training as representatives and assistant representatives overseas. It also attaches importance to having men with a scientific training participating in the higher direction and planning of its work. The article, however, points out frankly that it is difficult for anyone serving the Council overseas to remain an active specialist in a scientific subject, unlike the librarian, for example, who will continue to use his expertise.

The 27 librarians serving the Council overseas play an important part in the Council's work, but until recently a Council career in librarianship meant a series of moves from country to country without advancement. Prospects have now improved and, apart from the upgrading of posts, some librarians have moved to posts of general responsibility. The article describes briefly the nature of the work overseas, and a brief section on the sciences indicates how here it is concerned with promoting science contacts: in the more advanced countries this entails liaison work at the research and postgraduate level, but in the less-developed countries the emphasis is on help and advice in teaching science. A realistic analysis of rewards and drawbacks in Council service overseas notes the recent improvements in pay, allowances and conditions of service and refers to the easements for officers and their wives serving in distant posts, especially in regard to family and educational problems which have been permitted as an indirect consequence of the Plowden Report on Overseas Services. Nevertheless, in terms of prestige and emoluments the normal ultimate ceiling is lower than in the Diplomatic Service and the main attractions are intangible.

Receipts from earnings and donations of £626,000 brought the Council's income for the year to £9,260,000, and for 1964-65 the Government grant of £8,634,000 in 1963-64 has been increased to £10,010,000, that from the Department of Technical Co-operation rising from

£233,000 to £371,000, largely for the cost of administering the increasing number of graduate volunteers. The Council also administers some £2 million provided by technical assistance schemes by the same Department, and the report refers particularly to the exceptionally close working relations between the Department and the Council, which now has representatives in more than 80 countries. It is still impossible to keep pace with the demand for various kinds of assistance in teaching English, and, of the 60 additional experts to serve in key English language teaching posts in the Commonwealth which it is planned to fill by 1970, 8 were appointed in the autumn of 1963, 12 in the autumn of 1964, and 10 are to be appointed in 1965. The Council continued to develop its work in English language teaching by television and has posted television officers for this purpose to India, Iran, Kuwait, Nigeria and the Sudan.

Of more than 64,000 overseas students now in Britain, a fifth are at universities, and although projects for 1,665 places in hostels in London and 1,471 elsewhere had been agreed in September 1964, besides the 1,226 additional places already available, the need for such accommodation remains urgent. Apart from such hostel accommodation, the £3 million voted by Parliament for the Overseas Students Welfare Expansion Programme administered by the Council goes to expanding the services provided at the Council's centres for overseas students and trainees in Britain. The Council is subsidizing an experiment at the Department of Education in the University of Sheffield on the development of programmed instruction in English for overseas students of scientific subjects. At the Department of Education in the University of Birmingham it is assisting research on tests of proficiency in English for overseas students. The National Foundation for Educational Research is being assisted in a large-scale survey of the problems which these students face in adjusting themselves to conditions of study in Britain, particularly the effect of the language factor.

Of some 476 specialist tours and advisory visits overseas arranged by the Council in 1963-64, 71 were concerned with science and technology, 85 with medicine, and 134 with education. It is estimated that almost 35 per cent of visitors and scholars coming to Britain in 1963-64, and of advisory visits and specialist tours overseas, were concerned with science and medicine: they included 118 scholars, bursars and others from the United Arab Republic, while 20 scientific and medical specialists from Britain visited that Republic. Of 8,851 overseas visitors to Britain assisted by the Council, 1,986 were concerned with science and technology, 1,110 with medicine, and 2,263 with education. Of 82 university interchange visits under the Commonwealth Scheme, 50 were for university teachers on study leave, 22 for postgraduate research workers and 10 for distinguished scholars invited for short visits. Under the Foreign Scheme there were 142 short visits, 4 longer teaching visits and 104 visits by young research workers.

\* The British Council, Annual Report 1963-1964. Pp. vii + 107 + 12 plates. (London: The British Council, 1964.) 2s. 6d.

## THE DESIGN OF SPECIFICATIONS FOR DRUGS

THIS account is based on a document produced by the Science Committee (Pharmaceutical Analysis) of the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain. Recent advances in analytical techniques have revealed the presence in some drugs of hitherto unsuspected impurities with potent undesirable pharmacological effects. The Science Com-

mittee (Pharmaceutical Analysis) of the Department of Pharmaceutical Sciences has discussed the design of specifications as set out in the current British Pharmacopoeia and the British Pharmaceutical Codex, and it has formulated ideas for the future. The Committee's work has shown the need to apply a more critical approach to specifications for all drugs (see *Nature*, 203, 701; 1964).



The present conventional form of specification is in many instances unrealistic. This is particularly noticeable where in a group of drugs of a very similar chemical structure small differences in composition, or in the spatial arrangements of the molecule, may alter the potency considerably. Such slightly different compounds can be produced during synthesis so that impurities, generally of a lower potency but sometimes more toxic, remain in the commercially purified drug. These impurities are not necessarily detected by tests included in the specifications. The determination of the content of pure substances should be replaced by tests to limit the content of impurities. This change of emphasis is desirable even if an investigation of more fundamental physical properties were to enable a better assessment of the purity of a commercial sample of a drug to be made, or if methods could be devised to give more exact determinations of the percentage purity, particularly when pure reference substances are available for comparative assays. Moreover, tests for specific impurities can be applied to formulated products and thus give a better assessment of the quality of the drug used. Sensitive modern techniques make this practicable; formerly it was rarely possible.

**Stability tests.** In future, analytical assessment of the stability of the active ingredients of formulated products during storage must also be considered; limits for the content of decomposition products after a certain storage period should be included in monographs. For pharmaceutical purposes formulated products often contain other substances in much larger quantities than the active ingredients, and adequate standards for the purity of such substances must be specified, especially as many of these materials are not well-defined substances.

**Toxicity tests.** Toxicity tests should be made concurrently on the purest possible sample and on a sample of the same chemical at a certain stage of preparation. This stage should be where impurities, which the final purification should eliminate, are still present. Attention may also have to be given to products not refined by crystallization techniques, such as those purified by washing under controlled conditions or by the use of distillation or

sublimation methods. For any subsequent control purposes biological tests would seldom be required since, having established which of the impurities have to be controlled, these can be determined satisfactorily by chemical or physical methods.

**Non-toxic impurities.** A limit for non-toxic impurities such as non-active isomers or inactive hydrolysis products is also necessary, since they will diminish the potency of the material. The possible necessity of permitting a certain amount of impurity—even toxic impurity within clinically acceptable limits—in the interest of economy of manufacture should be recognized.

From the foregoing it follows that in future drug standards must include tests for all impurities that may be introduced during the manufacture of a drug. Clearly, each drug standard must include specific quantitative tests for possible impurities, with appropriate limits according to the toxicity of the impurity and the intended use of the drug. There should be a general test, such as phase-solubility measurement, thin-layer chromatography, gas-liquid chromatography, refractometry, etc., to limit total organic impurities. There should also be a screening test, for example, by emission spectrography, to detect any traces of toxic metal remaining in a purified drug as a result of the use of catalysts that a manufacturer might be reluctant to disclose, and limits should be laid down for any such contamination.

In order to formulate suitable specifications, the responsible committees must be provided with all relevant information. The disclosure of adequate details of the chemistry of a process and the practical details of each synthetic step, of the raw materials and solvents used, and some indication of probable impurities, will be essential. Although this would be best achieved by close co-operation between industry and the standardizing bodies, which has for the most part existed in the past, it may be necessary in fairness to all concerned to make such disclosure a legal requirement.

Once a drug has been standardized every new manufacturer would also have to comply with the full requirements of disclosure considered necessary when the drug was first approved.

## A CO-OPERATIVE APPROACH TO NON-DESTRUCTIVE TESTING

THE British National Committee for Non-Destructive Testing has given much consideration to the needs of industry in this field. Investigations by a working party under the chairmanship of Dr. E. G. Stanford have been described in a paper by Dr. A. Nemet<sup>1</sup>. Entrusted with the task of ascertaining the problems and difficulties in industry, the working party met representatives of three important engineering industries, the power generation industry, the aircraft industry, and the steel industry, and discussed these matters with them. The discussions revealed the keen interest of the different industries in non-destructive testing and in possible improvements of techniques. It was not difficult to find common ground within each industry and, in some cases, similarity of needs between industries which are in no way connected. There was clear indication of the wide scope for useful research and development work on methods of non-destructive testing and for co-operation between interested parties.

In November 1963, a second working party was appointed by the National Committee under the chairmanship of Dr. A. Nemet to examine further the needs of industry. The broad aims of the working party are: to confirm that a problem exists and is common in industry; to assess existing knowledge; to ascertain what bodies are doing relevant research; to ascertain what information is still required; to review means of utilizing this information as it is made available.

The working party is compiling a list of problems; it already includes a number of items such as investigation of fluorescent screens for high-energy radiations; attenuation effects of ultrasonic vibrations in solids; adhesive bonding appraisal, etc. It is hoped that university and college departments, Government laboratories, research associations and industrial organizations pursuing research or development work which may be helpful in this field may let the working party have an indication of their projects. It is also hoped that organizations using testing techniques may bring to the notice of the working party particular problems that they encounter or foresee.

Although the working party is limited, by its terms of reference, to finding and describing the most important problems, bringing together interested groups and making recommendations, it is already clear that there is much to be achieved by co-ordinated effort and by bringing the problems to the notice of Government and industrial organizations who could offer material help. Mention should be made of the valuable assistance and many courtesies extended to the working party by the Institution of Mechanical Engineers.

Correspondence should be addressed to the Secretary, The British National Committee for Non-Destructive Testing, The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

<sup>1</sup> Nemet, A., *The Engineer*, February 23, 1963.

## APPLICATION OF THIN FILMS TO THE PRODUCTION OF USEFUL ELECTRIC CIRCUITS

THE eleventh meeting of the Dielectrics Evaporation Group was held at the Institute of Physics and the Physical Society during October 20-21. The following papers were read: "Ion Beam Deposition", by B. A. Probyn; "Thermally-grown, Pyrolytic and Sputtered Silicon Oxides", by M. J. F. Gaze and A. M. Gundlach; "The Use of Alloy Systems in the Epitaxial Growth of Silicon", by Dr. S. Nielson; "Progress in Reactive Sputtering of Oxide Films", by E. Hollands; "Effects of Film Composition on the Temperature Coefficient of Nickel-Chromium Resistive Films", by B. Hendry; "Factors Affecting Properties and Performance of Thin-film Microcircuits", by J. L. Parmee; "Techniques for Obtaining Films of Uniform Thickness", by B. Wood; "The Role of the Active Device in Thin-film Circuits", by D. Salisbury; "A Bakable Multiple-mask Changer", by Dr. P. R. Stuart; "Some Notes on the Recent American Vacuum Society Congress", by C. Stoddart.

The tone of this meeting inclined rather more to the technological aspects of thin films than the previous ones. However, it is sometimes salutary for the worker in pure research to pay heed to the more practical application of his work. The theme was in fact the application of thin films to the production of useful electronic circuits. One fact which emerged very clearly was the need for thin-film active devices the preparation of which should be compatible with the passive circuit elements commercially available. It is somewhat depressing to note that only a small amount of fundamental research has been carried out in Britain and elsewhere on compatible thin-film active devices. It was pointed out by Mr. Parmee that the only solution to this problem at present, and for some time to come, must be to attach a semiconductor device to a thin-film passive circuit. Such devices are now available in flakes which do not need separate leads. He also advocated, using compound methods of construction (evaporated conductors, reactively sputtered silica dielectrics and separately attached active devices), that reliable miniature assemblies can be constructed on a commercial

alternative approach to the device problem which has enjoyed widespread and influential support is the deposition of silicon on some suitable substrate. It was made clear by Dr. Nielson that this process has not yet been perfected. Many practical difficulties remain on the fundamental problem of producing monocrystalline films. It seems unlikely that this type can succeed at temperatures sufficient to avoid damage to more normal passive

components of the thin-film active device does not exist in the so-called 'solid circuit'. In this the problem

is the fabrication of integral passive circuit elements. Mr. Gaze compared silica films prepared by various methods in this connexion. He has been successful in forming capacitor films by thermal oxidation and pyrolytic deposition. This success may have influenced his decision not to persist in experiments on reactive sputtering for which Mr. Parmee had reported such excellent results.

The process of reactive sputtering is, due to its complexity, scarcely understood theoretically, and it is hoped that more fundamental research in this field may throw some light on the mechanism of deposition. In the practical field Mr. Hollands reported results on the sputtering of mixed titanates and of zirconia. He has confirmed that with mixed titanates, while high breakdown strengths may be obtained, they are associated with permittivities far lower than are to be expected from the bulk material. This phenomenon has been observed in other investigations of such materials. It is interesting to speculate whether or not in practice such a correlation will always be found. In the case of zirconia it was shown that adequate protection from moisture might cause a four-fold increase in dielectric strength.

Much interest is still shown in work on the preparation of nickel chromium alloy resistive films, and greater understanding of their properties is now emerging as a result of the combination of electrical measurements with electron microscope examination.

Evaporation is still the customary method of deposition. Mr. Wood described a method of producing uniform deposits over a large area using a line source and baffle plates. He used an optical transmission method for assessing uniformity of deposit as well as an optical analogue to determine the optimum geometry of the system.

Mr. Hendry related the changes in electrical properties for nickel chromium films of varying composition and different thicknesses to the structures shown by electron microscopy. He invoked the present theories of conduction in continuous and aggregated films appropriate to the different cases.

The approach of the vacuum engineer characterized the description of the bakable multiple-mask changer designed and made at the National Physical Laboratory for the preparation of cryotron arrays. This is suitable for operation in vacuum equipment at pressures down to  $10^{-6}$  mm mercury, and can hold up to 24 masks.

The meeting was concluded with an account of some of the more interesting papers at the recent American Vacuum Society Symposium. It is not clear when these will be published.

It is hoped that the next meeting of the Dielectrics Evaporation Group will be held some time before Easter 1965.

J. R. BALMER

## MAPS OF THE PERSEUS REGION AT 600 AND 1,415 MEGACYCLES PER SEC

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MAPS of the Andromeda Nebula (*M* 31) region made at 600 and 1,415 Mc/s with the Ohio State University 260-ft. radio telescope have recently been published<sup>1</sup>. The maps covered 285 square degrees of sky between right

ascensions of 23h 40m to 02h 10m and declinations of 36° to 46° (N.). Since the telescope is of the meridian transit type and runs continuously, radiometric data were obtained during the same programme not only for the

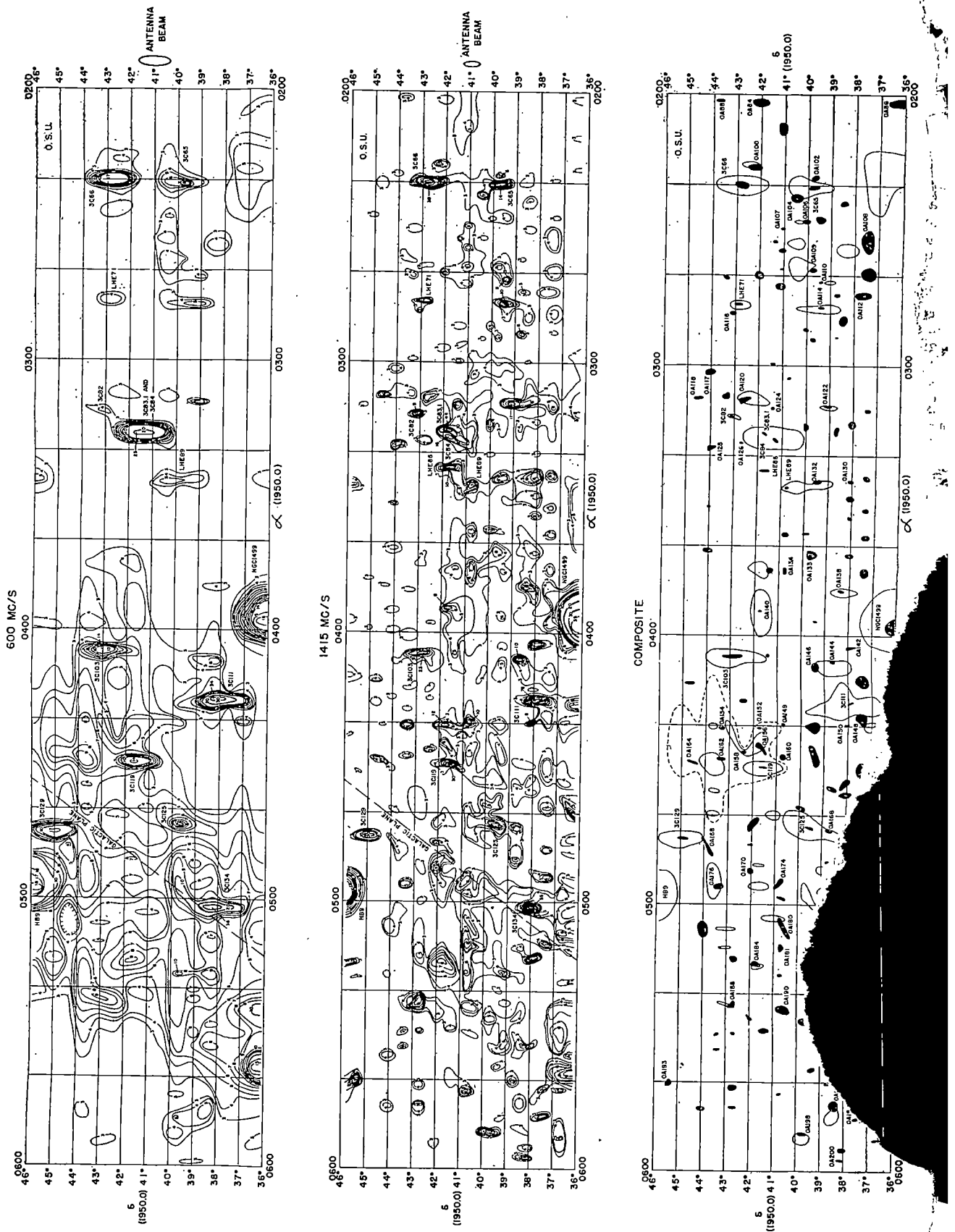


Fig. 1. Maps of the Perseus region made with the Ohio State University 280-ft. radio telescope at 600 Mc/s (top) and 1,415 Mc/s (middle). The bottom map is a composite one for comparing the positions of the most significant features of the other two maps by solid areas for 1,415 Mc/s and open contours for 600 Mc/s. The contour interval of the 600 Mc/s map is 0.25° K and of the 1,415 Mc/s map is 0.06° K antenna temperature



Andromeda region but for other areas in the zone between the declinations of 36° and 46°. The present article presents maps and results of observations with the Ohio State University telescope for a 450 square degree portion of that zone lying between right ascensions of 02h 00m and 06h 00m.

The 1,415-Mc/s receiver makes use of a liquid-nitrogen-cooled parametric amplifier with a noise temperature of 50° K. At 1,415 Mc/s the antenna beam-width is 11' × 36', the system temperature is 120° K, and with a 5-Mc/s bandwidth and a 12-sec integration time the root mean square noise temperature for one record is 0.025° K. The parameters of the 600-Mc/s system are 23' × 85', 650° K and 0.15° K, respectively. Two drift records were averaged for each declination, with the interval between adjacent declination scans being 20' at 1,415 Mc/s and 7' at 600 Mc/s. The region covered by this report contains approximately 4,000 beam areas at 1,415 Mc/s and 10 beam areas at 600 Mc/s.

The results of the observations are presented by the maps in Fig. 1. All are to the same scale. The top one gives antenna temperature at 600 Mc/s with contour spacings of 0.25° K. The middle map shows the antenna temperature contours at 1,415 Mc/s with intervals of 0.06° K. In both these maps the lowest two contours (1 and 2) are omitted, since they are closest to the root mean square noise temperature level and therefore probably the least significant. In addition, their absence greatly simplifies the appearance of the maps. Every tenth contour-level is indicated by a heavier line. Long-term residual drift was subtracted from the records so that the contour-levels are above an arbitrary zero level.

The bottom map is a composite map for comparing the positions of the most significant features of the upper two contour maps. In this composite map the 1,415-Mc/s sources are shown by solid areas and the 600-Mc/s sources by open contours. The size of the solid areas corresponds to the highest 1,415-Mc/s contour for a source, while the solid contours correspond to contour-level 4 (in a few cases) on the right half of the 600-Mc/s map and to somewhat higher levels on the left half in the vicinity of the galactic plane. Thus, the size of the solid and open areas show a correspondence. All positional information is epoch 1950.0.

An interesting region covered by these maps is that near the galactic plane. The position of the galactic equator has been given by Blaauw, Gum, Pawsey and Westerbasis. Indicated by a dashed line. The complex structure shown clearly in the 600-Mc/s map as it has reached the ninth contour level with a number of epitaxial features. An asymmetry of the ridge with respect to the galactic equator is also evident. In addition to a number of discrete sources near the galactic plane there arise even two clusters on the right-hand part of the continuous ridge and 03h 15m. These are most evident processes of the map. The dashed contour on the conveniently low temperatures a grouping near the galactic plane components. The sources listed in the revised third

The proper catalogue<sup>4</sup> in the area of our maps. All arise in the maps with the exception of 3 C 129.1. The sources 3 C 65, 66, 103, 111 and 119 as given by German and Long<sup>5</sup> were used as reference

Comparing our positions (at 1,415 Mc/s) with the values for the 3 C sources on our maps (3 C 83.1 and 84) yields a systematic difference of 1 sec of time in right ascension and 2 min of declination. These differences were regarded as small as to make any correction of our independently determined positions unwarranted. The standard deviation is 5 sec of time in right ascension and 1 min of arc in declination.

There are 9 additional sources on our maps in the list of Scott, Ryle and Hewish (SRH)<sup>6</sup>. Three of these are also listed by Long, Haseler and Elsmore (LHE)<sup>7</sup>. Comparing our positions with those in these lists (6 in one and 3 in the other) gives a systematic difference of 1 sec in time in

Table 1. SOURCES ON OHIO STATE UNIVERSITY 600-Mc/s AND 1,415-Mc/s MAPS OF THE PERSEUS REGION

Source	h	Position* (1950.0)		Dec. min	Flux density†	
		R.A. m	s		600 Mc/s	1,415 Mc/s
OA 100†	2	16	02	42	25	1.8
OA 102	2	18	42	39	44	1.4
3 C 66	2	20	08	42	48	15.7
3 C 65	2	20	32	39	43	7.4
OA 104†	2	22	42	40	25	1.7
OA 106	2	28	02	40	02	1.0
OA 107	2	28	44	41	03	1.2
OA 108	2	32	34	37	21	1.0
OA 109	2	38	42	39	44	1.4
OA 110	2	41	28	39	22	1.9
OA 112	2	44	22	37	39	1.0
LHE 71‡	2	46	15	42	56	3.7
OA 114	2	47	08	39	25	4.6
OA 116	2	48	28	43	05	1.2
OA 117	3	01	29	44	00	1.0
OA 118	3	07	18	44	32	1.0
OA 120	3	07	38	42	37	2.8
OA 122	3	09	05	39	05	4.6
OA 124†	3	09	35	41	23	1.7
3 C 82	3	11	21	43	05	3.7
3 C 83.1	3	15	12	41	45	10.8
3 C 84	3	16	41	41	20	6.2
OA 126	3	17	17	42	42	1.9
OA 128	3	18	25	44	00	1.2
LHE 86	3	23	21	41	45	2.4
OA 130	3	25	40	38	03	1.9
OA 132	3	26	01	39	24	4.6
OA 133	3	41	48	39	41	1.4
LHE 89	3	27	03	40	45	2.2
OA 134	3	45	30	40	46	1.7
OA 138	3	50	03	38	25	3.7
OA 140†	3	54	35	41	45	3.7
OA 142	4	02	34	37	53	1.0
3 C 103	4	04	38	42	47	12.0
OA 144	4	05	49	38	44	5.5
OA 146	4	07	03	39	22	1.2
3 C 111	4	15	06	37	56	28.3
OA 148	4	18	33	37	27	1.2
OA 149†	4	19	51	40	45	2.4
OA 150	4	19	56	38	02	1.9
OA 151	4	19	56	36	41	1.2
OA 152	4	20	32	41	45	3.0
OA 154	4	20	38	43	22	1.7
OA 156	4	24	17	41	45	1.4
OA 158†	4	25	55	42	25	1.8
OA 160	4	27	00	40	45	1.2
OA 162	4	27	24	43	22	1.8
OA 164	4	28	11	44	33	1.2
3 C 119	4	29	19	41	35	12.9
3 C 125	4	42	51	39	32	5.6
OA 166	4	43	26	38	42	1.4
3 C 129	4	45	16	44	54	10.0
OA 168	4	47	05	43	51	1.0
OA 169	4	50	18	38	42	1.4
OA 170	4	52	37	42	02	2.2
OA 172	4	54	24	37	22	1.4
OA 174	4	55	05	40	45	1.4
OA 176	4	56	07	43	22	1.8
3 C 134	5	01	17	38	02	25.9
OA 178	5	02	35	37	45	1.4
OA 180	5	03	24	40	33	1.2
OA 181	5	09	42	40	42	1.9
OA 182	5	12	56	38	02	1.9
OA 184	5	13	23	41	45	12.1
OA 186	5	16	08	39	03	1.4
OA 188	5	22	22	42	42	3.7
OA 190	5	23	42	40	35	1.2
OA 192	5	32	41	38	02	1.8
OA 193	5	40	12	45	22	2.2
OA 194	5	45	25	38	25	2.7
OA 196	5	48	13	37	22	1.4
OA 198	5	51	47	39	42	2.7
OA 200	5	57	36	38	02	1.4

\* Probable error for OA sources: ± 10 s in R.A., ± 10' in dec.

† In units of 10<sup>-26</sup> W m<sup>-2</sup> (c/s)<sup>-1</sup>.

‡ In Scott, Ryle and Hewish list (ref. 6) (178 Mc/s).

§ LHE: Long, Haseler and Elsmore source (ref. 7) (408 Mc/s).

Note: Map sources OA 84, 86, and 88 are listed in ref. 1.

right ascension and 1 min of arc in declination, with standard deviations of 7 sec of time in right ascension and 4 min of arc in declination.

Those sources which appear at both frequencies in approximately the same position, or rise several contour intervals above the background at only one frequency, are considered to have a high probability of being real sources and are assigned OA (Ohio List A) numbers, provided they have not been previously named. These 59 new sources are shown with their OA numbers on the composite map. The positions and flux densities of all named sources found on the maps are listed in Table 1. The positions given are those measured at 1,415 Mc/s (epoch 1950.0), inasmuch as these are probably more accurate. Flux densities for the OA sources are probably correct to within ± 25 per cent. Adding the 59 OA sources of the present map to those previously published<sup>1</sup> brings the

published total to 110 O.A. sources in 715 square degrees of sky.

Three relatively strong sources appear at the extreme edges of the maps. These sources are NGC 1499 (California Nebula) at about 04h R.A. and 36° dec., HB 9 at about 05h R.A. and 46° dec., and an unnamed source at about 05h 45m R.A. and 36° dec. Since only small parts of these sources are within the map limits, they are not listed in Table 1.

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<sup>3</sup> Blaauw, A., Gum, C. S., Pawsey, J. L., and Westerhout, G., *Mon. Not. Roy. Astro. Soc.*, **121**, 123 (1960).

<sup>4</sup> Bennett, A. S., *Mem. Roy. Astro. Soc.*, **68**, 163 (1962).

<sup>5</sup> Conway, R. G., Kellerman, K. I., and Long, R. J., *Mon. Not. Roy. Astro. Soc.*, **125**, 281 (1963).

<sup>6</sup> Scott, R. F., Ryle, M., and Hewish, A., *Mon. Not. Roy. Astro. Soc.*, **122**, 95 (1961).

<sup>7</sup> Long, R. J., Haseler, J. B., and Elsmore, B., *Mon. Not. Roy. Astro. Soc.*, **125**, 213 (1963).

## GAMMA-RAY SPECTRA ANALYSED BY COMPUTER PROGRAMME USING THE PEAK AREA METHOD

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AFTER considerable time had been devoted to manual analyses of more than 500 gamma-ray spectra, a computer programme was formulated, and it is now used routinely in the instrumental neutron activation analysis of meteorites<sup>1</sup>. In particular, our present research problem involves the determination, via instrumental neutron activation analysis, of the abundances (or concentrations) of selected chemical elements in meteorites and in separated meteoritic minerals and chondrules, etc. A known quantity of meteorite and appropriate reference standards are irradiated by thermal neutrons; afterwards, measurements are made of the gamma-rays of the radioactivities from the meteorites and reference standards of Na (15-h <sup>24</sup>Na), Mn (2.56-h <sup>56</sup>Mn), Cu (12.8-h <sup>64</sup>Cu), Cr (28-day <sup>51</sup>Cr), Fe (45-day <sup>59</sup>Fe), Sc (85-day <sup>46</sup>Sc), and Co (5.2-yr. <sup>60</sup>Co).

Typical gamma-spectra (see Figs. 1 and 2) result from the interaction of one or more gamma-rays in a scintillation detector, usually a 3-in. × 3-in. NaI(Tl) crystal coupled to a pulse-height analyser, usually 200 to 512 channels. The information from the analyser may be visually inspected via an X-Y plotter and also printed out on tape.

The abundances may be manually calculated by comparison of peak heights (corrected for radioactive decay) of selected prominent gamma-rays from known quantities of irradiated meteorites and reference standards.

The mathematical formulation of this computer programme follows the brief description given below; other methods of analysis have been given by Heath<sup>2</sup>. Peak areas of a selected gamma-ray (or rays) of both the irradiated reference standard and of the specimen are determined as follows. The ambient background spectrum is first subtracted, the subtraction being adjusted for differences in counting time for background, specimens, and reference standards. Background spectra are usually not subtracted from the reference standards, since counting rates of standards usually exceed the ambient background by many orders of magnitude. The Compton continuum, represented as a trapezoid under a given peak, is next calculated.

To calculate the concentration in p.p.m., the following items are included in the computer formulation: quantities of the specimen and reference standards expressed in g and  $\mu$ g units, respectively, and duration of counting and median counting times for specimen and standard, respectively. The printed result in p.p.m. also includes the standard deviation due to counting statistics.

Occasionally, the right-end plateau of a given peak in a specimen has such a steep drop-off that a straight line

initiating at the left minimum of a given peak may not be tangent-fitted to the Gaussian equation describing the right plateau. For calculation of such spectra, the  $\Delta$ -channel found for the corresponding reference standard peak is applied to the peak in the specimen. This  $\Delta$ -channel interval for the reference standard peak is the number of channels between the left-end minimum and the point where the tangent meets the right-end plateau Gaussian

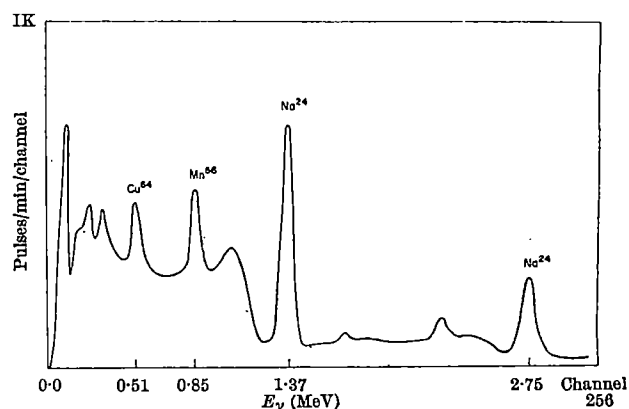


Fig. 1.  $\gamma$ -Ray spectrum of Mokoia (0.257 g) after irradiation at  $2 \times 10^{11}$  neutrons/cm<sup>2</sup>-sec for 5 min and decay of 26 h (energy calibration at 12 keV/channel; 3 in. × 3 in. NaI(Tl) crystal)

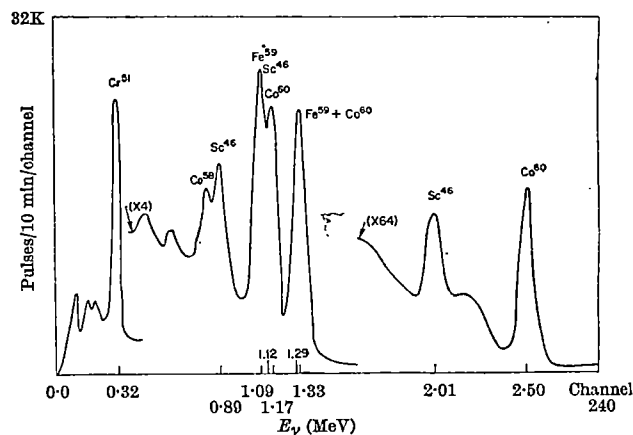


Fig. 2.  $\gamma$ -Ray spectrum of Mokoia (0.257 g) after irradiation at  $2 \times 10^{12}$  neutrons/cm<sup>2</sup>-sec for 30 min and decay of 2 weeks (energy calibration at 12 keV/channel; 3 in. × 3 in. NaI(Tl) crystal)

equation. Multiplication of the number of  $\Delta$ -channels and the average of the channel data corresponding to these two extreme channel abscissa yields a fair approximation for the subtraction of the Compton continuum.

A check on the identity of radionuclides may be made by comparing the peak areas of two or more gamma-rays or by following the radioactive decay of a given peak area. The former has been provided for in the computer formulation. As an example, 2.56-h  $^{56}\text{Mn}$  is identified by requiring identical ratios of the peak areas of the 0.85-MeV and 1.81-MeV gamma-rays in the specimen and in the Mn reference standard. Peak areas of both gamma-rays are calculated as described above. Other identification examples are 15-h  $^{24}\text{Na}$  via ratios of the peak areas of the 1.37-MeV and 2.75-MeV gamma-rays, and 85-day  $^{45}\text{Sc}$  via ratios of the peak areas of the 0.89-MeV and 2.01-MeV sum gamma-rays.

When a composite peak contains two or more gamma-rays, interfering peak areas must be subtracted. Generally, this is done by selecting a peak for the interfering element which is itself free of interference in the spectrum of the specimen and determining its area,  $A$ . The area,  $B$ , of this peak in a reference standard of the interfering element is then determined, together with the area,  $C$ , in the same reference standard of the interfering line.  $AC/B$  is then subtracted from the composite line in the specimen spectrum. An illustration follows. In the composite twin peaks of  $^{56}\text{Fe}$ ,  $^{46}\text{Sc}$ , and  $^{60}\text{Co}$ , the peak areas of the 1.12-MeV gamma-ray of  $^{46}\text{Sc}$  and the 1.17-MeV and 1.33-MeV gamma-rays of  $^{60}\text{Co}$  must be subtracted to obtain the residual peak area of the 1.10-MeV and 1.29-MeV gamma-rays of  $^{56}\text{Fe}$ . The amount of  $^{46}\text{Sc}$  interference is simply found by multiplying the ratio of the 1.12-MeV peak area to the 2.01-MeV sum peak area of the  $^{46}\text{Sc}$  reference standard by the peak area of the 2.01-MeV sum peak area of  $^{46}\text{Sc}$  in the specimen. Similarly, the  $^{60}\text{Co}$  interference equals the product of the ratio of the 1.17-MeV and 1.33-MeV peak areas to the 2.50-MeV sum peak area and the 2.50-MeV sum peak area of  $^{60}\text{Co}$  in the specimen. Subtraction of these two quantities from the total composite peak area yields the residual  $^{56}\text{Fe}$  twin peak area. The standard deviation of the residual peak area includes all standard deviations of the interfering peak areas.

The abundances of the seven elements of interest are usually calculated in about 20 or more specimens in a single computer analysis against one set of reference standards. The programme also computes the mean concentration and the associated standard deviation of each element for a given set of specimens.

The peak-area method is relatively insensitive to gain shifts of the order of a few per cent. The programme also need be concerned only with peak-area information and not with the general Compton continuum with its satellite pair-peaks, backscatter peaks, etc. The peak areas of associated gamma-rays assume relevancy only under subtractive circumstances. Special cases arise when a gamma-ray of an unwanted radionuclide contributes to a particular peak area and no other gamma-ray(s) of that unwanted radionuclide exists. In most cases, differences in decay half-life will show up as two half-lives if the concentration values are plotted against spectral counting times.

**Numerical method.** The concentration of an element in a sample is found by comparing the area of a specified gamma-ray peak with that of a known standard that has been irradiated and counted under similar circumstances. The programme works with the raw counting data in the form of counts per channel for the sample, the standard, and the background. Other data needed by the programme are the counting time and the mean time-interval from a given time (such as the end of irradiation) for each spectrum, the mass of the irradiated standard and sample, the half-life of each standard element, and a first guess at the location of the channels which bound the peak to be studied. The background

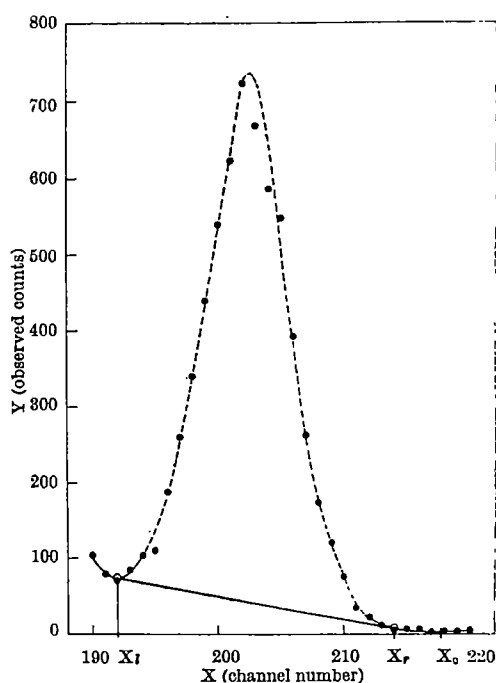


Fig. 3. A typical example of a  $^{60}\text{Co}$   $\gamma$ -ray sum peak at 2.50 MeV with binomial equations fitted to the left minimum and right plateau. The solid curves are the approximating functions around the left end,  $X_1$ , and the right end,  $X_r$ . Circles are the real data points, and  $X_0$  is the first approximation point of the right end.

spectrum is subtracted from the sample and/or the standard spectrum at the user's option, and the Compton continuum is subtracted from both the sample spectrum and the standard spectrum before the areas are compared.

In order to compute the peak area, the programme first finds the low-energy bound of the peak,  $X_1$ , and the high-energy bound of the peak,  $X_r$  (see Fig. 3). The channel counts near  $X_1$  and  $X_r$  are approximated by a second-order polynomial and a Gaussian, respectively, as shown in Fig. 3 by the solid curves. The peak area is the area surrounded by the peak and the straight line drawn between the points  $(X_1, Y_1)$  and  $(X_r, Y_r)$ . The Compton continuum is the areas of the trapezoid under this straight line.

The low-energy bound of the peak is called the left end of the peak. It is assumed that the channel counts near the left end can be approximated by a second-order polynomial of the form:

$$Y = a_1 X^2 + b_1 X + c_1 \quad (1)$$

where  $a_1$ ,  $b_1$  and  $c_1$  are the constants which give the least-squares deviation of  $Y$ 's from the actual counts.  $X$  is a channel number and  $Y$  is a channel count.

The user of the programme has to specify the channels within which the real left-end point lies. The programme first finds the channel number which has the smallest count among the specified channels. Then, using this channel as a midpoint and using the two channel numbers on each side of it, the programme finds the coefficients in equation (1). The minimum point of equation (1),  $X_1$ , is considered to be the left end of the peak:

$$X_1 = -\frac{b_1}{2a_1} \quad (2)$$

where  $a_1$  must be positive.

To check that the point  $X_1$  is an acceptable left end of the peak, the programme looks to see if  $X_1$  lies within the channel numbers that have been used to compute the coefficients in equation (1) and if the coefficient  $a_1$  is positive. If not, the programme uses one more channel on each side of the midpoint to find a new set of coefficients and a new  $X_1$ . The programme repeats this procedure



until an acceptable  $X_l$  is found, or stops further computation if an acceptable  $X_l$  is not found after all the specified channels are used.  $Y_l$ , the channel count corresponding to the channel  $X_l$ , is found by substituting  $X_l$  into equation (1).

The high-energy bound of the peak is called the right end of the peak. The channel counts near the right end are approximated by a Gaussian function of the form:

$$\ln Y = a_2 X^2 + b_2 X + c_2 \quad (3)$$

where  $a_2$ ,  $b_2$  and  $c_2$  are the constants which give the least-squares deviation of  $\ln Y$ 's from the logarithms of the actual counts.  $X$  is a channel number and  $Y$  is a channel count. In the polynomial fit, the user of the programme has to specify the channels that are to be used to compute the coefficients in equation (3). If the number of the specified channels is less than or equal to nine, the programme uses all the specified channels to compute the coefficients in equation (3). If the number (which is restricted to be an odd number) is greater than nine, it uses the central nine channels of those specified to compute the coefficients. Then the programme checks the sign of the coefficient  $a_2$ . If  $a_2$  is positive, the programme proceeds with the tangent method; if  $a_2$  is zero or negative, the programme has the following choice: First, if all the specified channels have already been used to compute the coefficients in equation (3), the programme proceeds with the  $\Delta$ -channel method as an alternative. Secondly, if not all the specified channels have been used to compute the coefficients, the programme uses two more channels to obtain a new set of the coefficients,  $a_2$ ,  $b_2$  and  $c_2$ .

In the tangent method, the right end of the peak,  $X_r$ , is defined to be the point where the straight line drawn from the left-end point ( $X_l$ ,  $Y_l$ ) is tangent to equation (3). For the first approximation to  $X_l$ , a minimum point of equation (3),  $X_0 = -(b_2/2a_2)$ , is used if  $X_0$  lies within the points that have been used to find the coefficients in equation (3). If the minimum point does not lie within these points, the extreme right-end point, again called  $X_0$ , is used as the first approximation. Using this  $X_0$  value, the real right end (the tangent point  $X_r$ ) is found as follows:

By differentiating equation (3), we get

$$Y' = (2a_2 X + b_2) \exp(a_2 X^2 + b_2 X + c_2)$$

At  $X = X_r$ ,

$$Y'_{X=X_r} = (2a_2 X_r + b_2) \exp(a_2 X_r^2 + b_2 X_r + c_2) \quad (4)$$

But  $Y'_{X=X_r}$  must be equal to the slope of the tangent line—starting at the point ( $X_l$ ,  $Y_l$ ) and drawn to the point ( $X_r$ ,  $Y_r$ )—which has the tangent of  $(Y_r - Y_l)/(X_r - X_l)$ . Therefore:

$$\frac{Y_r - Y_l}{X_r - X_l} = (2a_2 X_r + b_2) \exp(a_2 X_r^2 + b_2 X_r + c_2)$$

Substitution of  $Y_r = \exp(a_2 X_r^2 + b_2 X_r + c_2)$  into the above equation gives:

$$[(X_r - X_l)(2a_2 X_r + b_2) - 1] \exp(a_2 X_r^2 + b_2 X_r + c_2) + Y_l = 0 \quad (5)$$

Equation (5) is solved iteratively by the Newton-Raphson method to find  $X_r$ . That is:

$$X_{r,n+1} = X_{r,n} - \frac{f(X_{r,n})}{f'(X_{r,n})}$$

where  $X_{r,n}$  is the  $n$ th approximation of  $X_r$  (note that  $X_{r,1} = X_0$ , our first approximation),

$$f(X) = [(X - X_l)(2a_2 X + b_2) - 1] \exp(a_2 X^2 + b_2 X + c_2) + Y_l \quad (6)$$

and

$$f'(X) = (X - X_l)[(2a_2 X + b_2)^2 + 2a_2] \exp(a_2 X^2 + b_2 X + c_2) \quad (7)$$

To check that the point  $X_r$  is an acceptable right end of the peak, the programme looks to see if  $X_r$  lies within the points that have been used to compute the coefficients in equation (3). If not, the programme has the following choice: First, if all the specified channels have already been used to compute the coefficients in equation (3), the programme uses the  $\Delta$ -channel method as an alternative. Secondly, if not all the specified channels have been used to find the coefficients previously, the programme uses two more points to get a new set of coefficients  $a_2$ ,  $b_2$ , and  $c_2$ , and repeats the procedure in entirety.

If the tangent method fails to find an acceptable right end of the peak, the  $\Delta$ -channel method is used as an alternative. The  $\Delta$ -channel is defined to be the number of channels which is added to the left-end point,  $X_l$ , to find the right end,  $X_r$ . The user of the programme has to specify the  $\Delta$ -channel for the standard if the  $\Delta$ -channel method is to be used to find the right end of the peak of the standard in case the tangent method fails. But the programme automatically uses the  $\Delta$ -channel method for the sample if the tangent method fails to find an acceptable right end. The  $\Delta$ -channel of the sample is assumed to be equal to  $X_r - X_l$  of the corresponding peak of the standard spectrum.

*Area of the peak and its standard deviation.* The total area under the peak,  $C$ , with the low-energy bound  $X_l$  and the high-energy bound  $X_r$  is computed by the trapezoidal rule, and its standard deviation,  $\sigma_C$ , is given by  $\sqrt{C}$ . The Compton continuum,  $B$ , is defined to be the trapezoid under ( $X_l$ ,  $Y_l$ ) and ( $X_r$ ,  $Y_r$ ), and its standard deviation,  $\sigma_B$ , is given by  $\sqrt{B}$ . Note that  $Y_l$  is found by substituting  $X_l$  into equation (1), and that  $Y_r$  is found by substituting  $X_r$  into equation (3). The peak area of interest here,  $A$ , is found by subtracting  $B$  from  $C$ , and its standard deviation:

$$\sigma_A = \sqrt{\sigma_C^2 + \sigma_B^2}$$

This work was supported in part by the National Aeronautics and Space Administration under contract NASw-843.

<sup>1</sup> Schmitt, R. A., and Smith, R. H., *Abundances of Na, Sc, Cr, Mn, Fe, Co, and Cu in 18 Meteorites and 146 Individual Chondrules* (General Atomic Report GA-4997, Feb. 29, 1964).

<sup>2</sup> Heath, R. L., in *Proceedings of Conference on the Utilization of Multi-parameter Analyzers in Nuclear Physics* (Columbia University Report CU(PNPL)-227 (NYO-10595).

<sup>3</sup> For the complete details of the Fortran IV programme for the IBM-7044, see the Appendixes of General Atomic Report GA-5233.

## COVALENT RADII OF CERTAIN METAL IONS AND THE LENGTH OF THE METAL-METAL BONDS

By M. J. BENNETT and PROF. R. MASON

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**C**OVALENT radii of metal ions in unusual oxidation states. The availability of a number of accurate X-ray analyses of bond lengths in organometallic molecules appears to provide values for radii of metal ions of formal

oxidation state rarely met in transition metal complexes *per se*. The data required for the present analysis are summarized in Table 1; it is to be noted that in  $\pi$ -cyclopentadienyl derivatives, assignment of the formal oxida-

Table 1. BOND LENGTHS IN SEVERAL ORGANOMETALLIC MOLECULES

Metal	Molecule	Ref.	Bond	Bond length (Å)
Molybdenum	$\{\pi\text{-C}_5\text{H}_5\text{MoC}_2\text{H}_5(\text{CO})_3\}_2$	1	$\text{MoI} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.37 (avg.)
		2	$\text{MoI} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.40
	$\{\pi\text{-C}_5\text{H}_5\}_2\text{MoH}_2$ $\{\pi\text{-C}_5\text{H}_5\}_2\text{Mo}(\text{CO})_2$	3	$\text{MoIV} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.29 (avg.)
		2	$\text{MoI} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.35 (avg.)
Manganese	$\{\pi\text{-C}_5\text{H}_5\}_2\text{Mn}(\text{CO})_3$ $\{\pi\text{-C}_5\text{H}_5\}_4\text{Mn}(\text{CO})_3$ diars	4	$\text{MnI} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.155 (avg.)
		5	$\text{MnI} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.15 (avg.)
Cobalt	$\pi\text{-C}_5\text{H}_5\text{C}_5\text{H}_5\text{C}_5\text{H}_5\text{Co}$ $\pi\text{-C}_5\text{H}_5\text{C}_5\text{H}_5\text{C}_5\text{H}_5\text{Co}$	6	$\text{CoIII} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.07 (avg.)
		7	$\text{CoIII} \rightarrow \text{C}(\text{C}_2\text{H}_5)$	2.07 (avg.)

tion state of the metal ion relies on the  $\pi\text{-C}_5\text{H}_5$  anion being regarded as a six-electron donor. This procedure is entirely arbitrary but is acceptable provided self-consistency is maintained.

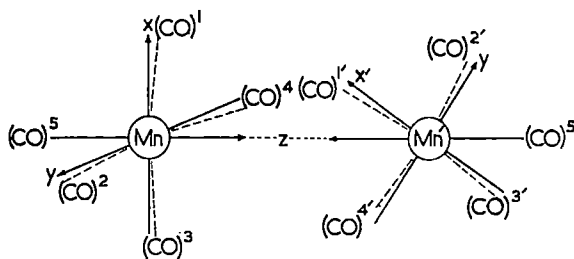
This limited survey immediately suggests that the 'covalent' radius of a cyclopentadienyl carbon is essentially identical ( $\pm 0.03$  Å) with that of a simple tetrahedral  $\sigma$ -bonded carbon atom. This is evidenced by the equality of molybdenum-carbon bond lengths in  $\pi\text{-C}_5\text{H}_5\text{MoC}_2\text{H}_5(\text{CO})_3$  (ref. 1) apart, that is, from the  $\text{Mo} \rightarrow \text{C}(\text{O})$  bond lengths, and by the bond lengths in the  $\text{CoIII}$  examples. The octahedral covalent radius of  $\text{Co}^{2+}$  can be estimated as  $1.30 (\pm 0.03)$  Å from the bond lengths in  $\text{CO}(\text{NH}_3)_3(\text{NO}_2)_2\text{Cl}$  (ref. 8), using a chlorine radius of  $1.00$  Å and a value of  $0.70$  Å for nitrogen, and as  $1.27$  Å from azidopentamine cobalt(III) azide<sup>9</sup>. The bond lengths of  $2.07$  Å in the  $\pi\text{-cyclopentadienyl}$  derivatives therefore suggest a 'covalent' radius for a cyclopentadienyl carbon as  $0.77\text{--}0.80$  Å. Clearly the simplicity of this 'hard-ball' approach implies a certain lack of generalization, but for certain metal configurations, in particular  $d^6$  with the exception of  $\text{Fe(II)}$ , the agreement is satisfyingly good. As such, we can calculate effective metal radii and these are listed in Table 2. Kilbourn and Powell<sup>11</sup> give a value of  $1.31$  Å for the manganese radius in *bis*(pentacarbonylmanganese) diphenyltin. Depending on one's view of the bonding in this complex, the oxidation state of the transition metal ion is  $+1$ ,  $0$  or  $-1$ . In all these cases, however, some shortening of the Mn-Sn bond distance may take place through  $d\pi\text{--}d\pi$  bonding, so that it is not surprising that the Kilbourn and Powell value is smaller than we suggest.

Table 2. RADII OF MOLYBDENUM AND MANGANESE IONS

Metal ion	Radius	Comments
$\text{MoIV}$	$1.52 \pm 0.03$ Å	Formally eight co-ordinate
$\text{MoI}$	$1.60 \pm 0.03$ Å	Formally seven co-ordinate
$\text{MnI}$	$1.38 \pm 0.03$ Å	Formally octahedral, identical with value derived from $[(\text{CO})_5\text{MnBr}]_2$ (ref. 10) using bromine radius of $1.14$ Å

**The length of metal-metal bonds.** The observed manganese-manganese bond length of  $2.93$  Å in  $\text{Mn}_2(\text{CO})_{10}$  (ref. 12) is not substantially greater than would be expected. A calculated bond length of  $2.76$  Å is obtained for  $\text{Mn(I)} \rightarrow \text{Mn(O)}$  and we should clearly anticipate a greater value for  $\text{Mn(O)} \rightarrow \text{Mn(O)}$ . Judged by bond length differences in other organometallic molecules, the change in metal radius following unit change of oxidation state is of the order of  $0.05$  Å, so that the sum of radii for  $\text{Mn(O)}$  is  $2.86$  Å. This value is only  $0.07$  Å less than the observed result and, while this difference must not be taken too literally in view of the unsophisticated methods by which it was obtained, it does seem clear that Cotton and Monchamp<sup>13</sup> were correct in finding it "difficult to believe" that a metal-metal bond stretched by about  $0.5$  Å could be as strong as  $34$  kcal/mole. The earlier suggestion<sup>3</sup>, that the Mo-Mo bond in the supersandwich  $\{\pi\text{-C}_5\text{H}_5\text{Mo}(\text{CO})_3\}_2$  is long, as a result of steric strain, has been questioned some time ago<sup>1</sup> on the basis of the similarity of its structure to that of  $\pi\text{-C}_5\text{H}_5\text{MoC}_2\text{H}_5(\text{CO})_3$  where such possible steric interactions must be absent.

**The nature of the metal orbitals in  $\text{Mn}_2(\text{CO})_{10}$ .** While, as was mentioned earlier, the precise value by which the

Fig. 1. Diagrammatic representation of the carbonyl arrangement in  $\text{Mn}_2(\text{CO})_{10}$ 

observed bond length in  $\text{Mn}_2(\text{CO})_{10}$  is greater than the sum of metal radii is open to doubt, it does seem clear that, taken with the structure of  $\{\pi\text{-C}_5\text{H}_5\text{Mo}(\text{CO})_3\}_2$ , we wish to explain a difference of the order of  $0.1$  Å. In  $\text{Mn}_2(\text{CO})_{10}$ , the two  $\text{Mn}(\text{CO})_5$  moieties are in a staggered conformation so as, one may presume, to minimize intramolecular repulsions between the non-bonded carbonyl groups. It is therefore surprising to find that the distances between these groups are decreased from what they would be in a perfectly symmetrical situation by the separate carbonyl groups bending towards one another (Fig. 1). Moreover, it is unlikely that such distortions can be explained as originating from, say, crystal packing effects, because an identical situation has been described as existing in  $\text{Mn}(\text{CO})_5\text{H}$  (ref. 14). We can investigate the effect of these small angular distortions on the Mn-Mn  $\sigma$  bond by considering the  $\text{Mn}(\text{CO})_5$  radical; the character of the orbital directed along the 'z' axis, housing the unpaired electron, is examined for two situations: (i) the manganese lies exactly in the plane defined by the four carbonyl groups, and (ii) the manganese and four carbons are non-coplanar in the sense that the angles between the orbital  $\chi_z$ , directed along the 'z' axis, the manganese and the four carbons are  $85^\circ$ —a value which represents the trend of the experimental results in  $\text{Mn}_2(\text{CO})_{10}$  and  $\text{Mn}(\text{CO})_5\text{H}$ .

The numbering of atoms is shown in Fig. 2.

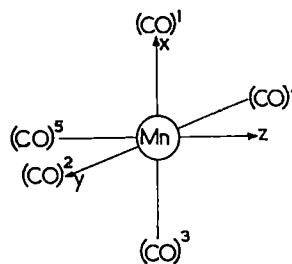


Fig. 2

To obtain some idea of the qualitative changes involved in going from the symmetrical to the unsymmetrical structure, we assume all carbonyl groups are equivalent. For the symmetrical structure the overlap matrix for the angular part of the wave functions only is as shown in Table 3.

Table 3

Metal orbitals	Ligand orbitals				
	$\varphi_1$	$\varphi_2$	$\varphi_3$	$\varphi_4$	$\varphi_5$
$s$	1	1	1	1	1
$p_z$	0	0	0	0	$-\sqrt{3}$
$d_{z^2}$	$-\sqrt{5}/2$	$-\sqrt{5}/2$	$-\sqrt{5}/2$	$-\sqrt{5}/2$	$\sqrt{5}$
$p_x$	$\sqrt{3}$	0	$-\sqrt{3}$	0	0
$d_{xz}$	0	0	0	0	0
$d_{yz}$	0	0	0	0	0
$p_y$	0	$\sqrt{3}$	0	$-\sqrt{3}$	0
$d_{x^2-y^2}$	$\sqrt{15}/2$	$-\sqrt{15}/2$	$\sqrt{15}/2$	$-\sqrt{15}/2$	0
$d_{zz}$	0	0	0	0	0

Table 4

Metal orbital	Ligand orbital				
	$\varphi_1$	$\varphi_2$	$\varphi_3$	$\varphi_4$	$\varphi_5$
$s$	1	1	1	1	1
$p_z$	$\sqrt{3} \cos 85^\circ$	$\sqrt{3} \cos 85^\circ$	$\sqrt{3} \cos 85^\circ$	$\sqrt{3} \cos 85^\circ$	$-\sqrt{3}$
$d_{z^2}$	$\sqrt{5}/2(3 \cos^2 85^\circ - 1)$	$\sqrt{5}/2(3 \cos^2 85^\circ - 1)$	$\sqrt{5}/2(3 \cos^2 85^\circ - 1)$	$\sqrt{5}/2(3 \cos^2 85^\circ - 1)$	$\sqrt{5}$
$f_x$	$\sqrt{3} \sin 85^\circ$	0	$-\sqrt{3} \sin 85^\circ$	0	0
$d_{xz}$	$\sqrt{15} \sin 85^\circ \cos 85^\circ$	0	$-\sqrt{15} \sin 85^\circ \cos 85^\circ$	0	0
$p_y$	0	$\sqrt{3} \sin 85^\circ$	0	$-\sqrt{3} \sin 85^\circ$	0
$d_{yz}$	0	$\sqrt{15} \sin 85^\circ \cos 85^\circ$	0	$-\sqrt{15} \sin 85^\circ \cos 85^\circ$	0
$d_{x^2-y^2}$	$\sqrt{15}/2 \sin^2 85^\circ$	$-\sqrt{15}/2 \sin^2 85^\circ$	$\sqrt{15}/2 \sin^2 85^\circ$	$-\sqrt{15}/2 \sin^2 85^\circ$	0
$d_{xy}$	0	0	0	0	0

The orbitals  $d_{xy}$ ,  $d_{xz}$  and  $d_{yz}$  are now orthogonal to the five ligand  $\sigma$  orbitals  $\varphi_1 \dots \varphi_5$ , and a further orthogonal orbital, in which the unpaired electron must be placed, can be readily constructed from a linear combination of the metals ' $s$ ', ' $p_z$ ' and ' $d_{z^2}$ ' orbitals. Orthogonality is satisfied by the wave function:

$$\chi = \sqrt{5}/2 s + d_{z^2} + \sqrt{15}/2 p_z$$

which on normalization gives:

$$\chi_z^1 = 0.456s + 0.408d_{z^2} + 0.791p_z \quad (1)$$

The equivalent orbital matrix can be set up for the unsymmetrical structure (Table 4).

As before,  $d_{xy}$  is orthogonal to the ligand orbitals but  $d_{xz}$  and  $d_{yz}$  must be mixed with  $p_y$  and  $p_z$  to become orthogonal and hence the other non-bonding orbitals. The required linear combinations are:

$$\psi_2 = \frac{d_{xz} - \sqrt{5} \cos 85^\circ p_x}{(1 + 5 \cos^2 85^\circ)^{1/2}}$$

and:

$$\psi_3 = \frac{d_{yz} - \sqrt{5} \cos 85^\circ p_y}{(1 + 5 \cos^2 85^\circ)^{1/2}}$$

giving the three orthonormal non-bonding orbitals as:

$$\chi_1 = d_{xy}$$

$$\chi_2 = 0.982d_{xz} - 0.191p_x$$

and:

$$\chi_3 = 0.982d_{yz} - 0.191p_y$$

The fourth orthogonal orbital is built up as before from the  $s$ ,  $p_x$  and  $d_{z^2}$  orbitals:

$s$	1	1	1	1	1
$p_x$	0.151	0.151	0.151	0.151	-1.732
$d_{z^2}$	-1.093	-1.093	-1.093	-1.093	2.236

and the required normalized orbital is:

$$\chi_z^{11} = 0.378s + 0.803p_x + 0.460d_{z^2} \quad (2)$$

We can see therefore that the angular distortions of the carbonyl groups from strict square pyramidal symmetry about the manganese ion lead to an increase in the ' $p$ ' and ' $d$ ' character in the orbital directed along the axis of the metal-metal ' $\sigma$ ' bond in  $Mn_2(CO)_{10}$ . While in this molecule the sense of the distortion would be thought to have the consequence of increasing repulsions between the non-bonded carbonyl groups, repulsion terms will be effectively reduced by: (i) the Mn-Mn bond length being greater as a result of the new hybrid orbital having greater ' $p$ ' and ' $d$ ' character, and (ii) the mixing of ' $p$ ' character

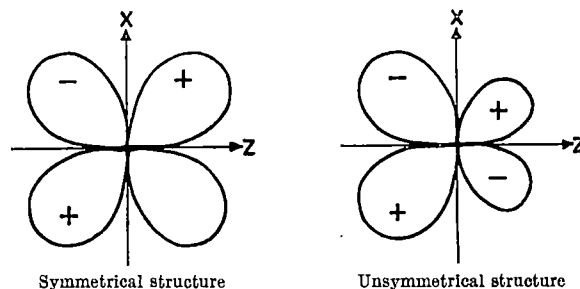


Fig. 3

into the non-bonding orbitals  $d_{xz}$  and  $d_{yz}$  decreases repulsions between these orbitals (Fig. 3).

A far less satisfactory calculation can be made for the 'supersandwich'  $\{\pi-C_5H_5Mo(CO)_3\}_2$  since the assumption of equality of ligand groups around the metal ion cannot be made in any reasonable sense. An application of the maximum overlap criterion to the structure of  $\pi-C_5H_5C_2H_5-Mo(CO)_3$  (ref. 1) does, however, indicate that the metal orbitals used for bonding to the doubly degenerate  $e$  orbitals of the cyclopentadienyl anion, which formally are the degenerate  $d_{xz}$  and  $d_{yz}$  orbitals (or hybrids), are not degenerate. They contain differing amounts of ' $d$ ' and ' $p$ ' character, a feature which explains the observed lack of five-fold symmetry in the bonded cyclopentadienyl ligand<sup>15</sup>.

Two points need emphasis. The derivation of metal radii from the  $\pi$ -cyclopentadienyl complexes is a simplification which must not be relied on to provide accurate values; rather, it is the trend among a related class of compounds which is instructive. A shortcoming of our overlap analysis is clearly connected with the use of the angular part of the metal wave functions only; this is not serious here, we feel, since the method attempts to derive differences between structures which are very similar. The method cannot be used to obtain precise orbital coefficients until such factors as the  $\pi$ -bonding of the ligands, effective nuclear charge of the metal and so on are taken into account.

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# TENSILE STRENGTH OF INDIVIDUAL COMPRESSION WOOD FIBRES AND ITS INFLUENCE ON PROPERTIES OF PAPER

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A LIMITED amount of evidence is available to indicate that compression wood fibres produce pulps which are significantly low in most strength properties compared with pulp produced from normal wood<sup>1,2</sup>. The influence is particularly striking in the case of the tearing resistance of papers. Tearing resistance has been related in the past to fibre length and to fibre stiffness<sup>3-5</sup>, while the breaking length, burst factor and fold endurance are determined primarily by the bonding capacity of the fibres and, as such, by the flexibility of the fibres as determined by their diameter, cell wall thickness and density.

Within the last few years the hypothesis has been presented that the tensile strength of the individual fibres is important in determining most of the strength properties of the paper<sup>6-9</sup> with a possibly greater significance with regard to tear than to breaking length, burst and fold<sup>6,8</sup>. It was considered that this hypothesis could be readily tested on the known large differences in some of the strength properties between normal wood pulp and compression wood pulp. This procedure is preferred to the selection of different species, for, although this would provide a wide range in tear and breaking length, it would introduce larger variations in fibre length, diameter, wall thickness and density than is obtained in the comparison of normal and compression wood of a single species. In addition, new information on the relative strength of normal and compression wood fibres would be obtained.

The compression wood in four logs, each from a different species, was separated from the normal wood and pulped separately, using a kraft liquor. Each log contained approximately 30 growth rings and, since the compression wood was generally restricted to the outer rings, the first 15 rings from the pith of the normal wood was discarded, thus rendering the pulp samples more comparable in anatomical and chemical properties. Since the lignin content influences both the fibre strength<sup>9</sup> and the physical properties of the paper<sup>10</sup>, all the pulps were cooked to a

Kappa number of 25. This entailed cooking the compression wood either for a longer period or with an increase in the percentage of active alkali from 20 to 25.

The pulp properties of the normal and compression wood are presented in Table 1. The tear factor (T.f.) of the compression wood pulp is substantially lower (30 per cent) than the normal wood pulp. The breaking length (B.L.) and burst factor (B.f.) of normal and compression wood pulps are comparable, but compression wood pulp does not develop so well on beating and there is an appreciable difference in the strength properties of the beaten pulps. The air-resistance factor (A.R.f.) of both unbeaten and beaten pulps from the compression wood is higher by about 50 per cent; this is not reflected in the freeness.

Fifty fibres from each of the nine samples were mounted on paper tags with ethylhydroxyethyl cellulose by a method slightly modified from that of Hartler, Kull and Stockman<sup>11</sup>. The fibres were strained in a controlled environment on an Instron tensometer with a rate of loading of 0.005 in./min. Approximately 50 per cent of the fibres failed near the point of gluing, but a 't' test indicated no significant difference in the strengths of fibres failing in the free zone and near the glue. The cross-sectional area of the cell wall was determined on 50 fibres removed from each of the same fibre suspensions as were the fibres that were strength-tested. This technique is considered to be adequate, since the prime consideration is the relative cell wall area of the various fibre samples.

The tensile strengths are recorded in Table 1, and the values for normal wood fibres agree well with the limited amount of published data<sup>7,12</sup>. The strength of the compression wood fibres is appreciably lower (46 per cent) than that of the normal wood and, of the fibre properties measured, only the degree of polymerization of the cellulose, as indicated by viscosity measurements (Tappi Standard T206 with 0.5 per cent solution), varies in a

Table 1. COMPARISON OF FIBRE AND PAPER PROPERTIES FROM NORMAL AND COMPRESSION WOOD

Species	Fibre properties						Cooking		Pulp properties											
	l (mm)	d (μ)	w (μ)	2w d	Vis- cosity (cP)	Tensile strength (kg/mm <sup>2</sup> )			Unbeaten					Beaten						
							Time (min)	Alkali (%)	Bulk	T.f.	A.R.f.	B.f.	B.L.	C.S.F.	Bulk	T.f.	A.R.f.	B.f.	B.L.	
<i>Tsuga hetero- phylla</i>	N	2.44	27.2	2.70	0.181	9.09	50.0	195	20	1.66	167	10	47	6,470	670	1.34	85	660	95	12,500
	C	2.26	26.8	3.08	0.230	6.58	28.9	150	25	1.62	120	20	50	6,540	610	1.30	84	1,200	86	11,450
<i>Picea sit- chensis</i>	N	3.45	30.5	3.19	0.208	10.64	70.5	90	20	1.90	172	3	56	8,170	690	1.42	130	650	98	12,850
	C	3.24	31.2	3.52	0.226	7.35	37.4	90	25	1.61	120	12	58	8,200	660	1.37	110	1,100	83	10,950
<i>Pseudot- suga</i>	N	2.83	31.4	4.23	0.270	9.71	46.6	90	20	2.05	175	2	34	5,720	700	1.52	158	520	82	10,550
	C	2.78	31.3	4.11	0.263	6.49	34.8	100	25	1.82	135	4	47	6,306	690	1.48	124	1,013	75	9,270
<i>taxifolia</i> <i>Picea abies</i>	N	2.72	36.4	3.33	0.210	10.87	54.8	105	20	1.77	151	15	56	8,450	680	1.40	112	1,112	91	13,020
	C	2.64	31.8	4.05	0.255	8.00	35.6	210	20	1.66	108	18	51	7,760	700	1.36	90	1,528	70	10,900
	C	2.64	34.0	3.71	0.219	7.30	38.7	80	25	1.67	124	19	53	8,100	680	1.40	100	1,164	77	10,830
Average	N	2.86	3.14	3.36	0.217	10.00	55.5			1.84	166	7.5	48	7,220	655	1.42	121	736	91	12,230
	C*	2.73	3.06	3.64	0.239	6.94	34.6			1.68	123	13.8	52	7,244	663	1.38	103	1,165	80	10,650
% Dif- ference†		-4.6	-2.5	+8.0	+9.6	-36	-46			-9.1	-30	+59	+8.0	+0.3	-3.3	-2.9	-16	+45	-13	-11

N, normal wood; C, compression wood; l, fibre length; d, fibre diameter; w, wall thickness of fibre. C.S.F., Canadian Standard Freeness.

\* Two samples of *P. abies* were averaged before calculating the mean of four samples.

† Difference/mean per cent.

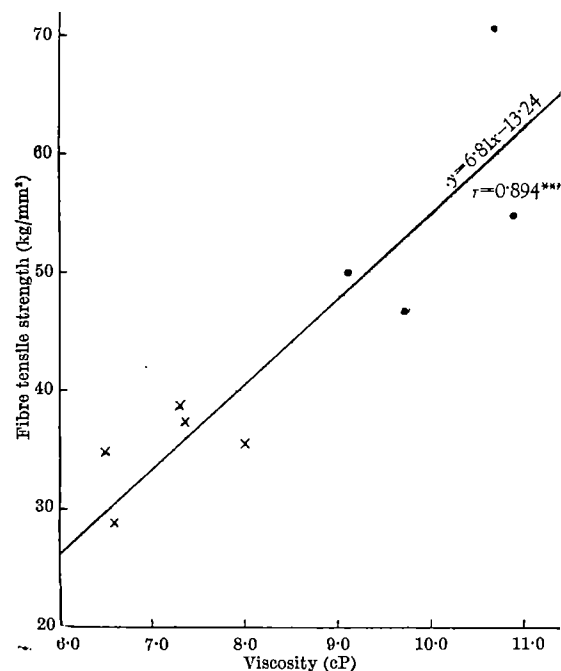


Fig. 1. The relationship between the tensile strength of individual fibres of normal (●) and compression (×) wood and the viscosity of their cellulose. \*\*\*, Significant at the 0.1 per cent level

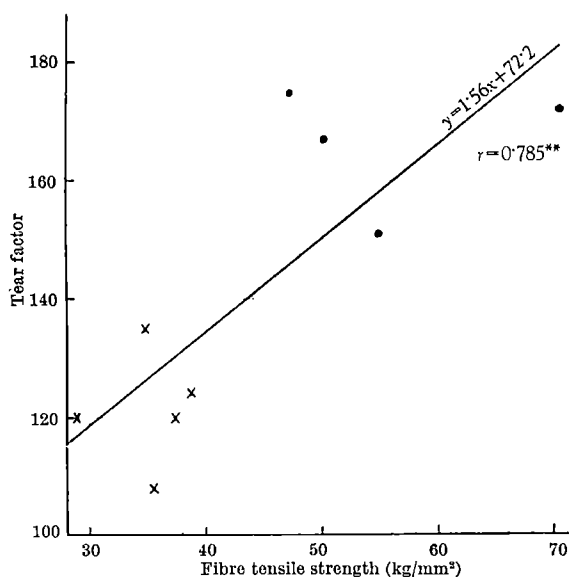


Fig. 2. The dependence of the tear factor of unbeaten pulp on the tensile strengths of the individual fibres. ●, Normal wood; ×, compression wood. \*\*, Significant at the 1 per cent level

manner which may explain the difference. The relationship between the fibre tensile strength and the viscosity of the cellulose is presented in Fig. 1, while Fig. 2 shows the relationship between the tearing resistance of the paper and the tensile strengths of the individual fibres.

Bonding can only increase the breaking length and burst where there is adequate fibre strength, and it would appear that this latter property is the limiting factor in the development of the beaten strength of the compression wood pulp (Table 1). Since the breaking length and burst of the unbeaten compression wood pulp are similar to those of the normal wood, it would appear that, at least in long-fibred low-density pulps, tearing resistance is more sensitive to variation in fibre strength than are breaking length and burst.

The considerably higher air-resistance of the compression wood paper is probably associated with factors modified during sheet formation, such as the degree of fibre collapse, since the freeness of the pulp suspensions is unaffected, and it is extremely unlikely that fibre strength *per se* influences the resistance of the fibre web to the passage of air.

It could, however, be argued, from the experimental evidence which has been described, that the decrease in viscosity, which resulted in a decrease in the tear factor of the compression wood, is the result of the more severe cooking conditions of the compression wood samples.

Evidence is available to indicate that degradation of the cellulose does occur in kraft cooking at 160°–170° C<sup>14,15</sup>, but it would appear that most of this occurs in the initial stages, after which the degree of polymerization decreases only slowly<sup>14–16</sup>. From the work of Ahlm and Leopold<sup>16</sup> on the cooking of loblolly pine (*Pinus taeda*), the decrease in degree of polymerization with increasing cooking time from 105 min to 210 min (see Table 1) was only 14 per cent, while McIntosh<sup>17</sup> records no difference during this increase in cooking time. Dadswell, Wardrop and Watson<sup>2</sup> found no difference in the strength properties of compression wood samples cooked for the same time but with active alkali concentrations of 20 and 25 per cent, and Stone and Clayton<sup>18</sup> record only a slight decrease in fibre strength with decrease in lignin content. Thus, while the possibility that the decrease in viscosity of the compression wood fibres is induced by the slight differences in cooking conditions cannot be eliminated, the evidence is such as to indicate the probability that the greater part of the viscosity difference is inherent. The nature of the investigation did not permit cooking time, liquor concentration and lignin content to be held constant simultaneously.

Substantiating evidence of the importance of fibre strength in determining the tearing resistance of paper is available from recorded work<sup>7,13</sup> and is supported by some of our own unpublished work. In sulphate pulps there is an increase in the number of fibres broken in the tear tests compared with breaking-length tests, inferring a closer and more critical relationship between fibre strength and tear than between fibre strength and breaking length.

It may be concluded from this limited investigation that there is a high probability that the tensile strength of individual compression wood fibres is considerably lower than that of normal fibres. This appears to be related to a decrease in the degree of polymerization of the compression wood fibres. Another possible explanation for the lower strength values of the compression wood fibres is the frequent spiral fissuring of the cell wall.

The results obtained support the view that individual fibre strength is of prime importance in determining the tearing resistance of paper. It is also significant in determining the breaking length and burst factor, but only in paper from beaten pulp.

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## DIFFUSION OF PHOSPHATE TO PLANT ROOTS

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SCHOFIELD<sup>1</sup> has pointed out the necessity of considering both capacity and intensity factors when assessing the availability of plant nutrients. Plant growth, however, is a dynamic process in which ion transport phenomena are involved, both within the soil and within the plant. Nutrient transport in the soil can occur as mass flow of the soil solution and also by diffusion. Opinion has been divided as to the relative importance of each of these mechanisms, particularly in regard to phosphate<sup>2-5</sup>. Strong evidence that diffusive flow can supply all the phosphate required by a growing plant has now been obtained.

The accumulation of phosphate by wheat plants has been followed as a function of time over a complete growth cycle, and the rate of uptake compared with a computed uptake if diffusion alone contributed. The soil used for both pot experiments and laboratory investigations was the Seddon sandy loam for which some data on phosphate retention have been obtained by Kanwar<sup>6</sup>. Phosphate additions of 150, 300 and 600  $\mu\text{g P/g}$  soil were made to bulk samples of soil by spraying on appropriate amounts of calcium phosphate solution, followed by drying and thorough mixing. Wheat plants were grown under conditions of controlled environment in small pots of these soils and the plant tops were collected and analysed at intervals of two weeks from sowing to maturity (22 weeks). The rate of phosphate uptake was almost constant during the period 2–12 weeks and the mean rates for the three phosphate-levels were 2.7, 5.6 and 12.0  $\mu\text{g P/plant/h}$ .

For the calculation of phosphate supplied to the root by diffusion, a physical model was adopted in which the root was considered to be a cylindrical sink towards which phosphate ions would diffuse under the influence of a concentration gradient induced by active uptake at the root surfaces; that is, flow would be radial at all times from an infinite unstirred medium of uniform initial phosphate concentration  $P_0$ . Since the concentration of phosphate near the root surface could not be measured, some estimate was necessary to enable mathematical calculations to be made. In this work the boundary condition imposed was that the phosphate concentration at the root surface decreased exponentially with time from the initial value  $P_0$ .

For this system a general diffusion equation can be written as follows, using cylindrical polar co-ordinates:

$$\frac{\partial C}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( r D \frac{\partial C}{\partial r} \right) \quad (1)$$

valid for  $a \leq r \leq \infty$ ,  $t \geq 0$ , where  $C = C(r, t)$  = concentration of phosphate for any time 't' at a distance 'r' from the centre of the root;  $D$  = diffusion coefficient;  $a$  = root radius.

As no published data were available concerning the diffusion of phosphate in soil, experiments were carried out to evaluate the diffusion coefficient for Seddon soil using different levels of added phosphate. The techniques described by us elsewhere<sup>7</sup> were used to determine the self-diffusion of <sup>32</sup>P in samples of soil to which  $\text{KH}_2\text{PO}_4$  solution had been added to give phosphate additions ranging from 100 to 2,000  $\mu\text{g P/g}$  soil. The soils were stored at 20 per cent water content for 7 days before the diffusion cells were assembled. The measured diffusion coefficient was calculated from the amount of <sup>32</sup>P transported across the boundary after 6 days' contact. The calculated

diffusion coefficients were very small, ranging from approximately  $2 \times 10^{-10} \text{ cm}^2/\text{sec}$  for the lowest addition to  $4 \times 10^{-8} \text{ cm}^2/\text{sec}$  after addition of 2 mg P/g soil.

Over this range there was a very marked curvilinear relationship between diffusion coefficient and level of phosphate addition, which could be described approximately by:

$$D = KP^2 \quad (2)$$

where  $D$  = measured diffusion coefficient of the phosphate ion in the porous medium;  $K$  = constant;  $P$  = concentration of added phosphate ( $\mu\text{g P/g}$  soil). Since the measured diffusion coefficient is not constant, it is not possible to give a general solution to the diffusion equation (1) given here, but numerical solutions can be obtained for specific initial concentrations and prescribed boundary conditions.

On substituting relation (2) into equation (1) a non-linear equation results which, subject to the imposed boundary conditions, was solved by numerical methods (using an IBM '1620' computer) to give the change in concentration at any point out from the surface of the root with time. Calculations were performed for three levels of added phosphate (150, 300 and 600  $\mu\text{g P/g}$  soil) and two different root dimensions (0.2 and 0.005 mm) corresponding to the observed radii of the main root and the root hairs. Subsequent integration of each set of results around the root axis gave the cumulative change in concentration per unit length of root with time, that is, the mass of phosphate which had moved from the soil to the root surface as a result of diffusion. The mean uptake rates for the different root dimensions at the 3 levels of phosphate are given in the table.

Table 1. PHOSPHATE UPTAKE RATES: COMPUTED AND MEASURED

Computed uptake ( $\mu\text{g P/m/h}$ )	Phosphate addition ( $\mu\text{g P/g}$ soil)		
	150	300	600
For main root	0.074	0.33	1.57
For root hairs	0.0084	0.034	0.13
For proposed model root*	0.494	2.03	7.97
Measured uptake ( $\mu\text{g P/plant/h}$ )	2.7	5.6	12.0
Required length of model root (m/plant)	5.5	2.8	1.5

\* Model root proposed had 50 mm of root hair per mm of main root.

To convert these data to total plant uptake a model of the nutrient-absorbing root must be used. On the basis of microscopic observation of the root systems of wheat plants grown in the Seddon soil (Fig. 1) a model root was adopted, in which every mm of main root has attached to it 50 root hairs, each of an average length of 1 mm, that is, each mm length of root is associated with 50 mm of root hairs. The total uptake rates per unit length of model root for each of the 3 levels of added phosphate are also given in Table 1, together with the mean uptake rate per plant determined in the pot experiment.

By comparing the computed rate of uptake per unit length with the measured uptake rate of the growing plant, the length of model root required to achieve the measured uptake can be calculated, assuming diffusive movement to the root surface were totally responsible. The calculated lengths shown in Table 1 must be recognized as minimum values of actively absorbing root under ideal moisture conditions. The lengths required (2–6 m per plant) are not excessive and may be even less if the root hair numbers or lengths are greater than the estimates used. Field measurements of root lengths of wheat plants indicate values ranging from 32 m (ref. 8) to 0.5 mile (ref. 9) when sown in drills, and up to 50 miles for isolated plants<sup>9</sup>. These suggest that the values

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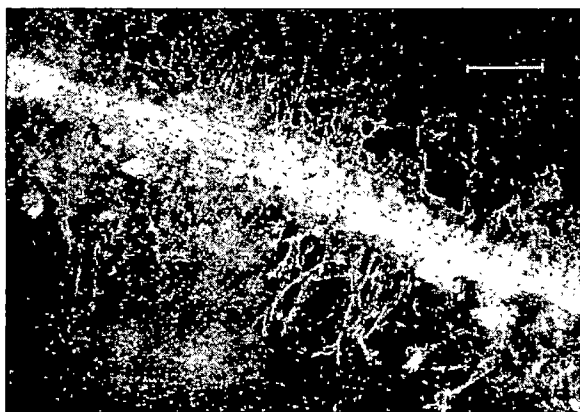


Fig. 1. Photomicrograph of primary root and associated root hairs of a wheat plant grown in Seddon soil after addition of  $150 \mu\text{g P/g}$  soil. Reference line is 0.5 mm

obtained would represent only a small fraction of the total root system.

At maturity the plants in the uptake experiment had a root volume of 7–12 c.c./plant which would correspond to 50–90 m of model root per plant. Fawcett<sup>10</sup> conducted

similar pot experiments and after 6 weeks growth found oven-dry root masses of 0.67 g per plant which would correspond to 15–17 m of model root. At the 6-week stage, the plants would be absorbing phosphate at the mean rate given in the table and so about 40 per cent of the calculated length would need to be active at the lowest P addition or only 7 per cent at the highest level. These results suggest that for the ideal model systems considered, diffusion alone could supply the phosphate absorbed by plants. In practice the conditions are less than ideal, for example, where the water content is less than saturated it is probable that the diffusion coefficient would be lower. On the other hand, it is also likely that older tissue continues to be active in uptake, although perhaps not so efficiently as newer tissue near the root tip.

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## THE MOLECULAR PHYSIOLOGY OF CITRATE

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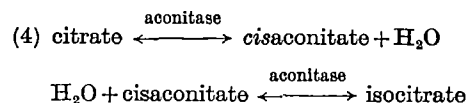
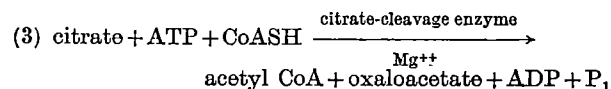
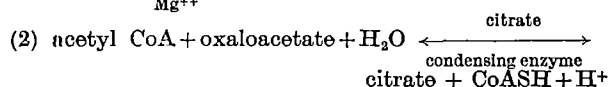
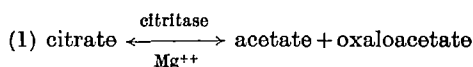
THE term molecular physiology might be applied to those metabolic investigations that relate to the function and control of biological compounds and the reactions they undergo. Thus for a particular substance an investigation of the intracellular and tissue distribution and of the enzymes that catalyse its reactions could yield information on its function. Using this approach, I have reviewed some of the recently reported information on citrate biochemistry in animal tissues.

Citrate has always occupied an important position in metabolism and several recent observations have added new dimensions to its role in living processes. A significant observation was that of Brady and Gurin<sup>1</sup>, who found that the rate of fatty acid synthesis by a crude soluble enzyme system was greatly stimulated by citrate. This observation eventually led to the recognition of three metabolic functions for citrate in addition to its role in the Krebs cycle and ATP production: a source of reducing power, a source of acetyl groups for biosynthetic pathways and a controlling (activating or inhibiting) substance for a number of enzymes.

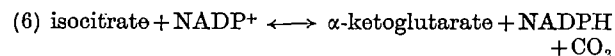
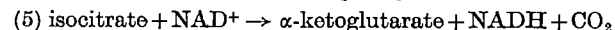
It is the purpose of this report (1) to review and discuss the data relating to these recently described functions, (2) to present some data concerning enzymes of citrate metabolism in bone, the tissue with the highest citrate content, and (3) to consider several of the problems of citrate metabolism and its control. No attempt will be made to review the literature concerning citrate metabolism in plants and bacteria.

### Metabolic Functions of Citrate

Only four enzymes are known to catalyse chemical conversions of citrate.



In addition, it is useful to consider the NAD-dependent isocitrate dehydrogenase and NADP-dependent isocitrate dehydrogenase as citrate-utilizing enzymes:



Citritase has thus far been found only in bacteria and will not be considered here.

Fig. 1 shows the intracellular distribution of enzymes known to be associated with citrate metabolism in animal tissues. The cytoplasmic citrate-cleavage enzyme is the most recent addition<sup>2</sup>. The occurrence of aconitase both in mitochondria and cytoplasm<sup>3</sup>, as well as the distinction between the mitochondrial NAD-requiring isocitrate dehydrogenase and the cytoplasmic NADP-requiring isocitrate dehydrogenase<sup>4</sup>, are recently accepted ideas. This unique localization pattern for citrate-metabolizing enzymes invites speculation concerning the function of citrate in the cytoplasm.

Let us first consider the evidence and reasoning that support the role of citrate as a hydrogen donor for biosynthetic reactions. The oxidation of citrate in the mitochondria leads to the production of ATP and not to the production of reducing hydrogen available for synthetic purposes. The dehydrogenases coupled to oxidative phosphorylation are NAD-coupled enzymes; once the electrons are transferred to NAD they are transferred in turn to  $\text{O}_2$  with the concomitant formation of ATP. No data are available concerning the transport of NAD(H) or NADP(H) out of mitochondria. Lehninger<sup>5</sup> has shown

Mitochondria	Cytoplasm
Citrate-condensing enzyme	Citrate-cleavage enzyme
Aconitase	Aconitase
NAD isocitrate dehydrogenase	NADP isocitrate dehydrogenase

Fig. 1. Distribution of some enzymes concerned with citrate metabolism

that NADH enters mitochondria very slowly, if at all, unless they are first treated with distilled water with resultant swelling and altered permeability. In addition, Purvis and Lowenstein<sup>6</sup> have shown that nucleotides are transported slowly into mitochondria. Since the reducing potential of mitochondrial metabolism is all converted to ATP and since reductive syntheses usually involve NADPH and occur in the cytoplasm, we must examine the cytoplasm for sources of NADPH. It is doubtful if it can still be claimed that the aconitase or isocitrate dehydrogenase found in the cytoplasm is mitochondrial in origin, so it is probable that these enzymes function to supply hydrogen for cytoplasmic syntheses. Another source of NADPH in the cytoplasm is from the oxidation of glucose-6-phosphate by the enzymes of the so-called 'shunt' pathway<sup>7</sup>.

One cytoplasmic biosynthetic pathway that requires a source of NADPH is the fatty acid synthesizing system. After the work of Brady and Gurin<sup>4</sup> it was shown by several laboratories that other NADPH-generating systems could partially replace the citrate-activating effect on fatty acid biosynthesis. However, in a crude system capable of generating as much NADPH from glucose-6-phosphate as from citrate, six times more fatty acids were made in the presence of citrate than in the presence of glucose-6-phosphate<sup>7,8</sup>. The greater efficiency of the citrate-reducing system may be due to the ability of citrate to activate an enzyme involved in fatty acid synthesis as will be discussed later on.

A second major function for citrate lies in its ability to supply acetyl groups for biosynthetic purposes in a reaction catalysed by the citrate-cleavage enzyme<sup>9</sup>. When the cleavage enzyme was first described<sup>10</sup>, it was believed that it might function as an energetically more economical way of forming citrate. In citrate formation catalysed by the citrate-condensing enzyme reaction, the energy contained in the acetyl-CoA bond is lost, whereas in the reverse of the citrate-cleavage enzyme reaction, the energy of that bond would be conserved as 1 mole of ATP. Later observations<sup>11</sup>, however, showed that though the free energy change for the cleavage reaction was zero, no synthesis of citrate could be detected under a variety of conditions.

The citrate-cleavage enzyme is found exclusively in the cytoplasm, and since it is unable to catalyse the formation of citrate, its function is probably related to its ability to form acetyl-CoA or oxaloacetate<sup>10</sup>. We have pointed out<sup>9,12</sup> that the source of acetyl groups for cytoplasmic syntheses of cholesterol and fatty acids has not been adequately investigated. For the most part, the cell produces its acetyl groups in mitochondria in the reaction catalysed by pyruvate oxidase and utilizes them for syntheses in the cytoplasm. The problem then resolves to the transfer of acetyl groups from mitochondria to cytoplasm. Four possible mechanisms are illustrated in Fig. 2.

The first mechanism shown in Fig. 2 is unlikely; transfer of acetyl-CoA *per se* across the mitochondrial membrane would entail the transport of free CoA back into the mitochondria and, as has already been pointed out here, the available evidence indicates that nucleotide transfer across the mitochondrial membrane is poor. In a discussion on acetyl group transfer, Lowenstein has commented that the rate of diffusion of CoA and its derivatives across the mitochondrial membrane is slow<sup>13</sup>.

The second possibility for acetyl group transfer involves deacylation of acetyl-CoA to acetate, followed by transport of acetate into the cytoplasm where it is reconverted to acetyl-CoA in the reaction catalysed by the cytoplasmic acetate-activating enzyme. The operation of such a mechanism has been suggested by Wieland and Weiss<sup>14</sup>, in connexion with their recent finding that the free acetate concentration of rat liver is 400  $\mu$ moles/g wet weight, 20 times that of acetyl-CoA.

A third possible route of acetyl group transfer is based on the activity of carnitine acetyltransferase: acetyl-CoA in the mitochondria forms acetyl carnitine which is transported into the cytoplasm where cytoplasmic acetyl-CoA is regenerated via the same enzyme<sup>15</sup>. This is an attractive hypothesis because, unlike the second mechanism, it involves no loss of energy. There is good evidence indicating that carnitine acetyltransferase plays a part in the transfer of acetate into mitochondria resulting in increased acetate oxidation on addition of carnitine<sup>16</sup>. Attempts to establish this mechanism as operating in the transfer of acetate out of mitochondria have not been conclusive. We have shown that acetate incorporation into fatty acids *in vitro* can be stimulated by addition of mitochondria<sup>17</sup>. However, when we used an *in vitro* system containing the soluble fatty-acid-synthesizing enzymes and mitochondria to test the effect of carnitine on the incorporation of labelled pyruvate into fatty acids, no effect was observed<sup>18</sup>. On the other hand, Bressler and Katz<sup>19</sup> have reported that the *in vivo* synthesis of long-chain fatty acids by guinea-pig liver and epididymal fat pad from pyruvate-2-<sup>14</sup>C is augmented five- to ten-fold by the feeding of carnitine.

The fourth mechanism illustrated in Fig. 2 is one in which the citrate-condensing enzyme and the citrate-cleavage enzyme act together as an acetyl group carrier. We have pointed out<sup>9</sup> that the function of this system is consistent with the intracellular and tissue distribution of the two enzymes. High concentrations of citrate-cleavage enzyme occur in those tissues that have a high capacity for acetyl group utilization: extracts of rat liver, with a high capacity for fatty acid synthesis, and rat brain, which uses citrate for acetylcholine synthesis, show moderately high specific activities of this enzyme<sup>11</sup>. Recently, Lowenstein's group<sup>20,21</sup> showed that altered nutrition in the rat changes the activity of its citrate-cleavage enzyme in liver and its capacity for fatty acid synthesis in the same direction. Along the same line,

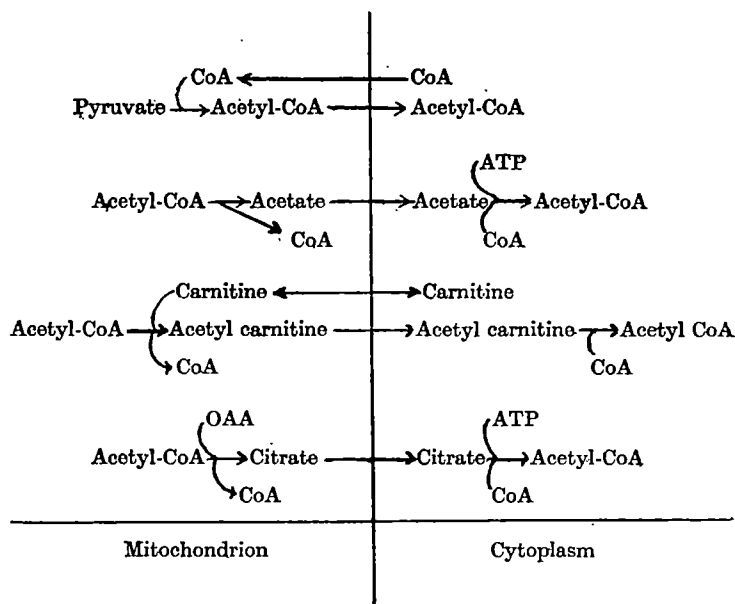


Fig. 2. Schematic representation of four possible mechanisms of acetyl group transfer out of a mitochondrion

Abraham<sup>22</sup>, in an investigation of the lactation cycle of the rat mammary gland, demonstrated that the rate of fatty acid synthesis and activity of the citrate-cleavage enzyme increased and decreased concurrently. Several observations, however, are in contradiction to this hypothesis involving citrate transport for acetyl group transfer. All direct evidence would indicate that the mitochondrial membrane is relatively impermeable to citrate in both directions. Schneider *et al.*<sup>23</sup> showed that the mitochondria contained 70 per cent of the citrate of the cell and that this concentration is not reduced by repeated washing of isolated mitochondria. It is frequently observed that citrate is one of the poorest substrates for mitochondrial oxidation<sup>24</sup>. In experiments in our laboratory no extra-mitochondrial citrate was formed in a system containing labelled pyruvate and pigeon liver mitochondria. Yet citrate does exist in the cytoplasm and some mechanism must place it there—perhaps in some tissues its chief source is from the blood. There is no direct evidence for any of these four mechanisms for transfer of acetyl groups out of mitochondria, and it may well be that all are operative to varying extents depending on the tissue and the physiological state of the animal.

Whatever the mechanism for the transfer of acetyl groups from mitochondria to cytoplasm, it is clear that citrate is as good a precursor for acetyl groups in tissues as is acetate. Lipton and Barron<sup>25</sup> first demonstrated this in experiments in which a crude brain extract catalysed the formation of acetylcholine from citrate, choline, ATP and a yeast factor (CoA). A number of experiments indicate that citrate is an excellent precursor of acetylcholine in brain<sup>26</sup> and in insects<sup>27</sup>. This conversion of citrate to acetylcholine was first thought to be mediated through a reversal of the reaction catalysed by citrate-condensing enzyme, but its requirement for ATP clearly indicates operation of the cytoplasmic citrate-cleavage enzyme in the system. At a suggestion of Dr. Charles West and Dr. Werner Seubert we were able to show that citrate carbon is incorporated rapidly into fatty acids in a pigeon liver system<sup>9</sup>. Similar observations have been reported by several laboratories<sup>28,29</sup>.

Although it has been generally assumed that the conversion of amino-acids such as glutamate to precursors of fat and carbohydrate is mediated by the mitochondrial enzymes of the Krebs cycle, recent data from several laboratories<sup>30-32</sup> indicate that extramitochondrial conversion of glutamate to fat or carbohydrate can occur. The results of isotopic experiments indicate conversion of glutamate to citrate with the subsequent enzymatic cleavage of citrate. By this pathway protein could be converted to fat or carbohydrate without involvement of the Krebs cycle enzyme system. It is interesting to note that the reaction catalysed by the NAD isocitrate dehydrogenase (mitochondrial) cannot be reversed whereas the reaction catalysed by the cytoplasmic NADP isocitrate dehydrogenase is reversible<sup>33</sup>, as is required by the proposed 'reversal' of the Krebs cycle.

The important observation that citrate can act as an activator for acetyl-CoA carboxylase<sup>34</sup> uncovered a third biological role for this compound. Vagelos *et al.* have presented convincing evidence that this activation is attended by a concomitant three-fold increase in the sedimentation coefficient of the enzyme<sup>35</sup>. It is not yet established whether this conformational change represents a physiologically occurring process. It has been reported<sup>36</sup> that tricarballoylate causes trimerization of the carboxylase but no concomitant activation. It is disturbing also that an unusually high concentration of citrate, 0.01 M, is required for activation.

Citrate alters the activity of at least two other enzymes, phosphofructokinase<sup>37,38</sup>, and isocitrate dehydrogenase<sup>36</sup>. The phosphofructokinase reaction is known to be a rate-limiting step in glycolysis, hence this interaction may have important implications in the control of metabolism. Purified phosphofructokinases from a number of sources

are inhibited by citrate. The inhibition occurs at physiological levels of citrate ( $2 \times 10^{-4}$  M) and is reversed by the addition of substrates and other activators of the enzyme. (Since this review was written, a number of reports have appeared that present additional data on the role of citrate in metabolic regulation. The article by J. R. Williamson, E. A. Jones and G. F. Azzone (*Biochem. Biophys. Res. Comm.*, 17, 696; 1964) summarizes this more recent work.) Isocitrate dehydrogenase has been shown to be activated by citrate; the activation is highly specific for citrate<sup>39</sup>.

### Citrate Formation in Bone Tissue

Of all the animal tissues, bone has the highest citrate content<sup>40</sup>. The relationships between bone, citrate and calcium have been intensively investigated and well reviewed<sup>41-45</sup> and will not be discussed at length here. Surprisingly few determinations of citrate-condensing enzyme activity in bone have been made<sup>46-49</sup>. In these the citrate-condensing enzyme activity reported represented the net synthesis of citrate from acetate and oxaloacetate. This net synthesis in turn is a measure of the difference between citrate formation (by an acetyl-CoA-forming system in combination with citrate-condensing enzyme) and the utilization of citrate in the preparations.

Aconitase and isocitrate dehydrogenase activities in various bones were also measured by Dixon and Perkins<sup>46</sup>, but recent work<sup>50</sup> has shown the activity of isocitrate dehydrogenase to be much higher than reported by them. We have, therefore, re-examined the citrate-condensing enzyme of bone using a sensitive enzyme assay method recently developed by us<sup>51</sup>, and we have attempted to measure for the first time the citrate-cleavage enzyme in bone. As can be seen in Table 1, our values for bone citrate-condensing enzyme are two orders of magnitude higher than the values (0.04 units/g) reported by the other workers in this field<sup>46</sup>. The previous work was done with slices of bone or homogenates, which probably explains the low values obtained; citrate-condensing enzyme occurs in the mitochondria, and, as can be seen (Table 2), when a Waring blender is used for more vigorous homogenization, higher activities are obtained. Even the values reported here may be too low since the extraction procedures used here are far from adequate, as can be seen by the low values for protein for shaft extracts. The high activity is even more striking when the relative scarcity of cells in bone compared to soft tissues is considered.

Dixon and Perkins<sup>46</sup> reported that in bone the citrate-condensing enzyme activity (citrate-condensing enzyme and acetate-activating enzyme) was much higher than the isocitrate dehydrogenase activity; they suggested this relationship

Table 1. CONDENSING ENZYME CONTENT OF RABBIT BONE AND MARROW

Bone region	Total protein, mg/g	Condensing enzyme	
		Specific activity, units/mg protein	Total activity, units/g
Tibia end	48	0.06	2.9
Tibia shaft	Not detectable	0	0
Tibia, marrow	30	0.07	2.3
Femur end	15	0.2	2.9
Femur shaft	Not detectable	0	0
Femur, marrow (end)	18	0.1	2.7
Femur, marrow (middle)	30	0.07	2.8

Rabbits were killed by fracture of the cervical vertebrae. Tibias and femurs were dissected from the animal and cleaned of most of the adhering tissue. The bones were then placed in liquid nitrogen. After they were frozen they were broken to give ends and shafts which were separated. The pieces of bone were freed completely of all soft tissue by scraping with a blade. The marrow was removed by washing in a stream of distilled water. In addition, the spongy bone of the ends was cleaned of marrow with a jet of compressed air. Only those shafts which were completely free of spongy bone were used for these analyses. After this cleaning procedure the bone chips were powdered in a mortar, keeping them frozen with liquid nitrogen. When a powder was obtained, an aliquot was weighed into a beaker and about 30 ml. of cold 20 per cent ethanol in 0.4 M potassium chloride per g of femur tissue or marrow (or 10 ml. 0.25 M sucrose for tibia) was added. This mixture was homogenized in an ice-cooled micro attachment to a Waring blender for a total of 5 min. The suspension was centrifuged at 20,000g for 20 min and a portion of the supernatant solution assayed for protein<sup>52</sup>, citrate-condensing enzyme<sup>51</sup>, and citrate-cleavage enzyme<sup>5</sup>. All enzyme units are expressed as  $\mu$ moles of product formed per min at room temperature.



Table 2. COMPARISON OF METHODS OF EXTRACTION OF CITRATE-CONDENSING ENZYME FROM RABBIT FEMUR ENDS

Extraction method	Total protein mg/g	Condensing enzyme specific activity, units/mg protein	Total activity, units/g
Alcohol-KCl (see Table 1) in Waring blender	15	0.2	2.9
0.03 M Tris-HCl-0.013 M Versene pH 8.0+homogenization 4 min in Waring blender	42	0.07	3.0
0.5 M Tris-HCl-0.4 M Versene pH 8.0. Let stand 30 min at 0°	23.3	0.07	1.7

See Table 1 for details.

could account for citrate accumulation in bone. However, later when Van Reen and Losee<sup>60</sup> found high isocitrate dehydrogenase activity in bone, it seemed difficult to explain bone citrate accumulation. A sensible picture is re-established with the values for citrate-condensing enzyme presented here: the citrate-forming capacity now is three times higher than the reported citrate-utilizing activity<sup>60</sup>. It is certainly true that the measurement of enzyme activity usually gives only the maximum enzyme capability for the extract, that is, it does not necessarily indicate the actual rate of tissue synthesis. However, whatever the figures obtained for enzyme activities, it is logical to assume that bone must have higher citrate formation than citrate utilization, since in this tissue the arterio-venous difference for citrate is positive and citrate accumulation is very high.

Very little citrate-cleavage enzyme was detected in bone tissue, a finding consistent with the idea that bone metabolism accounts for a net production of citrate. Citrate-cleavage enzyme is more unstable than the condensing enzyme and it is possible it was destroyed during the extraction, although a similar procedure was used without difficulty to measure the citrate-cleavage enzyme in a number of other tissues. Cleavage enzyme activity of less than 0.05 units/g would not have been detected by our methods; in one experiment we tested a more concentrated homogenate of bone marrow and found 0.03 units/g wet weight marrow.

Table 3. FATTY ACID SYNTHESIS BY BONE MARROW

Substrate	Additions	Fatty acids, total c.p.m.
Acetate 1- <sup>14</sup> C, 1 $\mu$ mole ( $5.0 \times 10^6$ c.p.m.)	—	23,500
Acetate 1- <sup>14</sup> C, 1 $\mu$ mole ( $5.0 \times 10^6$ c.p.m.)	Citrate, 10 $\mu$ moles	50,000
Citrate 1, 5- <sup>14</sup> C, 2.3 $\mu$ moles ( $5.3 \times 10^6$ c.p.m.)	—	2,000
Citrate 1, 5- <sup>14</sup> C, 2.3 $\mu$ moles ( $5.3 \times 10^6$ c.p.m.)	ATP, 5 $\mu$ moles CoA, 0.5 $\mu$ moles	1,800

Marrow from the femur and tibia of a rabbit was homogenized in a Potter-Elvehjem homogenizer in 3 volumes of Gurin's buffer<sup>1</sup>. The homogenate was centrifuged for 20 min at 30,000g and the residue discarded. Each tube contained 0.6 ml. of this supernatant fraction and the additions shown in a total volume of 0.75 ml. Incubations were for 1 h at room temperature. Fatty acids were isolated and counted as described previously<sup>12</sup>.

I found that although bone marrow can make fatty acids from acetate, as reported previously by Altman *et al.*<sup>63</sup>, it is unable to utilize citrate, a finding consistent with its low citrate-cleavage enzyme activity (Table 3). The results reported here differ in two ways from those of fatty acid synthesis in homogenates of liver. First, addition of citrate results in only a two-fold stimulation of acetate incorporation into fatty acids, whereas in liver the requirement for citrate is absolute. Secondly, citrate is a much poorer precursor of fatty acids in marrow than in acetate, whereas the two are about equally effective in liver. This is an apparent exception to the generalization made earlier that high concentrations of citrate-cleavage enzyme are associated with high capacity for acetyl group utilization, and thus seems to support the idea that all possible ways of generating acetyl groups are utilized by cells.

### Control of Citrate Metabolism

The compilation of data from the literature in Table 4 compares citrate, citrate-condensing enzyme and citrate-cleavage enzyme in a number of tissues. Considering that these data represent a collection from various laboratories and that the comparison of such different tissues as liver

and bone on a wet weight basis is difficult at best, I still feel that several relationships deserve to be noticed. These relationships may be fortuitous since the citrate data represent steady-state concentration and the enzyme activities represent maximum capacities. There is a rough correlation between the amount of citrate-condensing enzyme in the soft tissues of a rat and their reported  $Q_{O_2}$  values<sup>60</sup>. This is not surprising since the citrate-condensing enzyme activity represents the first step in the Krebs cycle and the  $Q_{O_2}$  represents the total activity of this pathway. Citrate concentrations in the soft tissues seem to be related to the difference between citrate-condensing enzyme activity and citrate-cleavage enzyme activity. Interestingly enough, when fluoroacetate is injected into animals<sup>60</sup>, then those tissues with high citrate contents and high citrate-condensing enzyme, such as kidney and heart, accumulate most citrate. This probably occurs because those tissues with high condensing enzyme form most fluorocitrate, the compound that inhibits citrate utilization by its inhibitory action on aconitase.

The source of blood citrate is not well known (there is no citrate-condensing enzyme in blood cells), but, according to investigations of arterio-venous citrate differences, it is quite likely that tissues such as intestine and bone and/or bone marrow are able to supply it<sup>45,61</sup>. Similar investigations have indicated that kidney is largely responsible for the utilization of blood citrate<sup>42,61</sup>. No measurements have been reported on the arterio-venous difference for citrate in the heart, but from the data presented in Table 4 one might expect a positive difference.

Table 4. COMPARISON OF CITRATE, CITRATE-CONDENSING ENZYME AND CITRATE-CLEAVAGE ENZYME OF SOME RAT AND RABBIT TISSUES

Species	Tissue	Citrate $\mu$ moles/g	Ref.	Citrate-condensing enzyme, units/g	Citrate-cleavage enzyme, units/g
Rabbit	Liver	0.15	40	2.0	0.10
	Kidney	0.31	40	13	0.23
	Heart	—	—	40	0.22
	Bone (end)	3.5	54	2.9	<0.05
Rat	Liver	0.15	37, 55	5.6	0.67
	Kidney	0.23	56	13	0.97
	Heart	0.39	54, 55	78	0.07
	Bone (end)	2.0	55, 57	1.5	<0.05

The references indicate the sources of the citrate data. Where several values exist, averages were taken and corrected to wet weight values. The citrate-condensing enzyme values were taken from ref. 58 and corrected to rates obtained with our new assay<sup>41</sup>. Citrate-cleavage activities were taken from ref. 2.

The citrate-levels of blood and tissues seem to be under a variety of controls. Administration of vitamin D is known to increase the citrate content of bone, serum and other tissues, and a deficiency of this vitamin results in lowering of citrate-levels<sup>40,62,63</sup>. Hydrocortisone and other steroids can partially reverse the effect of vitamin D<sup>64</sup> and parathyroid hormone has been claimed to reduce citrate-condensing enzyme of bone<sup>57</sup>. Earlier claims of increased citrate formation by bone after administration of vitamin D (ref. 47) or parathyroid<sup>67</sup> are now in dispute, since recent work has demonstrated that vitamin D decreases the utilization of citrate by kidney mitochondria<sup>65</sup>.

Increases in fat metabolism or fatty acid concentration, such as can be caused by fasting or diabetes, result in large increases in tissue citrate-levels<sup>37</sup>. The observation that palmityl-CoA inhibits citrate-condensing enzyme<sup>66,67</sup> gave rise to the suggestion that this interaction might serve as a control of citrate levels in tissues<sup>67</sup>. We have shown, however, that the interaction between citrate-condensing enzyme and palmityl CoA is not a specific one<sup>68,69</sup>, and we have pointed out that the result would be changes in citrate-levels opposite to those observed under various physiological conditions<sup>37</sup>. There is no clear evidence as yet which makes it possible to formulate molecular mechanisms that can account for changes in citrate concentration in animal tissues.

Finally, the ability of citrate to chelate metal ions is well known: it has a relatively high affinity for such cations as  $\text{Ca}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Mg}^{++}$  and  $\text{Fe}^{+++70}$ . There is little direct evidence relating this ability to metabolic function. It has been shown that Fe citrate is an excellent substrate in the metabolism of Fe transferrin<sup>71</sup>. It is possible that the demonstrated accumulation of  $\text{Ca}^{++}$  by mitochondria may involve citrate metabolism. The binding capacity of citrate for calcium has led to speculations that both the resorption and the formation of bone may involve the chelation of calcium by citrate. It is interesting that citrate has been shown to function better than most chelators in the removal of radioisotopes from bone<sup>72</sup>, and that animals the citrate-levels of which were increased by fluoroacetate treatment excreted more lead than untreated animals<sup>73</sup>.

### Conclusions

This brief synopsis of citrate metabolism is intended to illustrate the unique features of this compound and the surprising diversity of parts it can play in animal tissues. It is difficult to understand the significance of this multiplicity of function and control. Born<sup>74</sup> has said that remarkable scientific achievements have come from "the method of simplifying thought by stressing one aspect only of the facts". None the less, in biological investigations we must deal with systems that are exceptionally complex and we run the danger of choosing model systems that are too simple to advance our understanding. To understand cellular metabolism we must continually search for model systems the complexity of which approaches as closely as possible that of the cell. The models also should have been sufficiently examined to enable us to make some simple predictions and explanations of the more complex cellular systems.

One such model which may prove to be valuable in discussing and understanding metabolic control and regulation is that which has been developed in the field of information theory for the construction of automata. Workers in this field have shown that it is possible to construct reliable circuits, that is, those capable of reliable computation, from unreliable formalized 'neurones' if the network contains sufficient redundancy<sup>75-77</sup>. When we are confronted with a compound such as citrate which is apparently multifunctional and under a variety of controls, or when we consider which of the many available pathways is responsible for acetyl group transfer across mitochondrial membranes, we may do understanding a grave disservice by looking for 'the' function, or control, or rate-limiting step. This diversity of roles for compounds, these multiple controls and pathways, may represent a biological systems redundancy which is necessary for stable metabolic regulation.

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## DEXTRAN TURBIDITY: ACUTE DISTRESS-PHASE REACTION

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DEXTRAN, a glucose polymer synthesized by various organisms, is well known to form complexes with blood proteins. The  $\alpha$ - and  $\beta$ -lipoproteins and fibrinogen are particularly readily bound to dextran, but almost all the blood proteins have been described as binding with it in some degree<sup>1-4</sup>. Besides reacting chemically with dextran, serum may also react with it immunologically. Kabat and Berg<sup>5</sup>, Grabar<sup>6</sup>, Jacobsson and Wikström<sup>7</sup>, and Jacobsson and Zsiga<sup>8</sup> all observed that a small number of normal human sera contained precipitating antibodies to dextran. It has been shown that dextrans may cross-react with several bacterial antisera. These sundry ways of interaction between dextran and plasma are increased in psychiatric patients by the possibility of a reaction between dextran and psychotropic drugs<sup>9</sup>.

A chance observation in this laboratory showed heavy turbidity in the plasma of an acute schizophrenic patient after addition of dextran. Further investigation of this phenomenon showed that: (a) most human plasmas contain measurable amounts of dextran-precipitable material; (b) its mean level is significantly higher in patients with mental disorders than in control subjects; (c) it tends to be more pronounced in patients with the most intense acute symptoms; (d) it increases in response to emotional stress; and (e) the precipitate contains cryoglobulins and cryoprecipitins of more than one sort.

Groups of mentally ill in-patients were investigated at the Langley Porter Neuropsychiatric Institute (LPNI), Veterans Administration Hospital, Palo Alto (VAPA), Napa State Hospital (NSH), Agnews State Hospital (ASH), and San Francisco General Hospital (SFGH). Various personnel from the wards, laboratories and offices at the five institutions provided control groups. Each patient group was compared with the controls from the same hospital. The effects of emotional stress were investigated by examining plasma taken from a group of prisoners on the morning that they appeared before a Parole Board and again two weeks later.

Plasma was obtained from 10 ml. of blood which had been anticoagulated with 2 ml. citrate solution. A 6 per cent solution of dextran in 0.9 per cent saline was obtained from Cutter Laboratories, Berkeley; the primary source Pharmacia, Ltd., Sweden, who had synthesized the dextran from *Leuconostoc mesenteroides*, strain 'NNRL B512'. The dextran used had an average molecular weight of 75,000 with a lower limit of 25,000 and an upper limit of 200,000. Plasma and dextran were mixed and left overnight at 4° C. The precipitate which developed was thoroughly dispersed in a Vortex mixer and the optical density of the plasma was measured at 600 m $\mu$  in a Coleman spectrophotometer; the turbid mixture was then centrifuged and the optical density of the supernatant was re-measured in the same cuvette. The difference in the optical densities before and after centrifugation was taken as the measure of the precipitate. Most of the experiments reported here used a plasma : dextran ratio of 1 : 1 and gave results shown in Table 1. Later experiments used a plasma : dextran ratio of 1 : 2.5. As indicated in Fig. 1, the amount of precipitate is not stoichiometrically related to the amount of dextran added; a plasma : dextran ratio of 1 : 2.5 gave generally higher values.

Electrophoresis, immunoelectrophoresis, ultracentrifugation and immuno-diffusion investigations were made with standard techniques. The Mann-Whitney U test

Table 1. DEXTRAN TURBIDITY TEST IN DIFFERENT MENTAL HOSPITALS

Hospital	No.	Patients		No.	Controls		P
		Mean	S.E.		Mean	S.E.	
LPNI	82	129	15.9	47	76	9.3	< 0.05
VAPA	27	183	23.7	32	85	18.6	< 0.05
SFGH	41	139	22.8	29	90	13.2	0.05
NSH (1)	26	152	18.6	10	97	25.0	< 0.05
NSH (2)	33	211	20.6	20	194	28.0	

Table 2. DEXTRAN TURBIDITY STUDIES AT AGNEWS STATE HOSPITAL

	No.	Mean	P
Controls	17	204	0.08
Short-term drugs*	18	242	< 0.05
Long-term drugs*	20	192	< 0.05
No drugs	13	256	< 0.05

\* See text for definition of short-term and long-term drug therapy.

was used for the statistical analyses; the confidence-level was set at 0.05.

At all five hospitals the mean level of dextran turbidity was higher in the patients than in the controls (Tables 1 and 2). The difference was significant ( $P \leq 0.05$ ) except in the second group of patients and controls tested at NSH. A re-test again showed no difference, a result which cannot be easily explained except by postulating that the ward personnel used as controls were under unusually severe stress. The results in all groups of patients and controls showed considerable overlap.

The following evidence shows that the level of intensity of the symptoms is an important correlate of the level of dextran turbidity in mental disease. The patients were divided into groups according to the duration and amount of dosage of phenothiazine drugs; a short time was taken as less than 2 months and a low dose as less than 400 mg chlorpromazine or its approximate equivalent dose of other phenothiazines. Patients treated for a short time had higher levels of dextran turbidity than patients treated for a long time. This difference was statistically significant at VAPA and ASH, and approached significance at NSH ( $P = 0.06$ ) and LPNI ( $P = 0.08$ ). All the patients

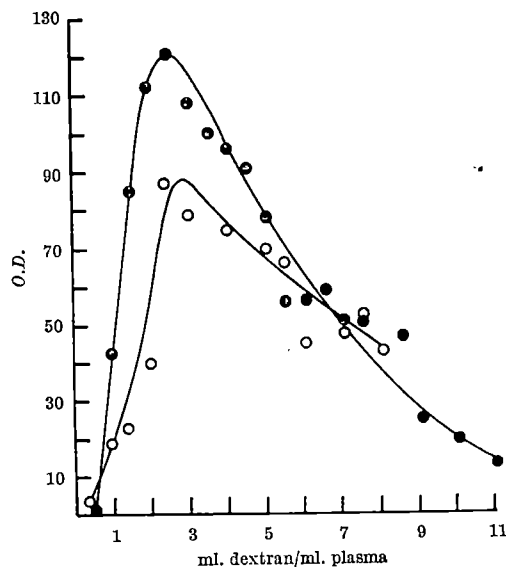


Fig. 1. Curves obtained from two subjects by adding increasing volumes of dextran to one volume of plasma



at SFGH were acutely ill and their blood was tested within a few days of admission; their mean level was significantly higher than in the controls. Subjects treated for a short time with a high dose, that is, those whose symptoms were acute and were also the most florid, had consistently higher mean levels than those treated for a long time with a high dose. These differences were marked: at VAPA 266 cf. 87 ( $P < 0.05$ ); at ASH 242 cf. 192 ( $P < 0.05$ ); at NSH 175 cf. 111 ( $P = 0.06$ ); and at LPNI 190 cf. 102 ( $P = 0.1$ ). In 9 patients at LPNI who were acutely ill, the mean level of turbidity was 233; three weeks later when the general intensity of symptoms had fallen, the mean level of turbidity was 137 ( $P < 0.05$ ).

The medication itself was not directly linked with the amount of dextran turbidity because: (a) patients on high doses of phenothiazines for a long period had consistently lower mean levels of turbidity than patients taking either high or low doses for a short time; (b) at SFGH, where the patients had been given either no tranquillizing drugs or only one or two small, sedating doses, the patients had significantly higher mean levels than the controls; and (c) at ASH, patients who had never had phenothiazines or had not been given them for several months had much higher mean levels than controls.

The high levels were not specific for any particular diagnostic category of mental disease, whether psychotic or psychoneurotic, functional or organic, nor were they specific to mental disease itself. So far, however, higher levels have been seen in mental disease than in most patients hospitalized with acute infections or with severe connective tissue diseases. Age and sex, both factors known to influence some serum proteins, did not seem to affect this reaction.

Emotional stress seems to be an important correlate of the amount of dextran turbidity. The level of turbidity fell in each of eighteen prisoners when plasma taken two weeks after a Parole Board hearing was compared with plasma taken on the morning of the hearing. The mean fall in the dextran turbidity of these plasmas was 66 per cent. Preliminary results in a few rats given repeated small electric shocks to the feet show that this stress causes a considerable rise in dextran turbidity over baseline values.

The wide variation of the values within all groups of patients and controls (Table 1) deserves comment. This was probably not a methodological error. For one plasma examined ten times, the mean value was 115 and the standard error of the mean 1.84. The wide variance in the subject groups was probably due to several substances being precipitated and also to an unfavourable ratio of dextran: plasma. When this ratio was changed from 1:1 to 2.5:1 the variance was lowered; as indicated in Fig. 1 this increased proportion of dextran causes maximal turbidity. We prefer to use this ratio in present-day investigations. The difficulties of reproducing turbidimetric estimations are well known. Yet the reproducibility was not improved after converting the reaction to a colorimetric one by washing the precipitate, dissolving it in 9 M urea and adding Biuret reagent. Seven plasmas were examined both in this way and, at the same time, turbidimetrically; a dextran: plasma ratio of 2.5:1 was used. The coefficient of variation (standard deviation divided by the mean) was 0.49 for the colorimetric method and 0.42 for the turbidity method.

Electrophoretic and ultracentrifugal investigations of a few plasmas showed that 19S protein levels were 38 per cent higher and 7S levels were 11 per cent higher in patients with very high levels of dextran turbidity than in patients with very low levels. There were no clear differences in the electrophoresis results in the two groups. Zone electrophoresis of proteins recoverable from the precipitate showed a single band in the  $\beta$ - $\gamma$  region with a marked trail from the origin. Ultracentrifugation showed that about two-thirds of the material was 7S class protein and the remainder 16S class protein; the exact proportions

are meaningless because of material lost during the purification procedure. Immuno-diffusion in agar investigations showed a strong precipitin reaction with a fibrinogen antiserum, a moderately strong reaction with a 7S antiserum, and a faint reaction with a  $\beta$ -2 macroglobulin antiserum. There were no precipitins formed with antisera to albumin;  $\alpha$ -2 macroglobulin; ceruloplasmin; Cohn's fractions III; Cohn's fractions IV, 5 + 6;  $\beta$ -2A; or  $\beta$ -lipoprotein.

A large proportion of the precipitable material was clearly fibrinogen: (a) there was a strong reaction between the proteins solubilized from the precipitate and a fibrinogen antiserum; (b) the precipitate was more intense in plasma than in serum—in 16 subjects whose plasma and sera were tested simultaneously, the mean serum level was only 26.4 per cent of the mean plasma level. The 16S material in the ultracentrifuge was probably largely polymerized fibrinogen; the 7S material was probably the serum component. The positive reactions in the immuno-diffusion investigations between the isolated precipitate and antisera against fibrinogen and 7S globulin showed immunological non-identity. It is known that dextran forms complexes with fibrinogen; (c) some of this fibrinogen could represent the portion described by Thomas, Smith and Von Korff<sup>10</sup> as cold precipitable in the presence of heparin. However, parallel investigations on 14 normal plasmas using both heparin, 0.1 mg per ml. blood, and dextran as precipitants showed a mean level of 65.7 precipitated by dextran and only 6.9 precipitated by heparin.

The presence of so much fibrinogen in the precipitate suggests the possibility that the increased levels of dextran turbidity in mental disease and emotional stress result from activation of various serum enzyme systems which cause changes in the internal structure of blood proteins. Plasmin, which affects the fibrinogen molecule, is activated by various agents including emotional stress<sup>11-13</sup>, physical stress<sup>14</sup>, adrenal steroids, adrenaline, acetylcholine and histamine<sup>15,16</sup>, and some evidence suggests that plasmin may be under reflex control<sup>17</sup>. Other serum enzymes are also activated by stress, which is known to influence a variety of blood proteins<sup>18</sup>.

Some of the precipitate might represent naturally occurring antibodies to dextran, reported to be present in small amounts in many human sera by Kabat and Berg<sup>5</sup> and Grabar<sup>6</sup>. In two patients, addition of increasing quantities of dextran to constant volumes of plasma caused precipitation in amounts resembling the classical precipitin curves for antigen-antibody reactions (Fig. 1). In both subjects, maximum precipitation occurred with a plasma: dextran ratio of 1:2.5 and increasing the proportion of dextran beyond this caused a decrease in the amount of precipitate.

The possibility of a cross-reaction between dextran and antibodies to bacteria, such as reported by Sugg and Hehre<sup>19</sup> and Neill and Abrahams<sup>20</sup>, was investigated by absorbing two plasmas with various antigens derived from *E. coli*, *Salmonella* and *Streptococcus* (Difco Laboratories). In one plasma there was a 52 per cent reduction in dextran turbidity after absorption with *E. coli* 'O' antigen 026. This is being further examined.

The dextran turbidity reaction provides a simple test which tends to give high values in states of mental distress. The complex reaction depends mainly on formation of precipitates between dextran and fibrinogen with contributions from other globulins. In mental illness, the reaction seems to be related to the level of intensity of the symptoms rather than merely to their severity. The amount of dextran turbidity is likely to be highest in the turbulent state of acute psychotic reactions or during chronic disease when the symptoms have shifted in intensity and have become more florid. The phenomenon is not restricted to any particular sort of mental disorder and seems analogous to the non-specific acute-phase reactants seen in systemic medical diseases.

Various non-specific stresses can affect blood proteins<sup>18</sup>; the preliminary findings suggest that the dextran turbidity reaction may be a non-specific one to any sort of emotional turmoil or stress. It seems likely that the increased levels in mental disease may result from the effects of stress on serum proteolytic enzyme systems which affect the internal structure of various plasma proteins including fibrinogen. The dissection of the components of this reaction might be an interesting avenue of research into mental illness.

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## SPARSOMYCIN MODIFICATION OF THE LETHAL ACTION OF IONIZING IRRADIATION ON *Escherichia coli*

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SPARSOMYCIN ( $C_{13}H_{21}N_3O_6S_2$ ) is an antibiotic of unknown structure<sup>1</sup> which has been reported to inhibit the growth of a variety of micro-organisms, *KB* cells in culture, and transplantable mammalian neoplasms in rodents<sup>2</sup>. In *Escherichia coli*, sparsomycin has been found to inhibit protein synthesis<sup>3</sup>. During the course of routine screening for irradiation modifiers, sparsomycin was found to protect slightly (at low concentrations) and to potentiate markedly (at high concentrations) the lethal action of ionizing irradiation on non-proliferating cell suspensions of *E. coli*.

*Escherichia coli* 'ATCC 9637' was used in most experiments. Stock cultures were maintained, inocula prepared and experiments performed in the following medium:  $NH_4Cl$ , 1.0 g;  $K_2HPO_4$ , 7.32 g;  $KH_2PO_4$ , 3.0 g;  $MgSO_4$ , 0.12 g; deionized water, 1 litre (after autoclaving at 15 lb. pressure for 30 min, 8 ml. of sterile 50 per cent glucose was added). For inocula, cultures were grown at 37° C for 18 h, washed three times in M/15 phosphate buffer (pH 7.0) and standardized to a reading of 0.48 optical density in a Bausch and Lomb 'Spectronic 20' spectrophotometer (660 mμ) with the buffer. Such suspensions usually contain approximately  $5 \times 10^8$  viable cell/ml.

In some experiments the following wild-type cultures of *E. coli* and derived resistant strains were used: *E. coli* 'B' (ORNL) and the derived irradiation-resistant strain designated *E. coli* 'B/r' (ref. 4); *E. coli* 'B' (Hill) and the derived strain of increased irradiation sensitivity<sup>5</sup> designated *E. coli* 'B<sub>s</sub>' (Hill) (these four cultures were kindly supplied by Dr. H. Adler, Oak Ridge National Laboratories); a strain from *E. coli* 'B' (Hill) as resistant to the alkylating agent, 1,3-bis(2-chloroethyl)-1-nitrosourea, and cross-resistant to ionizing irradiation<sup>6</sup>; the wild-type 'B' strain, *E. coli* 'ATCC 11303'; and five strains derived from *E. coli* 'ATCC 9637' which are individually resistant to nitrogen mustard, azaserine, 6-diazo-5-oxo-1-nor-leucine<sup>7</sup>, 2-fluoroadenine and 2-fluoroadenosine<sup>8</sup>.

The procedures used to determine irradiation-modifying activity were: for measuring effects on non-growing cells, washed cell suspensions of 5 ml. were exposed to irradiation from a cobalt-60 source with and without test compound. In most experiments duplicate samples were simultaneously handled. Following irradiation, the cells were suitably diluted in saline and 1-ml. aliquots of appropriate dilutions plated in triplicate. For measuring

effects on growing cells, cells were inoculated into the synthetic medium with and without various concentrations of sparsomycin and aliquots were irradiated immediately after inoculation and after 4 h growth. A second series of tubes was prepared in which the cells were allowed to grow for 4 h, sparsomycin was added, and aliquots were irradiated immediately and again after an additional 2 h growth in the presence of sparsomycin.

Irradiation was accomplished with the use of a flux-loaded <sup>60</sup>Co source (c. 1,000 c.) at a rate of 2.0 kr./min under our conditions of exposure. Tubes of cell suspensions were randomly placed in a circular device which rotates around the source. The tube holder is so constructed that each tube also rotates independently but at a constant known rate during the irradiation-exposure period. Ferric sulphate dosimetry showed constant exposure along the longitudinal axis of the tubes of cell suspensions.

A typical experiment showing modification of irradiation damage in non-proliferating *E. coli* by sparsomycin is presented graphically in Fig. 1. Concentrations of sparsomycin of 3–10 μg/ml. appear to protect slightly against irradiation damage. Thirty μg/ml. is essentially without effect and higher concentrations (100–1,000 μg/ml.) markedly potentiate irradiation damage. The protection afforded by low concentrations of sparsomycin is slight but consistent. In four individual experiments the observed protection falls beyond one standard deviation from the control-kill value.

The effect of sparsomycin on irradiation damage of several cultures of non-proliferating *E. coli* and derived resistant strains is summarized in Table 1. Sparsomycin, 10 μg/ml., slightly protects *E. coli* 'ATCC 9637', *E. coli* 'B' (Hill) and *E. coli* 'B ATCC 11303' against irradiation damage. Sparsomycin, 10 μg/ml., does not influence the response of *E. coli* 'B' (ORNL) to irradiation. This same dose of sparsomycin potentiates irradiation damage in the irradiation sensitive strain *E. coli* 'B<sub>s</sub>' (Hill). All the cultures examined were sensitive, in varying degrees, to the irradiation-potentiating effect of 100 μg sparsomycin/ml. Interestingly, cultures resistant to alkylating agents such as nitrogen mustard, azaserine, or 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were much less sensitive to the sparsomycin potentiating effect than were the parent cultures. Similarly, the irradiation-

Table 1. SPARSOMYCIN MODIFICATION OF THE DAMAGING EFFECTS OF IONIZING IRRADIATION ( $^{60}\text{Co}$ ) ON A SPECTRUM OF *Escherichia coli* AND DERIVED RESISTANT STRAINS (NON-PROLIFERATING)

Bacterium	% Survivors			Ratio % Survivors—20 kr.	
	20 Kr	20 kr. + sparsomycin*, $\mu\text{g}/\text{ml}$ .		% Survivors—20 kr. + sparsomycin, $\mu\text{g}/\text{ml}$ .	
		10	100	10	100
<i>Escherichia coli</i> 'ATCC 9637'	0.296	0.026	0.001	0.31	296.0
<i>Escherichia coli</i> 'ATCC 9637'/HN2†	12.5	15.8	1.0	0.79	12.5
<i>Escherichia coli</i> 'ATCC 9637'/FAS	0.98	1.75	0.012	0.56	31.67
<i>Escherichia coli</i> 'ATCC 9637'/FAD	0.3	0.57	< 0.00083	0.52	> 361.45
<i>Escherichia coli</i> 'ATCC 9637'/AZA	16.3	52.04	4.69	0.81	3.48
<i>Escherichia coli</i> 'ATCC 9637'/DON	0.257	1.057	< 0.0014	0.24	> 183.57
<i>Escherichia coli</i> 'B' (HIII)	0.1	0.22	< 0.00077	0.45	> 129.57
<i>Escherichia coli</i> 'B' (HIII)/BCNU	3.14	3.82	0.336	0.94	9.32
<i>Escherichia coli</i> 'B' (HIII)	0.45	0.065	< 0.0005	6.76	> 900.0
<i>Escherichia coli</i> 'B', 'ATCC 11303'	0.11	0.415	< 0.0005	0.26	> 220.0
<i>Escherichia coli</i> 'B' (ORNL)	0.167	0.153	< 0.00067	1.09	> 249.25
<i>Escherichia coli</i> 'B/r'	34.74	25.26	1.47	1.33	23.63

\* Sparsomycin is non-toxic at these concentrations under test conditions used.

† Resistance to a growth inhibitor indicated thus:

Name of parent culture/name of inhibitor to which strain is resistant.  
 HN<sub>2</sub> = Nitrogen mustard      AZA = Azaserine      r = Radiation  
 FAS = 2-Fluoroadenosine      DON = 6-Diazo-5-oxo-1-norleucine  
 FAD = 2-Fluoroadenine      BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea

resistant *E. coli* 'B/r' was less sensitive to the sparsomycin-potentiating effect than was its parent culture, *E. coli* 'B' (ORNL). The strain of *E. coli* resistant to the irradiation modifier 2-fluoroadenosine<sup>8</sup> was less sensitive to the sparsomycin potentiating effect than was either the parent culture or a sister culture resistant to the non-radiation modifier, 2-fluoroadenine.

The potentiating effect of sparsomycin is demonstrable over a range of irradiation doses (see Fig. 2). Irradiation kill incurred by doses of 2.5 kr. or lower does not appear to be enhanced by sparsomycin (100  $\mu\text{g}/\text{ml}$ ).

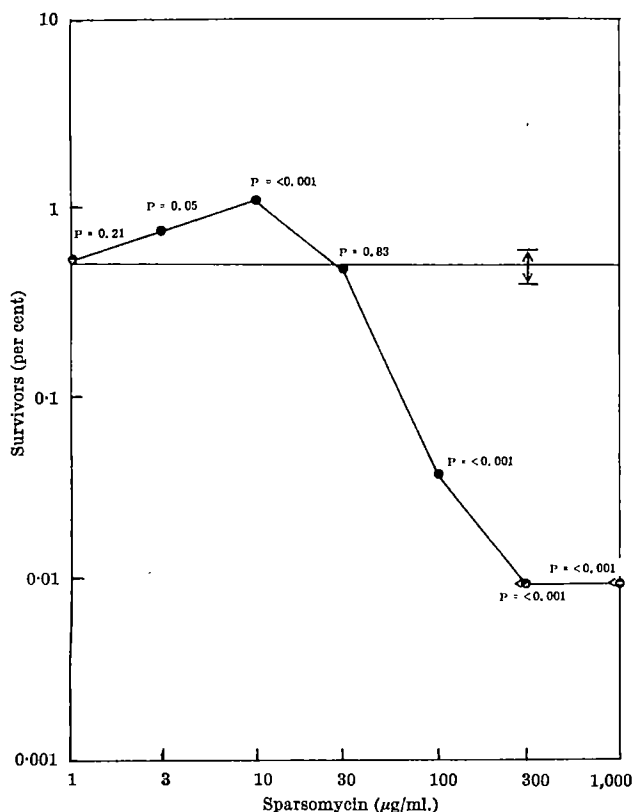


Fig. 1. Sparsomycin modification of the damaging action of ionizing irradiation ( $^{60}\text{Co}$ , 20 kr.) on *Escherichia coli* 'ATCC 9637' (non-proliferating). Horizontal line at c. 0.6 per cent survivors indicates per cent survivors to irradiation kill in the absence of sparsomycin with one standard deviation indicated. P = probability values of observed sparsomycin-irradiation effects

The effect of reported radiation protectors on the irradiation potentiating action of sparsomycin has been investigated. Cysteamine is without detectable effect on the sparsomycin potentiating effect (see Fig. 3). Cysteine<sup>9</sup> appears to protect slightly against the sparsomycin potentiating effect (Fig. 4) under these conditions.

Sparsomycin must be present during the irradiation period in order for its potentiating effect on non-proliferating *E. coli* to be demonstrated. Mixing suspensions of irradiated cells (20 kr.) with separately irradiated (20 kr.) sparsomycin (100  $\mu\text{g}/\text{ml}$ ) resulted in the same percentage survivors as were present in the control irradiated cell suspensions. Additionally, cells which had been suspended in sparsomycin (100  $\mu\text{g}/\text{ml}$ ) for 1 h, washed three times

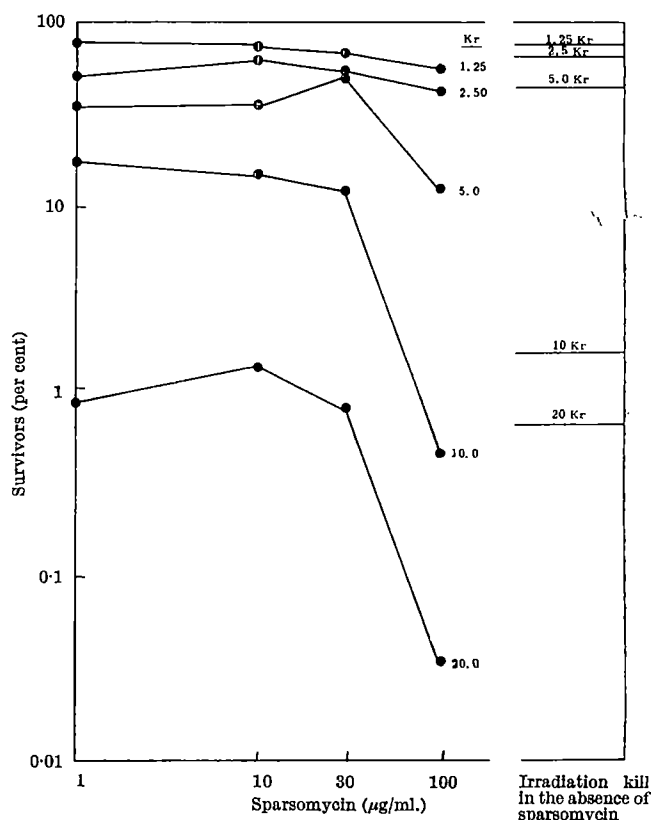


Fig. 2. The effect of irradiation dose on sparsomycin modification of irradiation damage in *Escherichia coli* 'ATCC 9637' (non-proliferating)



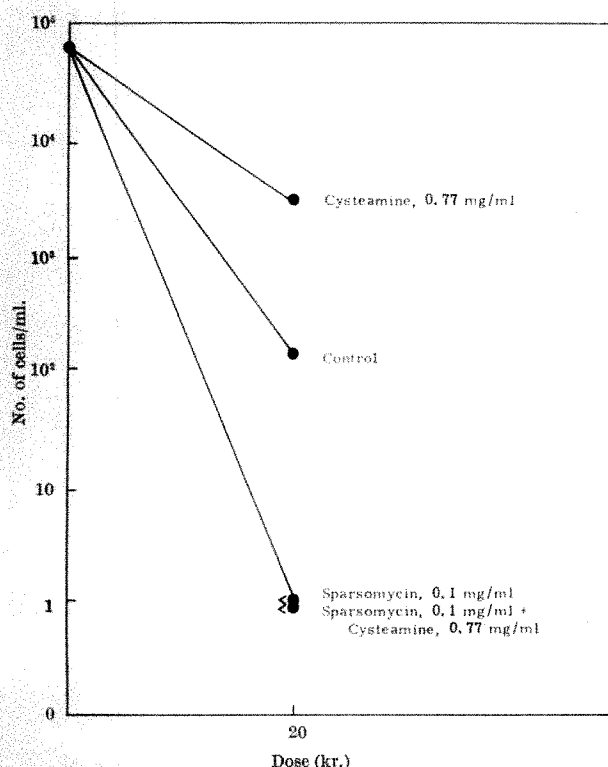


Fig. 3. The effect of cysteamine (0.77 mg/ml.) on the potentiation by sparsomycin of irradiation damage in non-proliferating cell suspensions of *Escherichia coli* 'ATCC 9637'

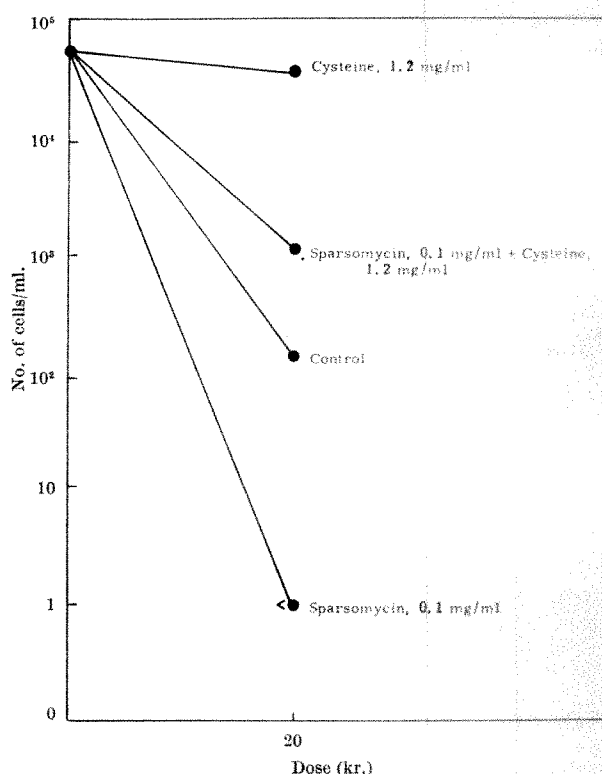


Fig. 4. The effect of cysteine (1.2 mg/ml.) on the potentiation by sparsomycin of irradiation damage in non-proliferating cell suspensions of *Escherichia coli* 'ATCC 9637'

by centrifugation and irradiated (20 kr.) were no more sensitive to irradiation than were control cells. Lastly, cells grown in non-toxic or partially toxic concentrations of sparsomycin (1 and 10  $\mu$ g/ml., respectively) were no more sensitive to irradiation when tested in buffer suspensions than were control cells.

Furthermore, an interesting sparsomycin-cysteine relationship has been observed: suspensions of irradiated cells (20 kr.) were very sensitive to a solution of cysteine (1.2 mg/ml.) and sparsomycin (0.1 mg/ml.) which had been separately irradiated (20 kr.). This solution was non-toxic to unirradiated cells. An unirradiated solution

of these two materials was non-toxic to both irradiated and unirradiated cells of *E. coli* (see Table 2). In experiments with growing cell (*E. coli* 'ATCC 9637') no effect by sparsomycin at non-toxic or partially toxic concentrations on irradiation damage has been observed.

As a potentiator of irradiation kill of non-proliferating *E. coli* cells suspended in buffer, sparsomycin is similar to chloral hydrate<sup>10</sup>, 2-fluoroadenosine<sup>8</sup>, and many other potentiators which must be present during the irradiation period and require moderately damaging doses of ionizing irradiation for the potentiating action to be observed. The decreased sensitivity to the sparsomycin potentiating action of strains of bacteria resistant to alkylating agents or ionizing irradiation is in agreement with earlier investigations with the radiation potentiator hadacidin<sup>11</sup> as well as with previously reported cross-resistance interrelationships between alkylating agents and irradiation in *E. coli*<sup>6,12,13</sup>.

The slight irradiation protection afforded by low concentrations of sparsomycin is obviously not clearly understandable. However, sparsomycin does contain sulphur in its structure. The observation that sparsomycin irradiated in the presence of cysteine yields a toxic product suggests the possibility that the sparsomycin structure is altered during irradiation. It may be noted that sparsomycin solutions following irradiation yield an odour similar to some sulphides.

The antibiotic sparsomycin demonstrates slight irradiation-protecting activity at low concentrations (10  $\mu$ g/ml.) and marked irradiation-potentiating activity at higher concentrations (100  $\mu$ g/ml.) in several strains of non-proliferating *E. coli*. Sparsomycin must be present during the irradiation period in order for the potentiating effect to be demonstrated. A dose of ionizing irradiation which in itself is not appreciably lethal for non-proliferating *E. coli* is not potentiated by sparsomycin. Strains of *E. coli* resistant to alkylating agents or ionizing irradiation are less sensitive to the sparsomycin potentiating effect than are the parent cultures from which they are derived.

Table 2. THE EFFECT OF SPARSOMYCIN AND CYSTEINE ON THE LETHAL ACTION OF IONIZING IRRADIATION ON SUSPENSIONS OF *Escherichia coli* 'ATCC 9637'

Effect of sparsomycin, cysteine, and sparsomycin + cysteine, irradiated and non-irradiated, on non-irradiated *Escherichia coli*

Compound(s)	% Survivors to irradiated compound(s)	% Survivors to non-irradiated compound(s)
Sparsomycin, 0.1 mg/ml.	97-92	98-96
Cysteine, 1.2 mg/ml.	101-00	101-04
Sparsomycin, 0.1 mg/ml. + cysteine, 1.2 mg/ml.	95-83	102-08

Effect of sparsomycin, cysteine, and sparsomycin + cysteine, irradiated and non-irradiated, on irradiated *Escherichia coli*

Compound(s)	% Survivors Cells and compound(s) irradiated separately, then combined	% Survivors Cells and compound(s) irradiated together
Control	0-36	—
Sparsomycin 0.1 mg/ml. Non-irradiated	0-125	—
Cysteine, 1.2 mg/ml. Non-irradiated	0-58	—
Sparsomycin, 0.1 mg/ml. + cysteine, 1.2 mg/ml. Non-irradiated	0-21	—
Sparsomycin, 0.1 mg/ml. Irradiated	0-135	< 0-001
Cysteine, 1.2 mg/ml. Irradiated	0-41	54-16
Sparsomycin, 0.1 mg/ml. + cysteine, 1.2 mg/ml. Irradiated	< 0-001	15-62

Preliminary data are given which suggest that sparsomycin in the presence of cysteine on irradiation yields a material toxic for irradiated *E. coli*. The radiation modifying effect of sparsomycin has not been observed in rapidly dividing *E. coli* cells.

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## COMPLEMENT-FIXATION TESTS IN TOXOPLASMOSIS WITH PURIFIED ANTIGEN

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THE complement-fixation test was first used in the diagnosis of toxoplasmosis<sup>1</sup>. The authors used as antigen the extracts of brain and spleen of infected rabbits. Since then a variety of other impure antigens has been used in the test and in consequence it has not been possible to compare results obtained in different laboratories. A pure suspension of *Toxoplasma gondii*, free from other cells, was used in this test<sup>2</sup>. The antigen has now been standardized by counting the parasites or by determination of the optical density of the suspension. Also, on freeze-drying the material in dextran it has been found that the properties of the antigen for complement fixation remain unimpaired. Some comparisons have been made of the results obtained by this method, by the dye test<sup>3</sup> and by the direct agglutination test<sup>4</sup>.

**Antigen.** This was obtained from the peritoneal exudate of cotton rats infected with the RH strain of *Toxoplasma gondii*. The nature of the preparation is shown in Fig. 1. It was treated with 1 per cent formalin in saline for 24 h, which was then replaced with a 1/10,000 solution of this substance or of merthiolate. The antigen used in the present experiments had  $7.5 \times 10^8$  parasites per ml. of suspension and its optical density in a Hilger Biochem absorptiometer using an Ilford filter RO 2 and 0.5 cm cell was 0.49. Aliquots of the suspension were prepared in



Fig. 1. Suspension of *Toxoplasma gondii* free from other cells, as used in complement-fixation test ( $\times 1,320$ ).

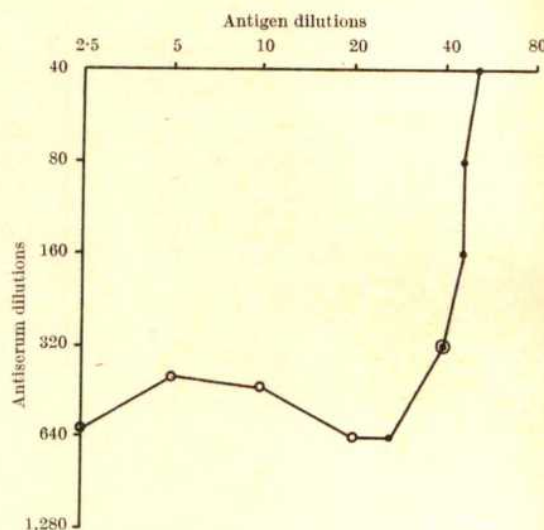


Fig. 2. The unit complement contour. This contour line denotes the antigen-antibody mixtures which fix exactly one unit of complement. Antigen: a purified suspension of *Toxoplasma gondii* containing  $7.5 \times 10^8$  parasites/ml. Antiserum, a known positive human serum. O, Points derived from the antigen contours; ●, points derived from the antiserum contours.

saline and in 6 per cent salt-free dextran. When freeze-dried in 2 ml. amounts and reconstituted by addition of distilled water the dextran mixture gave a satisfactory suspension. The properties of the freeze-dried suspension kept at 4° C were unimpaired when re-tested six months later.

**Complement fixation.** The antigen was used in a three-dimensional complement-fixation test<sup>5</sup> with a known positive serum with overnight primary fixation at 4° C. Antigen and antiserum contours were drawn, and from these contours the unit complement contour was constructed; this contour line denotes the antigen-antibody mixtures which fix exactly one unit of complement (Fig. 2). For a constant antigen, two-dimensional complement-fixation test, a dilution of 1 in 15 of this purified antigen was selected from the optimal range of antigen dilutions which gave a maximum serum titre. It contained  $5 \times 10^7$  parasites per ml. Fifteen human sera were tested using the plate method<sup>6</sup>. One plate ruled



Table 1. THE DESIGN OF A COMPLEMENT-FIXATION TEST

Plate constant: diluent					Plate constant: chosen antigen dilution					5 Row variable: doubling dilutions of serum
1	2	3	4	5	6	7	8	9	10	
										10
										20
										40
										80
										160
										320
										640
										1,280
										— Antigen control
C'(1)	C'(2)	C'(3)	C'(4)	—	C'(1)	C'(2)	C'(3)	C'(4)	—	

Column variable: 4 complement dilutions at 0.2 log<sub>10</sub> intervals. C'(4) contains less than 1 unit.

with 100 squares was used for each serum; the design of the test is shown in Table 1. The sera were inactivated at a dilution of 1 in 5 for 0.5 h at 56° C. The row variable on each plate was a set of 9 doubling dilutions of one of the sera starting with a dilution of 1 in 5. The tenth row was a control containing diluent but no serum. The column variable on each plate was a set of four complement dilutions spaced at 0.2 log<sub>10</sub> intervals. The first dilution in the set contained more than one unit of complement and the fourth dilution contained less than one unit. The set in columns 1, 2, 3 and 4 was repeated in columns 6, 7, 8 and 9. The fifth and tenth columns were controls containing diluent but no complement. A drop of diluent was added to each of the 50 squares on the left half of the plate and a drop of the selected antigen dilution was added to each of the 50 squares on the right half of the plate. The right half of the tenth row was the antigen control.

After overnight fixation at 4° C the titres of the sera were defined as the highest dilution which fixed

one unit of complement at the selected antigen concentration.

To test the feasibility of freeze-drying the antigen, a single contour at the selected level (1 in 15) was estimated with a known positive human serum. The contours are shown in Fig. 3.

In Table 2 are shown the titres determined on 15 human sera by the dye test, direct agglutination and by complement fixation.

Table 2. COMPARISON OF THE TITRES OBTAINED FOR *Toxoplasma gondii* ON 15 HUMAN SERA BY DIFFERENT SEROLOGICAL METHODS

Human serum	Dye test	Agglutination*	CF*
1	Neg.	8	7
2	Neg.	< 8	7
3	Neg.	8	5
4	Neg.	8	20
5	Neg.	8	< 5
6	128	128	80
7	128	256	80
8	32	128	40
9	256	1,024	40
10	256	256	20
11	4,096	2,048	640
12	4,096	8,192	> 1,280
13	16,000	8,192	1,780
14	1,024	1,024	1,280
15	2,048	2,048	1,280

\* Purified antigen from peritoneal exudate of cotton rats.

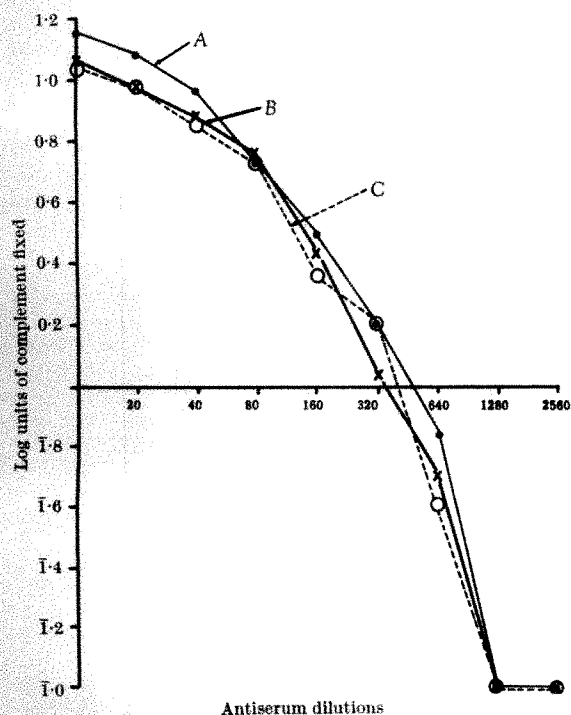


Fig. 3. Antigen contours. Antiserum, a known positive human serum. Antigens, A, standard antigen + equal volume of saline; B, standard antigen + equal volume of salt-free dextran; C, antigen dried with dextran and reconstituted.

There is a considerable degree of correlation in the results obtained by the different methods. It is usual to find the titres determined by complement fixation to be lower than those found in the direct agglutination or dye test. The complement-fixation test is more exacting to perform but is capable of a precision not always shared by the other tests. It also has the advantage that the antigen can be preserved by freeze-drying. This is not possible if the dye test is used because the parasites must be viable, and the agglutination test is also impracticable because the suspension of parasites after drying is auto-agglutinable.

Interest in *Toxoplasma gondii* was mainly zoological until 1939 (ref. 7), when it was isolated from children suffering from encephalomyelitis. Congenital and acquired forms of the disease are recognized and the parasitic attack is directed mainly against nervous tissue. Brain cord and eye are most affected in the congenital forms of the disease. Distribution is world-wide, and the interest of the public health authorities is evidenced by the provision of two reference laboratories in the United Kingdom where serological tests for the detection of *Toxoplasma* antibodies are performed.

Many of these tests have been devised and their number suggests that diagnosis of the disease by sero-



logical methods has not yet been perfected. The dye test and the complement-fixation reaction are probably most widely used. In the latter test the antigens have been so variable in origin that the results of tests from laboratories throughout the world could not be compared with any confidence. By the preparation of a pure antigen this difficulty has now been resolved. Further, the antigen can be freeze-dried and reconstituted and lends itself to accurate standardization by parasite counts, by nitrogen content or more simply by optical density measurements under standard conditions. It has been suggested that the World Health Organization should prepare a standardized serum for use throughout the world. It seems to us equally important that a standard antigen should be available for workers in this field, and its preparation is now feasible.

Earlier experiments on the immunization of rabbits with this pure antigen gave rise to a steady increase in the titre of antibody in serum in the direct agglutination and complement-fixation tests, indicating that a true antigen-antibody reaction was taking place. Since it is the same parasite which infects man, the results with human serum should be entirely specific. Although

widespread serological surveys have been carried out among populations in the past, correlation of the results with the clinical picture has been rare. The method of spread of the disease is still obscure and the acute disease is rarely detected except in cases occurring by accident in laboratories and they number less than a dozen. There is widespread occurrence of toxoplasmosis among sheep in Britain and, as in human populations, abortion, premature births and still-births are common. Isolation of the causative organism would be more satisfactory, but a serological test sufficiently sensitive and specific would give valuable information on the true prevalence of toxoplasmosis to-day. International co-operation is a desirable aim.

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## THE NEURONE SURFACE

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**R**ECENTLY we observed that isolated neurones lacked the typical electron micrographic image of a plasma membrane, and suggested that if one accepts that the neurone surface constitutes part of a liquid-crystalline phase system it should be possible to develop a medium in which the molecular orientation responsible for the electron dense image of a surface membrane is maintained<sup>1</sup>. The fact that we have now been able to devise such a medium emphasizes the interfacial characteristics of the neurone membrane as distinct from true solid, semi-solid membrane characteristics<sup>2</sup>. Since in this medium neurones are subjected to the same isolation procedure and hence the same mechanical stresses, the alternative view that the neurone membrane is a separable morphological entity appears unlikely. Recently there has been a revival of interest in visualizing cell membranes as part of multiphasic systems<sup>3,4</sup>, but supporting evidence from experiments on cells has hitherto been lacking.

The isolation of neurones in Ringer-Locke solution leads to extensive disruption of the surface, a membrane image being seen only where boutons are present. In the work recorded here, therefore, neurones prepared in Ringer-Locke solution served as the controls. The experimental media used were a 0.9 per cent solution of NaCl, and three solutions containing 0.25 mg, 0.55 mg and 1.1 mg of gangliosides per ml. of 0.9 per cent NaCl respectively. The pH of all media was 7.2-7.4. The first of these media was chosen since it was believed that the absence of bivalent ions would tend to increase the hydration of surface molecules. It has been shown, for example, that calcium ions decrease the water content of phospholipid membrane models<sup>5</sup>. Since the stability of particular liquid-crystalline systems is highly dependent on their degree of hydration, we believed that removal of the neurones from their supporting environment had led to a loss of hydration of surface molecules and a consequent disruption of their orientation. A number of polyanionic molecules were considered as possible constituents of the other media, but the gangliosides were chosen for preliminary investigations since their presence in brain tissues is well established and they have been shown to be involved in ion movements across cell membranes<sup>6</sup>. It is

known that gangliosides interact with inorganic cations<sup>7</sup>; therefore, in order to leave free anionic groups for interaction with neurone surface molecules they were added to the simple medium. The action of individual ganglioside molecules on surfaces may be expected to be at a maximum at the critical micelle concentration (CMC); therefore, the choice of the amounts of gangliosides used was made on the basis of the reported CMC in water and its alteration in NaCl solutions<sup>7</sup>. The gangliosides (a special order from Light-Koch Laboratories, Ltd.) were prepared according to Gammaack<sup>7</sup> and contained 26.5 per cent *N*-acetyl neuraminic acid. On thin-layer chromatography they were resolved into five major bands corresponding to mono-, di-, and tri-sialogangliosides<sup>8</sup>.

Neurones from the lateral vestibular nucleus of ox brain were isolated by the method previously described<sup>1</sup>. Ten to twenty isolated cells were placed in polythene planchets, the medium was withdrawn and the neurones were fixed, embedded, and sectioned for electron microscopy. Several batches of cells were prepared in each medium and most were fixed by osmication, but some were fixed with buffered glutaraldehyde. More than 2,000 sections from 50 neurones selected randomly were examined. The results reported here refer to effects on the soma and dendrite surfaces only. We have observed that the surface structure of the axon hillock and the initial segment of the axon appears to differ from that of the soma and dendrites, and this will be the subject of another communication.

Considerable maintenance of an electron dense surface lamina was achieved, the most striking results being obtained with the media containing 0.55 mg and 1.1 mg gangliosides per ml. 0.9 per cent NaCl. However, the weakest solution of gangliosides, and the 0.9 per cent NaCl alone, gave some retention of the image. In agreement with previous observations<sup>1</sup> all cells prepared in Ringer-Locke solution lacked a membrane image except where boutons remained attached. Fig. 1 shows a typical Ringer-Locke preparation in which the neurofilaments appear to be flying out from the surface. Fig. 2a shows part of the soma of a cell prepared in 0.9 per cent NaCl in which the membrane image is lacking but the surface does not show the complete disarray seen in Fig. 1. Fig.

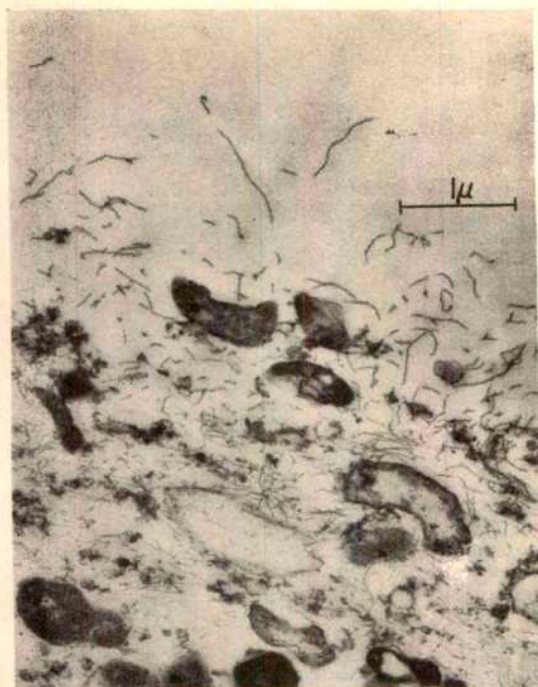


Fig. 1. Soma surface of cell prepared in Ringer-Locke solution

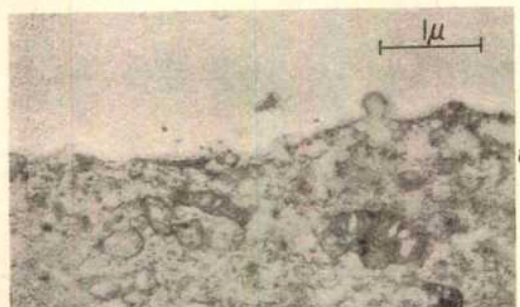
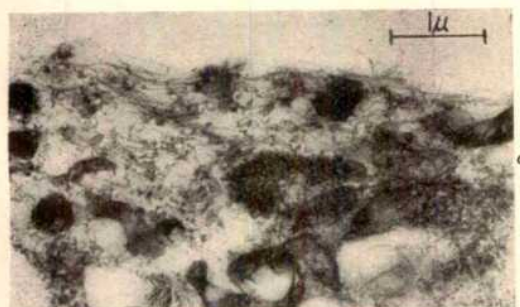


Fig. 2. Soma surface of cells prepared in 0.9 per cent NaCl. (a) Showing membrane lacking; (b) showing partial retention of membrane

2b also shows part of the soma of a neurone prepared in 0.9 per cent NaCl but one in which there appears to be considerable maintenance of surface structure. This effect was noted in approximately 20 per cent only of the 0.9 per cent NaCl samples. The addition of gangliosides to the media had a pronounced effect on retention of surface molecular orientation. More than 50 per cent of the neurones selected from the weakest ganglioside medium (0.25 mg/ml.), and 80–90 per cent of the cells examined from the stronger ganglioside solutions, showed considerable maintenance of the membrane image. In Figs. 3 and 4 are shown parts of the soma and dendrite surfaces of neurones prepared in 0.55 mg and 1.1 mg gangliosides

per ml. 0.9 per cent NaCl respectively. In general, when the membrane image was maintained it appeared as a continuous dense line 50–80 Å thick (Figs. 3 and 4a). In osmium tetroxide fixed material electron microscopy usually reveals a single dense line 60–80 Å thick at cell

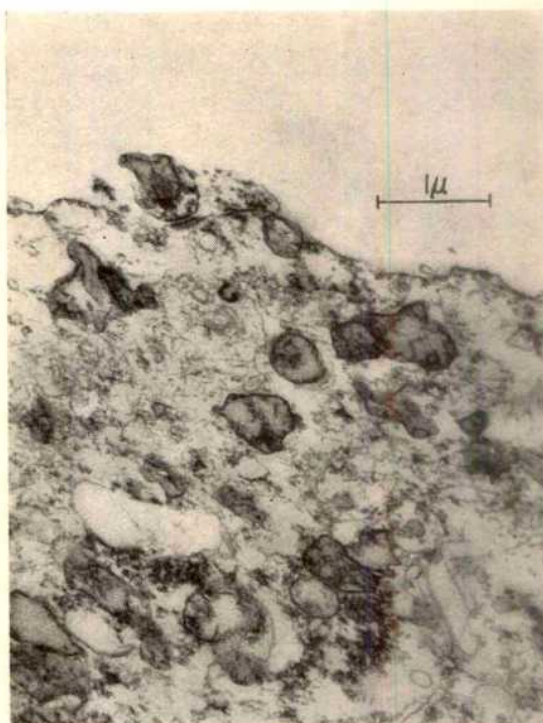


Fig. 3. Soma surface of cell prepared in 0.55 mg gangliosides per ml. 0.9 per cent NaCl

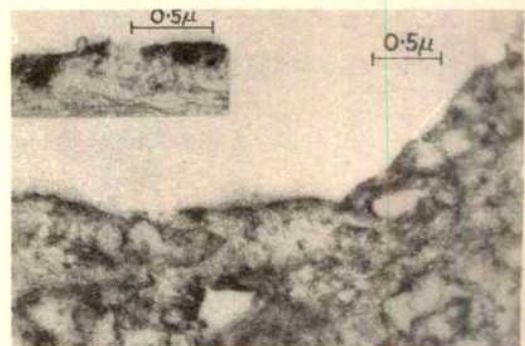
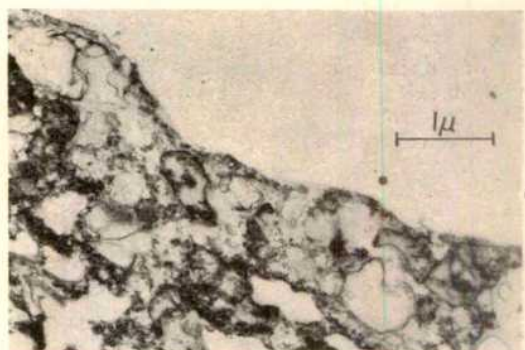


Fig. 4. (a) Soma surface of cell prepared in 1.1 mg gangliosides per ml. 0.9 per cent NaCl; (b) soma surface of cell prepared in 1.1 mg gangliosides per ml. 0.9 per cent NaCl showing precipitation on cytoplasmic side. Inset: membrane of dendrite showing heavy precipitate



surfaces<sup>9</sup>. It has not been possible at this stage to determine whether permanganate fixation would reveal the triple surface structure described by Robertson<sup>10</sup> since it was found that reaction of potassium permanganate with gangliosides led to incomplete fixation. It is hoped to overcome this difficulty in further investigations. In places the surface line was broken or appeared to have 'bubbles', examples of which may be seen in Figs. 3 and 4. In several samples of cells prepared in the strongest ganglioside solution there appeared to be precipitated material on the cytoplasmic side of the membrane (Fig. 4b). This effect was seen most frequently on dendritic surfaces and may indicate a special property of some dendrites. Some of these observations may be explained by variations in the angle between the cell surface and the plane of sectioning. Such effects have been discussed in detail by Goldberg and Green<sup>11</sup> in relation to the sectioning of fibroblasts from tissue cultures. In a number of respects the type of structure seen at the surface of neurones isolated in the presence of gangliosides and fibroblasts from cultures is similar.

The results obtained are indicative of phase phenomena in operation at the neurone surface. The isolated neurones are free from glia and are to a considerable extent stripped of boutons. Thus removed from the influence of the opposing phases containing polyanionic molecules and placed in a medium like Ringer-Löcke containing bivalent ions, the surface molecules of the neurone suffer a loss in hydration and a disruption of their orientation with consequent loss of the electron dense image. In these circumstances the neurones appear to exist in a gel-like state<sup>1</sup>. The isolation of the neurones in the ganglioside solutions simulated the natural opposing phase systems sufficiently well to maintain certain features of the usual membrane image. It may be surprising that the isolation of neurones in the absence of bivalent ions was sufficient to maintain the membrane image in some cases; however, this was anticipated for two reasons. First, during the isolation procedure it is likely that some polyanionic molecules such as gangliosides remain associated with the neurone surface and in the absence of bivalent ions their effect on surface molecules is not completely lost. Furthermore, the absence of calcium may be expected to favour water retention<sup>5</sup>. It may indeed be anticipated that the maintenance of the electron dense image of a surface membrane of any isolated cell or sub-cellular particle will depend largely on the relative affinity of stabilizing polyanionic molecules for the isolated phase. Thus, since gangliosides are concentrated in the nerve endings<sup>12,13</sup> they retain the membrane image on isolation<sup>14</sup>.

It is now possible to visualize how the surface lipoprotein molecules of neurones and their supporting glia and boutons are stabilized as opposing liquid-crystalline phases in such a way that the resultant interface provides the functional basis of an excitable membrane. It appears that the polyanionic gangliosides located predominantly in the nerve endings<sup>12,13</sup> serve two major functions. By virtue of their hydrophilic properties they regulate the degree of hydration of surface molecules and hence the stability of liquid-crystalline phases. Furthermore, they prevent coalescence of the phases by having affinity for both and by providing an interfacial barrier of ionic charges which is enhanced by their ability to interact with cations. Mucopolysaccharides located in the glia may be visualized as serving similar functions. Thus, the phases represented by the electron dense images of the neurone, glia and bouton membranes and the non-electron dense interface together form the functional unit.

The proposed system is endowed with considerable lability since any event which alters the ion- and water-binding states of interfacial polyanions would alter the attractive forces between the long-chain molecules of the lipoproteins in opposing phases, probably their phase form, and the diffusion properties of the system. It is to be expected that the magnitude of the non-electron-dense

interface seen between neurones and their supporting glia and boutons will differ with the composition and tonicity<sup>15</sup> of the preparative and fixative solutions. It follows that we do not support the view that there is appreciable extracellular space or 'gap substance'<sup>16</sup> between neurones and their surrounding elements since the hydrated polyanionic molecules at the interface are visualized as an integral part of the membrane system. Wyckoff and Young<sup>17</sup> by electron microscopy first showed that there are no large spaces or intercellular matrix between nervous elements and they concluded that exchanges between neurones and capillaries probably take place through the glial cytoplasm. On the basis of the present theory it must be concluded that their postulations are correct. Lumsden<sup>18</sup>, Sjöstrand<sup>19</sup>, de Robertis<sup>20</sup>, and Karlsson and Schultz<sup>15</sup> have also reported results and expressed opinions which are in agreement with Wyckoff and Young's original statements.

The complete physiological integrity of the proposed system is undoubtedly dependent on many factors. It is to be expected that the presence of substrates involved in oxidative phosphorylation is intimately involved. The present interpretation of the results is dependent on the physical limits set for the cell surface. If a third phase or 'gap substance' is visualized it may be proposed that the use of media free of calcium or containing calcium chelating agents decreased the adhesion between the opposing 'membranes' of the neurones and their surrounding glia and boutons, thus favouring retention of the neurone membrane. Further investigations designed to clarify this point are in progress. It appears, however, that the role of gangliosides may well be dominant. Several reports on the properties of gangliosides clearly indicate their importance in the control of nervous activity. They appear to be acceptors for serotonin in the central nervous system<sup>21</sup> and have been shown to interact with chlorpromazine<sup>22</sup>. Recently, Burton *et al.*<sup>23</sup> have suggested that gangliosides are involved in the transport of acetylcholine to the pre-synaptic membrane. McIlwain<sup>6</sup> has demonstrated that the action of gangliosides in restoring excitability to isolated cerebral tissues is related to ion movements across cell membranes. The results and hypotheses presented here clarify the mechanisms involved.

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## NEURONE—SATELLITE CELL RELATIONSHIP

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SINCE the first recognition of macroglia as distinct from neurones by Virchow and the first suggestion by Holmgren that these cells are concerned with the metabolic support of neurones, much confirmatory evidence for the latter concept has accumulated. Apart from the intimate cell contacts and membrane relationships revealed by light and electron microscopy, there is morphological and experimental evidence that the number of perikaryal satellite cells is related directly to the functional load imposed on the perikaryon by the maintenance of varying lengths of axon and by nervous activity (see review by Hamberger<sup>1</sup>). Microchemical methods have demonstrated that the perikaryon and its satellite cells are complementary, at rest or in nervous activity, in respect of RNA base composition, RNA and protein content, and enzymatic activity<sup>2</sup>. The results suggest that the perikaryon and its satellite cells form a metabolically linked cyto-physiological unit. However, analysis of this linkage has been hampered by the use of an experimental model with a mixed population of oligodendrocytes and astrocytes. The problem of mixed samples is aggravated by the lack of agreement among electron microscopists on the identification of glia-cell types<sup>3</sup>.

Again, investigations of the neurone-satellite cell relationship are incomplete if only the perikaryon of the neurone is considered. Yet work on the axon and its satellite cells has been limited in range if not quantity. The intimacy of this relationship in peripheral nerve has recently been brought home by the description of paranodal columns of Schwann-cell cytoplasm which are packed with mitochondria<sup>4</sup>, and of highly regular nodal processes of Schwann-cell cytoplasm which have a specific internal structure and end in close apposition to specific reception areas on the unit membrane of the nodal axon<sup>4</sup>. Since the satellite-cell columns contain perhaps sixty times the number of mitochondria in the subjacent axoplasm, they may be an important site for the production of energy-rich compounds. These may be transported to the axonal membrane, via the nodal processes, to provide the energy to drive, for example, the ionic pump-mechanism; the nodal processes may also facilitate interchange of other materials between the satellite-cell cytoplasm and the axon<sup>5</sup>. Whereas the number of satellite cells related to the perikaryon depends on functional considerations<sup>1</sup>, the number of satellite cells related to the axon in myelinated peripheral nerve fibres depends on morphogenetic and growth processes: since the number of satellite cells and internodal segments is determined at the onset on myelination<sup>6</sup>, the effects of functional load on such a fibre may be reflected in the degree of specialization and complexity of its nodal and paranodal apparatus. Biochemical and metabolic investigations of peripheral nerve have seldom considered the role of the satellite cells; results of such investigations have usually been interpreted as intrinsic to axons or to fibres as a whole. However, the enzymatic activities of the sheath and axoplasm of squid giant fibres seem to be complementary<sup>6</sup>. No comparable specific paranodal apparatus or nodal processes have been described in the central nervous system, but again the axon and the satellite cells show complementary enzymatic activity in certain human fibre tracts<sup>7</sup>.

Investigations on the optic nerve head of the cat have cast light on some of these problems. In the cat, as in man, the fibres of the optic nerve appear to be myelinated only behind the lamina cribrosa. In and anterior to the

lamina all the fibres are non-myelinated, as judged by light microscopy. Silver investigations revealed that, whereas the post-laminar part contains both astrocytes and oligodendrocytes, the laminar and prelaminar parts of the nerve contain only astrocytes<sup>8</sup>. This permitted the identification of astrocytes by electron microscopy and the correlation of the ultrastructural and light microscopical characteristics of three glia-cell types<sup>9</sup>. The first type was found in association with non-myelinated fibres in the prelaminar region where it corresponded to the small (spider) astrocyte: its extensive processes and its perikaryon contained abundant closely packed fine fibrils, but other cytoplasmic inclusions were not conspicuous. A second type was found in the immediately prelaminar or laminar regions and corresponded to the typical astrocyte: its ultrastructural characteristics were intermediate between those of the other two types; associated with it were a number of finely myelinated fibres, unrevealed in this situation by light microscopy. A third type was found only in the post-laminar region in association with more-heavily myelinated fibres, and thus corresponded to the oligodendrocyte: neither its smaller and less numerous processes nor its perikaryon contained fibrils; the denser perinuclear cytoplasm was less voluminous, but ribosomes and orientated endoplasmic reticulum were prominent. So far as the fibres were concerned, no specific organized paranodal apparatus was seen outside the myelin sheath. In this respect paranodal or juxta-terminal sections of fibres of the central nervous system<sup>10</sup> resemble those of similar-sized fibres of the peripheral nervous system<sup>11</sup>. Other differences would appear to relate to a division of labour among the satellite cells of the central nervous system. In myelinated peripheral nerve fibres, regardless of size, the internodal axon is invested with a myelin sheath and the nodal axon is supplied with specific processes by the same Schwann cell: the nodal processes are embedded in gap substance and the whole complex is surrounded by basement membrane<sup>4</sup>.

In the central nervous system, on the other hand, the internodal axon is invested with a myelin sheath by one or more glial processes<sup>12</sup> and the nodal axon is related to other glia or nerve processes<sup>10</sup>. The dual sheath-supporting and node-supporting roles of the Schwann cell would appear to be responsible for the specialized nature of the nodal and paranodal apparatus of peripheral nerve. The dichotomy of these roles in the central nervous system is associated with a simpler node-form: when glial processes are related to a central node they are irregular, are not embedded in gap substance and are not surrounded by a basement membrane<sup>13</sup>; no specific internal structure, no particularly intimate apposition to axonal membrane and no specific reception areas on the membrane have been seen. The relatively unspecialized nature of the central node and the occurrence of variants<sup>14</sup> make it very difficult to distinguish nodal axons from non-myelinated axons in transverse sections<sup>10</sup> and relative counts of mitochondria in nodal axon and nodal glia have not been possible. However, a reciprocal relationship between the numbers of internodal axonal mitochondria and the thickness of the myelin sheath was noted as fibres were traced back from the prelaminar region of the optic nerve. This was reflected in the histochemical activity of the mitochondrial enzymes, succinic and malic dehydrogenase, in the axons. Activity was highest in the non-myelinated prelaminar parts of the axons and fell off as they were traced back and

acquired myelin sheaths of increasing thickness. On the other hand, in the macroglia the response was predominantly oligodendrocytic, and thus post-laminar and associated with myelinated fibres. This suggestion that the citric acid cycle operates primarily within the axon of the non-myelinated part of the cat optic nerve, and within the satellite cells of the myelinated part, favours the concept of the oligodendrocyte as an energy-donor<sup>15</sup>, specifically at the central node. The response to histochemical tests for lactic dehydrogenase was also predominantly oligodendrocytic, suggesting that these cells have a capacity for anaerobic glycolysis: other evidence suggests that glia cells (mixed samples) may resort to this under conditions of intense nervous activity<sup>2</sup>. The response for  $\alpha$ -glycerophosphate dehydrogenase, on the other hand, was predominantly astrocytic, suggesting active lipid metabolism in these cells. It has been suggested that glia cells, which have a high lipid content (mixed samples), may resort to utilizing lipoproteins as an energy source<sup>13</sup>, but activity may well be concerned with phospholipid membrane production and turnover. These and other results, which will be reported in detail elsewhere, provide further evidence of the ultrastructural and enzymatic differentiation of satellite-cell types and of the complementary relationship between neurones and satellite cells. Histochemical investigations are being extended both in range of enzymes and sites for examination.

An understanding of the neurone-satellite cell relationship demands that all its vagaries be explored—perikaryal and axonal, central and peripheral. However, the interpretation of certain morphological differences revealed at different sites should be approached with caution. Despite their electron density, the polysaccharide-rich nodal gap substance and basement membrane of the peripheral node surely present no barrier to metabolites when particles as large as 150 Å can traverse the cornea<sup>16</sup>, with its chemically similar, but many times thicker, membranes: indeed, the matrix of the peripheral node may favour rather than hinder the movement of ions and facilitate

metabolic exchange at discrete intervals along the peripheral nerve fibre. From observations on the leech<sup>17,18</sup>, it would seem unlikely that satellite cells play an active part in the signalling activity of the nervous system or that they are directly concerned with the action potential mechanism of nerve cells. It is also unlikely that satellite-cell cytoplasm forms a significant part of the local electrotonic circuit which activates neighbouring nodes: gap substance and basement membrane are much more likely to be involved. Certainly, in the leech, the glia cell is not involved in the diffusion and probably not in the active transport of ions: the 150 Å intercellular spaces shown by electron microscopy serve as pathways for the flow of current and are utilized for the transport of ions and molecules as large as sucrose<sup>17,18</sup>. Progressive elimination of possible roles directs attention more than ever to the metabolic and myelinogenic aspects of the neurone-satellite cell relationship.

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## SURVIVAL OF FROZEN CHONDROCYTES ISOLATED FROM CARTILAGE OF ADULT MAMMALS

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FOR many years plastic surgeons have been successfully using autografts and homografts of fresh, living fibrocartilage in reconstructive surgery of the head, neck and chest in man<sup>1</sup>. Recently, living hyaline cartilage has been successfully homografted into joints of animals<sup>2-5</sup>. Similar techniques may be applicable in the treatment of human arthritis. One difficulty is to obtain fresh human cartilage when it is needed. The chondrocytes only survive for about one week when cartilage is stored *in vitro* at temperatures just above 0° C. Grafts of cartilage in which the cells are dead give less satisfactory results<sup>1</sup>. As long ago as 1957, Gibson<sup>6</sup> expressed the need for a method of banking fibrocartilage for long periods at low temperatures in a potentially viable state. A bank of hyaline cartilage may also soon be needed.

During the past 15 years various types of cells and tissues from adult mammals have been stored at low temperatures in a state of suspended animation in media containing glycerol<sup>7</sup>. So far, cartilage from adult mammals has not survived freezing in the presence of glycerol. For example, Curran and Gibson<sup>8</sup> found that the chondrocytes were killed after cooling cartilage to -15° C in saline containing 15 per cent glycerol. On the other hand, Heyner<sup>9,10</sup> found that cartilaginous bones from 17- and 18-day-old embryonic rats survived cooling to -79° C

in the presence of 15 per cent glycerol, provided that the matrix had previously been partially removed by tryptic digestion. Viability of the cartilage cells was assessed by elongation of the rudimentary bones in organ culture and by histological studies. Cartilage from older embryos or adult mammals does not survive similar treatment.

Recently, dimethyl sulphoxide (DMSO) has proved to be superior to glycerol in protecting certain adult mammalian cells and tissues, including human and bovine erythrocytes<sup>11</sup>, bone marrow<sup>12</sup>, the corneal endothelium<sup>13,14</sup> and smooth muscle<sup>15</sup> during freezing to, storage at, and thawing from, low temperatures. An essential feature of glycerol and DMSO is their low toxicity<sup>16</sup>. Their molecules must be in contact with and actually penetrate the living cells which are to be frozen and thawed; then, after thawing, they must be removed before the cells and tissues will resume their normal functions. DMSO diffuses into and out of cells and through capillary walls and tissue spaces more readily than does glycerol<sup>17</sup>. It has always proved more difficult to freeze tissues than to freeze isolated cells without loss of viability; tissues from adult mammals have a higher cell mortality than comparable embryonic tissues frozen, banked and thawed by the same technique. This may be due, in part, to the nature of the intercellular material which hinders equi-



bration of the component cells with protective substances in the medium both before cooling and during the process of separation of water as ice. The matrix of cartilage is composed predominantly of a mucopolysaccharide ground substance containing a variable proportion of collagen and elastic fibrils. If cartilage were to be kept alive during banking at low temperatures it would be essential to ensure the presence in and around the living chondrocytes of a protective agent such as DMSO. The concentration of this substance should not in itself be harmful, but it should be adequate to prevent damage due to electrolytes during the processes of freezing and thawing<sup>15</sup>.

An obvious way of attacking the problem was to start by liberating the chondrocytes from adult cartilage. Their viability before and after freezing in different media could then be assessed. Several methods have been described for isolating the cells from minute fragments of embryonic cartilage of birds and mammals by tryptic digestion<sup>18-20</sup>. Their ability to incorporate radioactive sulphur (<sup>35</sup>S) and to manufacture collagen and mucopolysaccharide in tissue cultures was tested. Hitherto, however, no method has been established for isolating chondrocytes in large numbers from pieces of fresh adult cartilage, although an ingenious technique for obtaining them in small numbers from fixed sections by microdissection has been described<sup>21</sup>. New methods had to be developed. My object was to remove the ground substance and fibrils in which the cells are embedded by successive treatment with different proteolytic enzymes at concentrations and under conditions which would not damage the cells.

The first enzyme selected was papain. When activated by cysteine, or any agent which reduces disulphide, papain removes mucopolysaccharides from cartilage and other tissues as shown by Thomas<sup>22</sup> and by Tsaltas<sup>23</sup>. The second enzyme was collagenase from *Cl. histolytica*, which digests native and denatured collagen and was purified and freeze-dried by MacLennan and his co-workers<sup>24,25</sup>. It was obtained from the Sigma Chemical Co., St. Louis, Mississippi. The third enzyme chosen was pronase (grade B), a protease obtained from *Streptomyces griseus* by Nomoto and his colleagues<sup>26</sup>, which was used to remove the zona pellucida of developing mouse eggs<sup>27,28</sup> and has also been used instead of trypsin to disperse mammalian cells in cultures<sup>29</sup>. Pronase was obtained from Calbiochem, Inc., Los Angeles.

The experimental method was as follows: rabbits, dogs and monkeys were the animals used. Strips of fibro-cartilage (2.0 × 0.4 × 0.1 cm) were cut from the pinna, and fragments of hyaline cartilage (less than 1 mm thick and less than 3 mm in length and width) were sliced from the articular surfaces of the larger joints. Human fibro-cartilage from a damaged meniscus from a knee joint, and hyaline cartilage removed at operation from an ankle joint were cut into similar-sized pieces. The strips of fibro-cartilage or 80-100 mg of hyaline cartilage were suspended in 4 ml. of Gey's balanced salt solution adjusted to pH between 7.0 and 7.4 by bubbling with air containing 5 per cent CO<sub>2</sub>. 0.4 ml. of a 1 per cent solution of crude papain was added to give a final concentration of 1 mg/ml. A solution of cysteine was added to give a final concentration of 0.003 M. After incubation for 1 h at +37° C the fragments of cartilage were washed. Some were fixed, embedded and sectioned histologically. They were stained with alcian blue and kernectrot and examined by ordinary illumination, or stained with euchrysin 3R and examined by fluorescence microscopy as previously described<sup>30</sup>. They did not show the staining reaction or the fluorescence characteristic of mucopolysaccharide ground substance. Other pieces of cartilage from animals and man were digested with papain, washed three times with 10 ml. of Gey's solution, and incubated with collagenase (0.2 mg/ml.) in Gey's solution at pH 6.8-7.4 for 16-20 h at +37° C. After this treatment blobs and strands of gelatinous material still remained. Under the phase contrast microscope the chondrocytes

could be clearly seen surrounded by amorphous material and delicate strands resembling elastic fibrils. A solution of pronase was then added to the medium containing collagenase, to give a final concentration of 0.2 mg pronase per ml. Incubation was continued for a further period of 16-24 h at +37° C. Sterile instruments, glassware and solutions, and aseptic techniques, were used throughout the enzyme treatment. Antibiotics were included in the media (penicillin 100 units per ml.; streptomycin 100 µg per ml., mycostatin 50 units per ml.). At the end of the 1.5-2 days' incubation the medium was turbid and contained minute visible particles but no fragments recognizable as cartilage. Prolonged digestion with trypsin (0.2 per cent) or any of the three enzymes used alone was less effective in disintegrating cartilage. The tubes were centrifuged, the supernatant fluid was discarded and the deposit resuspended in 10-15 ml. of Gey's solution. Centrifugation and re-suspension were repeated. After centrifuging for a third time the deposit was resuspended in 0.25 ml. of homologous serum or Gey's solution. Drops of the washed and concentrated suspension were mounted on slides coated with a thin film of 1 per cent agar in Ringer's solution. The preparations in serum were sealed and examined by phase contrast microscopy at +37° C. Those in Gey's solution were treated with an equal volume of euchrysin (1 in 50,000) and examined by fluorescence microscopy. Large numbers of intact isolated chondrocytes were seen. Those obtained from fibrocartilage contained one or more vacuoles and were often binucleate. Those from hyaline cartilage contained many granules and few if any vacuoles. When viewed by phase contrast microscopy at +37° C they took up amoeboid forms; when filmed by time-lapse cinematography they made active movements for periods varying from 6 h with chondrocytes from rabbit hyaline cartilage to 24 h with chondrocytes from the dog, monkey and man<sup>31</sup>. When stained with euchrysin and viewed by fluorescence microscopy their nuclei fluoresced a vivid yellowish green,

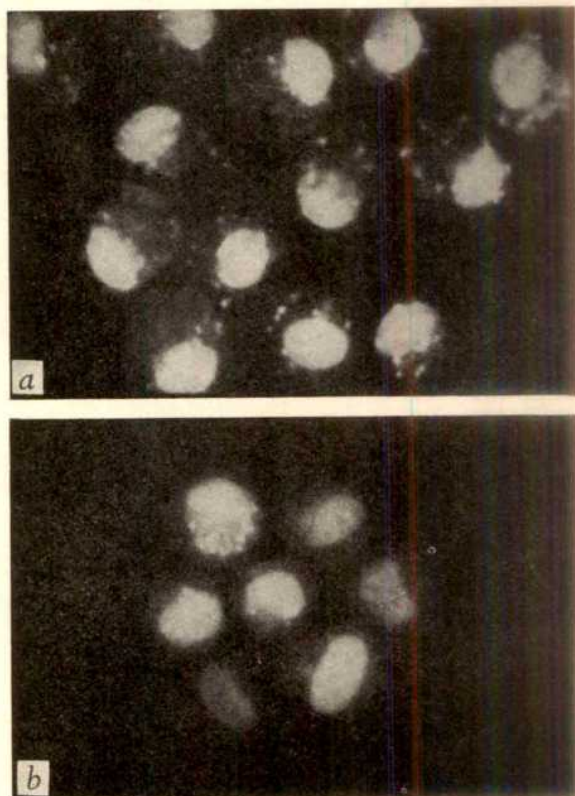


Fig. 1. Freshly isolated chondrocytes. a, From dog hyaline cartilage (× 1,500); b, from human hyaline cartilage, after treatment with 10 per cent DMSO (× 1,500)



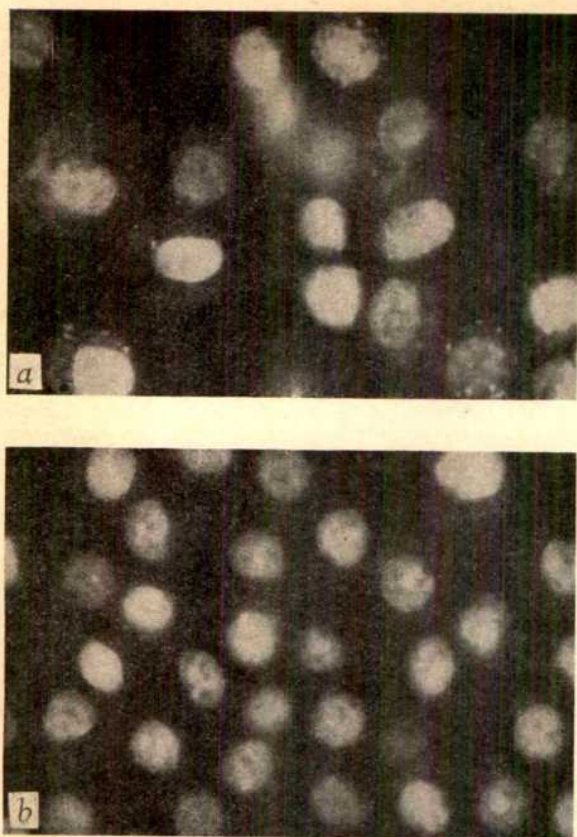


Fig. 2. Chondrocytes after freezing to and thawing from  $-79^{\circ}\text{C}$ . *a*, From dog hyaline cartilage ( $\times 1,500$ ); *b*, from human hyaline cartilage ( $\times 1,250$ ).

the nuclear sex chromatin body fluoresced bright yellow in cells from female animals, while minute granules fluorescing a brilliant orange-red could be seen in the cytoplasm (see Fig. 1, *a* and *b*). The cytological details varied in the chondrocytes from fibro- and hyaline cartilage of the four species examined. Some naked nuclei and cellular debris could be seen both by phase contrast and by fluorescence microscopy. They were most numerous in the preparations of rabbit chondrocytes and comparatively rare in preparations of dog and human chondrocytes. In recent experiments on rabbit cartilage the concentrations of enzymes have been doubled. With the larger fragments of dog cartilage the concentration of papain has been increased to 4 mg/ml. and the concentrations of collagenase and pronase increased tenfold; the incubation period was reduced to 1.5 h with collagenase and 5 h with pronase. There were many isolated cells in suspension and no obvious increase in the numbers of dead cells, but some partially digested fragments remained.

The main object was to determine whether isolated chondrocytes would survive freezing and thawing. Washed deposits of the cells obtained from hyaline cartilage of the different species were suspended in 1-ml. volumes of medium containing 10 per cent v/v DMSO, 40 per cent rabbit serum and 50 per cent Gey's solution. The suspensions were sealed in ampoules and cooled slowly to  $-79^{\circ}\text{C}$  at the rate of approximately  $1^{\circ}\text{C}/\text{min}$  to  $-20^{\circ}\text{C}$  and  $4^{\circ}\text{C}/\text{min}$  between  $-20^{\circ}\text{C}$  and  $-79^{\circ}\text{C}$ . They were kept at  $-79^{\circ}\text{C}$  for periods varying from one hour to one week. The ampoules were then immersed in water at  $+40^{\circ}\text{C}$  to thaw their contents rapidly. The suspensions were diluted gradually with 1 ml. of serum and then with 8 ml. of Gey's solution to reduce the concentration of DMSO to 1 per cent. They were centrifuged and the deposit was resuspended in 0.25 ml. of serum or Gey's solution for examination by phase contrast microscopy or fluorescence microscopy.

A high proportion of the thawed chondrocytes from all four species were normal in appearance under the phase contrast and fluorescence microscopes (Fig. 2, *a* and *b*), but in each instance there were more naked nuclei and disintegrated cells than in preparations of unfrozen chondrocytes. In some cells the cytoplasmic granules were larger and more heavily stained than normal. Time-lapse cinematography showed that the intact frozen cells were highly active for 4–6 h at  $+37^{\circ}\text{C}$  (ref. 31). In preparations of frozen dog, monkey and human chondrocytes, active cells were present after 8 h and occasionally after 24 h at  $+37^{\circ}\text{C}$ . Comparatively few of the frozen rabbit chondrocytes survived for 8 h.

The viability of fresh and frozen rabbit chondrocytes was also tested by homografting on to cancellous bone in prepared defects either on the outer table of the iliac bone or on the head of the humerus. Six weeks later the grafted areas were removed at necropsy and processed histologically. The sections were stained with alcian blue and kernelectrot. They showed large numbers of apparently normal chondrocytes arranged in columns and surrounded by well-stained matrix, whether the isolated cells implanted had been frozen or not<sup>32</sup>.

The survival of homografts of fresh untreated cartilage is thought to be due to a protective effect of the mucopolysaccharides in the matrix<sup>33,34</sup>. The rapid re-formation of matrix may have been responsible for the absence of plasma cells and lymphocytes and other signs of a homograft reaction in the vicinity of our grafts of chondrocytes.

Methods are now being worked out for treating the articular surfaces of long bones of rabbit and dog with DMSO so as to preserve the viability of the chondrocytes during freezing, storage and thawing from low temperatures without disrupting the tissue.

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## GROWTH OF CARCINOID TUMOURS IN TISSUE CULTURE

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IN recent years considerable knowledge has accumulated concerning the clinical course, pathology, and biochemistry of carcinoid tumours<sup>1</sup>. Much of this work has concerned the biochemical pathway of 5-hydroxytryptamine (5-HT) formation, and the effect of various 5-HT antagonists on this system. Examination of the metabolism of the tumour would be facilitated by its establishment in continuous tissue culture, free of *in vivo* variables. Moreover, as the embryonic origin of the argentaffin cell is disputed<sup>2</sup>, it would be of interest to observe the morphology of carcinoid cells *in vitro*. The results of our preliminary attempts to grow metabolically active carcinoid tumour cells in tissue culture are reported in this article.

Tumour tissue was obtained aseptically and placed immediately in balanced salt solution (BSS) (pH 7.2). The tissue was rinsed three times in BSS, minced into 1-mm cubes, and placed in collagenase (Worthington Biochemicals, 0.2 mg/ml. in BSS) for 5 h or 0.2 per cent trypsin (Difco 1:250 in BSS) for 40 min at 36.5° C. The specimen was shaken briskly for a short period to disperse the cells, and the supernate containing the cells was centrifuged at 60g and discarded. The cells were resuspended in growth medium and counted with a haemocytometer, and appropriate numbers of cells were plated in 60 × 15 mm Petri dishes (Falcon Plastic Co.). All cultures were incubated at 36.5° ± 0.3° C in a humidified continuously changed atmosphere of 2.5 per cent carbon dioxide in air. Growth media consisted of: (A) 70 per cent basal growth medium 199 (ref. 3) + 15 per cent foetal calf serum (Hyland Laboratories) and 15 per cent human cord serum (Microbiological Associates); (B) 80 per cent basal growth medium 199 + 10 per cent foetal calf and 10 per cent adult human serum (type O or A Rh-positive, from a local blood bank); (C) 80 per cent Eagle's medium<sup>4</sup> minus phenol red and 10 per cent foetal calf and 10 per cent adult human serum. Penicillin (0.1 mg/ml.), streptomycin sulphate (0.1 mg/ml.) and mycostatin (0.05 mg/ml.) were added to growth media.

The supernatant fluid obtained after removal of particulate matter by centrifugation at 2,000g for 10 min was extracted after protein precipitation and assayed for 5-HT by fluorimetry<sup>5</sup>. Cell suspensions in BSS were diluted 1:5 with distilled water, frozen and thawed three times, and then assayed for 5-HT. After protein precipitation of fluid and lysed cells with 50 per cent re-distilled trichloroacetic acid and chromatography on 'Decalco' resin<sup>6</sup>, histamine was determined by fluorimetry<sup>7</sup>. 5-Hydroxyindoleacetic acid (5-HIAA) was determined by fluorimetry after protein precipitation<sup>8</sup>. The first cell line was obtained at autopsy from a portal lymph node almost totally replaced by carcinoid tumour. The cellular morphology initially resembled that of a fibroblastic-like cell (Fig. 1). The majority of the cells were large and oblong to rounded in shape. At the time of the fourth sub-culture, a portion of cells were added to growth medium B. During the first two sub-cultures in medium B, the cells decreased in number and became very granular and large (Fig. 2), similar to cells in hormonally active human hydatid moles<sup>9</sup>. The cells then reverted to a fibroblastic-like appearance.

The Masson-Fontana stain<sup>9</sup> for argentaffin granules with appropriate controls was negative on sub-cultures 2 and 6. Secretion of 5-HT into growth medium was not found in the primary or third sub-culture, but a five-fold increase in supernatant 5-HT was seen in the medium from cells in sub-culture 4 in growth medium B versus no increase in the medium from cells of the same sub-culture incubated in growth medium A (Table 1).

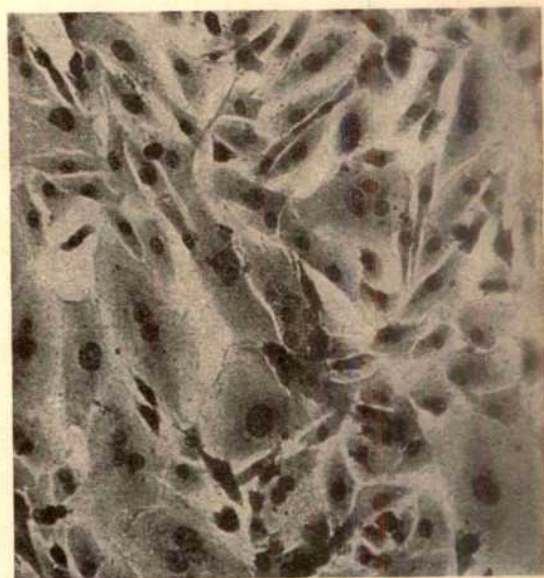


Fig. 1. Carcinoid cells of line 1 in sub-culture 2 in medium A. (Wright's stain; × 96)

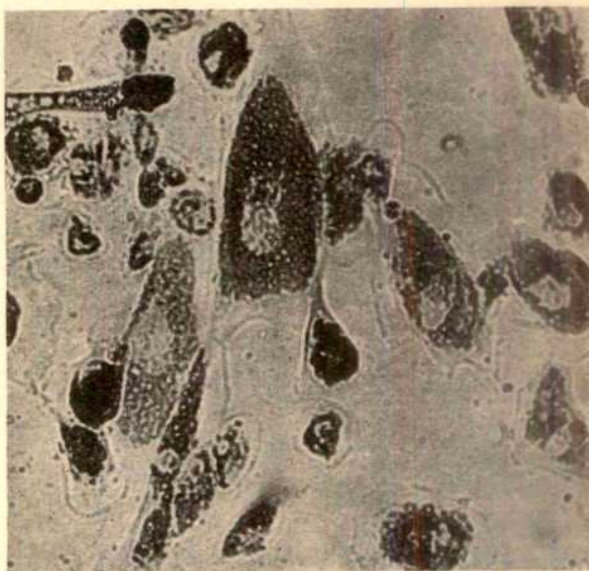


Fig. 2. Carcinoid cells of line 1 in sub-culture 5 in medium B. Note marked granularity of cells. (Unstained; × 152)

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Table 1. HORMONAL ACTIVITY OF CARCINOID CELL LINE 1

Medium	Sub-culture	5-HT concentrations ( $\mu\text{g/ml.}$ or per $10^6$ cells)				Cells
		Control*	from cells	Medium containing 5-HTP	Medium containing 5-HTP	
A	Primary	0.32	0.09	—	—	—
A	2	—	—	—	—	0.00
A	3	0.58	0.21	0.27	—	—
A	4	0.41	0.24	—	—	—
B	4	0.18	1.00	—	—	—
B	5	—	—	—	—	8.2
B	6	0.11	0.07	0.12	0.11	0.07
B	7	0.20	0.02	0.10	—	—

All media were placed on cell cultures 48 h prior to assay, except for passage six, which was 72 h, and passage seven, which was 96 h.

\* Indicated medium incubated at  $37^\circ\text{C}$  without cells.

Growth media containing graded amounts of 5-hydroxytryptophan (5-HTP) were incubated with cultures containing 50,000 cells in the sixth sub-culture for 72 h, without enhancing 5-HT production (Table 1). Cells from the second sub-culture contained no 5-HT whereas substantial amounts were found in the fifth sub-culture (Table 1). Histamine has been found previously in carcinoid tumour tissue<sup>10</sup>, and assay of cell lysates demonstrated 8.3 and 20  $\mu\text{g}$  of histamine per million cells in sub-cultures 2 and 5 in medium A and B respectively. No histamine was found in the growth medium from cells in sub-culture 5 in medium B, and histamine was absent in control fibroblastic-like cells from foetal human skin and intestine and from a human benign breast lesion.

Lymph nodes partially replaced by carcinoid tumour were obtained at the time of the laparotomy from a second case of the carcinoid syndrome. The cells readily established in culture were typically fibroblastic-like in appearance. Growth medium incubated with the cells from sub-culture 2 showed no increase in 5-HT or histamine over that of the control fluid concentrations. An argentaffin stain on cells of the first sub-culture was negative. Although morphologically similar to the other cell lines, these cells may well have been derived from connective tissue associated with the tumour.

Tumour nodules obtained at autopsy from the liver of a third patient were the source of the third cell line. Complete monolayers of cells morphologically similar to fibroblastic-like cells grew out in 11 days. Although morphologically similar to those from the other two



Fig. 3. Carcinoid cells of line 3 in sub-culture 3. Note argentaffin-positive granules. (Masson-Fontana stain,  $\times 1,120$ )

biopsies, typical argentaffin-positive granules were present in sub-cultures 1 and 3 (Fig. 3). Fluid used to grow the cells in sub-cultures 1 and 2 did not contain increased amounts of 5-HT or 5-HIAA (Table 2), but cells from sub-cultures 2 and 3 contained appreciable amounts of 5-HT (Table 2). Control fibroblastic-like cells from foetal human intestine, lung and skin in sub-culture 2 and from a human benign breast biopsy in sub-culture 2 contained no measurable 5-HT. Fluids from these fibroblastic-like cultures had no increase in 5-HT content over control fluids.

Table 2. HORMONAL ACTIVITY OF CARCINOID CELL LINE 3

Medium	Sub-culture	5-HT concentration ( $\mu\text{g/ml.}$ or per $10^6$ cells)		5-HIAA concentration ( $\mu\text{g/ml.}$ )	
		Control*	from cells	Control*	from cells
B	Primary	0.27	0.06	—	—
C	1	0.31	0.28	1.9	0.16
C	2	0.31	0.09	8.6	0.16
C	3	—	—	2.8	0.09

Media from primary culture placed on cell cultures 48 h prior to assay; those from sub-cultures 1 and 2, 72 h.

\* Indicated medium incubated at  $37^\circ\text{C}$  without cells.

The presence of 5-HT in cell lines 1 and 3, and of argentaffin-positive granules in 3, strongly suggests that these originated from carcinoid cells. Since it is known that argentaffin-positive granules may not be found in hormonally active tumours, it was not unexpected to find that the first cell line contained no such granules. Increased 5-HT was noted only once in the fluids used to grow the three carcinoid cell lines, despite addition of 5-hydroxytryptophan to the medium. Thus the factors leading to 5-HT secretion or release *in vitro* remain as obscure as the hormonal or environmental factors *in vivo*, although it is recognized that epinephrine administration can precipitate increases of plasma 5-HT in patients with carcinoid tumours<sup>11</sup>.

The 5-HT and histamine contents of the cells were considerably higher than those found in a murine mast cell in continuous culture by Day and Green<sup>12</sup>. Of interest in their investigations was the variable 5-HT and histamine activity *in vitro*, both qualitatively and temporally, over a two-year period. Our limited results indicated a similar variability in activity. The dissimilarity in *in vitro* behaviour among the cell lines correlates with known *in vivo* activity of carcinoid tumours, which are recognized to vary greatly in their metabolic activity and clinical manifestations<sup>1</sup>. Although the origin of argentaffin cells is disputed<sup>2</sup>, carcinoid tumours in the broad sense have been classified as being fore-, mid- and hind-gut in origin, favouring an entodermal origin<sup>13</sup>. Each of the carcinoid cell lines grown *in vitro* was derived from metastasis from a small bowel primary tumour. The cells were fibroblastic in appearance, suggesting that they were derived from mesenchyme.

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## LETTERS TO THE EDITOR

## ASTROPHYSICS

Possible Magnetospheric Phenomena  
associated with Neutron Stars

WITH the discovery of X-ray sources in the sky<sup>1,2</sup>, speculation has arisen that they might be associated with neutron or hyperon stars formed during the internal collapse which triggers off supernova explosions (probably of type I). Rates of cooling of neutron star models have been calculated by Morton<sup>3</sup>, Chiu and Salpeter<sup>4,5</sup>, and Tsuruta<sup>6</sup>. It appears (J. Bahcall, personal communication) that the importance of the early cooling by emission of neutrinos from the 'Urca' process has been underestimated in the foregoing investigations. With rough allowance for this effect, the calculations of Miss Tsuruta indicate that a neutron star will rapidly cool to  $3$  or  $4 \times 10^8$  °K, but that after  $10^5$  years its surface temperature will still be about  $2 \times 10^8$  °K.

During the earlier part of 1964 evidence unfavourable to the neutron star hypothesis for X-ray sources accumulated. Thus Bowyer, Byram, Chubb and Friedman<sup>7</sup> showed from a lunar occultation measurement that the diameter of the X-ray source associated with the Crab Nebula is about one light year, and there appeared to be a deficiency of soft X-rays from it. At the symposium on "Neutron Stars and Celestial X-ray Sources", held at the Goddard Institute for Space Studies during March 1964, Giacconi and Friedman both reported crude spectral estimates which suggested that the strongest X-ray source, in Scorpius, if thermal, would have a temperature of about  $1$  or  $2 \times 10^7$  °K—much too hot to be interpreted as a neutron star.

Many more measurements were reported at the second Texas Conference on Relativistic Astrophysics in December 1964. Friedman, for example, reported that the soft X-ray flux from both the Crab Nebula and the Scorpius source has been greatly underestimated; his newer determination of the equivalent thermal temperature of the Scorpius source was  $2 \times 10^6$  °K. He also reported that ten X-ray sources had now been identified and that these formed a distribution flattened toward the galactic plane. Also at the December 1964 Texas Conference Giacconi reported that the angular diameter of the Scorpius source is less than  $8$  min of arc. However, Clark, at the same Conference, reported that the Crab Nebula emitted a significant flux of  $\sim 30$  keV X-rays, consistent with the synchrotron emission picture of Woltjer<sup>8</sup>. Fisher, again at the second Texas Conference, reported that the X-ray energy spectrum from the Scorpius source contained too large a flux of higher energy X-rays to be consistent with a pure thermal spectrum of  $2 \times 10^6$  °K.

It is the purpose of the present communication to suggest that the discrete X-ray sources may be neutron stars with an associated magnetosphere. The X-ray spectrum would thus consist of a thermal component emitted from the photosphere and a non-thermal synchrotron component emitted by trapped electrons accelerated in the magnetosphere.

Magnetic fields are commonly associated with stars. Woltjer<sup>8</sup> has pointed out that neutron stars may contain magnetic fields with strengths up to  $\sim 10^{14}$  gauss, which would be formed during the compression of matter which forms the neutron star. This compression occurs during the hydrodynamic collapse of a pre-supernova star. Colgate and White<sup>9</sup> have found that a degenerate neutron core starts to build up in such a collapse, and additional matter descending on this core releases large amounts of gravitational potential energy. The deposition of this energy forms a strong shock wave which ejects the outer layers of the star. We must expect that the internal magnetic lines of force would be drawn radially outward

in this explosion. However, the rotation of the remaining neutron star would twist the lines of force in the inner region so that they would have to reconnect to form a self-contained magnetosphere.

The surface temperature of a neutron star is comparable with the kinetic temperature in the solar corona, but its radius is orders of magnitude less than that of the Sun. Hence the stellar wind associated with a neutron star will be negligibly small compared with the solar wind, according to the hydrodynamic model for coronal expansion<sup>10</sup>, unless much higher kinetic temperatures are produced in a corona around the neutron star. It should not be ruled out that the mechanism to be discussed here might produce these higher kinetic temperatures, in which case there could also be a bremsstrahlung component in the X-ray emission. The heating of the solar corona appears to be produced by generation of acoustic, gravity and hydromagnetic waves by turbulence in the convective layers below the solar photosphere. It is clear that no similar convective region can exist in a neutron star<sup>6</sup>.

However, the neutron star is capable of storing gravitational potential energy in the form of radial oscillations. Such oscillations will have a period in the millisecond range (F. J. Dyson, personal communication). The shock wave which ejects matter in the supernova explosion will eject only the outer layers; the inner layers will be accelerated outward by the shock but will fall back on to the neutron star. It seems likely that a substantial amount of energy may thus be stored in the resulting radial oscillations. The gravitational binding energy of a neutron star is a sensitive function of the mass<sup>6</sup>, but it may typically amount to several per cent of the rest mass energy. Hence it may be possible to store  $\sim 10^{52}$  ergs as vibrational energy in such a star. This is 5 or 6 orders of magnitude greater than the thermal energy content of a neutron star at the end of the initial rapid neutrino cooling stage<sup>6</sup>.

The radial oscillations will generate hydromagnetic waves at parts of the magnetic field which emerge from the photosphere at some angle to the normal. These waves will traverse the magnetosphere and can accelerate electrons. If this picture holds for the Crab Nebula, then evidently the electrons escape from the magnetosphere into the radial magnetic field system of the surrounding expanding envelope. The electrons will initially emit X-rays by the synchrotron process, but their synchrotron lifetime for X-ray emission is only about one year<sup>8</sup>. This would account for the observation that the region of X-ray emission in the Crab Nebula is smaller than the region of optical synchrotron emission.

If these considerations are correct, it is evident that many other non-thermal phenomena will be associated with the mechanical energy of vibration of neutron stars, and hence that extensive theoretical investigations of such phenomena may be rewarding.

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## PHYSICS

## Release of Gravitational Energy in General Relativity

THE clarity of Prof. McCrea's<sup>1</sup> recent treatment of the Schwarzschild singularity and of his discussion on the release of gravitational energy in general relativity opens up the possibility of a slightly different point of view. The present contribution may be of some academic and pedagogic interest.

It is assumed, following McCrea, that a free particle of rest mass  $m$  is projected inwards from infinity towards a spherical body of mass  $M$  and radius  $R$ . The free particle strikes the surface of the mass  $M$  and a fraction of its energy is released and then radiated away as a single particle (either a photon or a neutrino) of zero rest mass.

Failing a rational theory of the two-body problem we are compelled to use the Schwarzschild exterior metric for  $M$ , and to assume that the mass  $m$  is infinitesimally small in comparison with  $M$ . In many practical applications this procedure is justified. But if  $M$  approaches its limiting critical radius it is doubtful whether in the limit the neglect of the effect of the infinitesimal mass  $m$  is always possible. The following treatment takes into account the effect of  $m$  in an approximate way.

The Schwarzschild exterior metric is:

$$ds^2 = (1 - 2M/r)dt^2 - (1 - 2M/r)^{-1}dr^2 - r^2(d\theta^2 + \sin^2\theta d\phi^2)$$

with  $c = G = 1$ . Consider a free particle of infinitesimal rest mass  $m$  having an energy at infinity of:

$$E_m(\infty) = \alpha m, \alpha \geq 1 \quad (1)$$

and moving in the direction of the central mass  $M$ . Relative to an observer on the surface of  $M$  the free particle arrives with an energy of:

$$E_m(R) = E_m(\infty) (1 - 2M/R)^{-\frac{1}{2}} \quad (2)$$

After striking the surface, the energy in excess of some amount, say  $\beta m$ , is converted into a particle of zero rest mass of energy:

$$E_0(R) = E_m(R) - \beta m \quad (3)$$

In general,  $\beta \geq 1$ . When the constituent particles of  $M$  are stationary the free particle can come to complete rest, and in this event  $\beta = 1$ . Otherwise,  $\beta > 1$  on the average, where  $\beta m$  is the mean energy of the constituent particles at the boundary. The radiated particle of zero rest mass now moves outwards to infinity following a null-geodesic and ultimately has an energy of:

$$E_0(\infty) = E_0(R) (1 - M'/R)^{\frac{1}{2}} \quad (4)$$

Combining equations (1), (2), (3) and (4), it follows:

$$E_0(\infty) = E_m(\infty) \left\{ (1 - 2M/R)^{-\frac{1}{2}} - \beta/\alpha \right\} (1 - 2M'/R)^{\frac{1}{2}} \quad (5)$$

The argument so far is identical with McCrea's with the exception of the modification in equation (4), where  $M'$  is used instead of  $M$ . Let us suppose for the moment that  $M = M'$ . Furthermore, let us suppose that in principle it is possible for  $2M \rightarrow R$ ; then in the limit, it follows from equation (5) that:

$$E_0(\infty) = E_m(\infty) \quad (6)$$

This means, when the mass  $M$  has the critical value, the whole energy of the free particle is converted into radiation.

But in actual fact the free particle falls towards a mass of  $M$ , and the radiated particle escapes to infinity from a mass of  $M' = M + \beta m$ . So long as  $2M' \ll R$ , the infinitesimal increase in  $M$  is of no importance. However, when  $2M' \rightarrow R$ , in the limit it follows:

$$E_0(\infty) = 0 \quad (7)$$

and this result holds no matter how small  $\beta m$  is made. It is seen that the addition of the infinitesimal contribution to the central mass is of considerable importance when the central mass is close to its critical value.

If the mean density of  $M$  obeys an equation of the form:

$$\rho = \text{constant} \times M^\mu \quad (8)$$

then equation (5) can be rewritten as:

$$E_0(\infty) = E_m(\infty) \left[ \left\{ 1 - \left( \frac{M}{M_c} \right)^\nu \right\}^{-\frac{1}{2}} - \frac{\beta}{\alpha} \right] \left\{ 1 - \left( \frac{M + \beta m}{M_c} \right)^\nu \right\}^{\frac{1}{2}} \quad (9)$$

where  $M_c$  is the limiting Schwarzschild mass and:

$$\nu = \frac{2 + \mu}{3} \quad (10)$$

When  $\mu = -2$ , equation (9) does not apply, since the ratio  $2M/R$  remains constant. For  $\mu \leq -2$ , the mass  $M$  can be increased indefinitely and the Schwarzschild singularity is never attained. However, this represents an unrealistic relation between mean density and mass, and in fact only positive values of  $\mu$  should be considered.

Imagine that the mass  $M$  is built up, step by step, by releasing in succession  $n$  free particles of initial energy  $\alpha m$ , each of which contributes  $\beta m$ . Hence,  $M = n\beta m$ , after the  $n$ th particle has arrived. Also let  $M_c = N\beta m$ , where  $N$  is the maximum possible number of contributions. For the  $n$ th particle, equation (9) is:

$$E_0(\infty) = E_m(\infty) \left[ \left\{ 1 - \left( \frac{n-1}{N} \right)^\nu \right\}^{-\frac{1}{2}} - \frac{\beta}{\alpha} \right] \left\{ 1 - \left( \frac{n}{N} \right)^\nu \right\}^{\frac{1}{2}} \quad (11)$$

Consider now the final stages of the building-up process, in which  $n = N - \Delta N$ , and  $\Delta N$  is a positive integer small compared with  $N$ . That is,  $\Delta N = \dots 3, 2, 1, 0$ . Equation (11) is now, for  $\nu > 0$ :

$$E_0(\infty) = E_m(\infty) \left\{ \left( \frac{\Delta N}{\Delta N + 1} \right)^{\frac{1}{2}} - \frac{\beta}{\alpha} \left( \frac{\Delta N}{N} \right)^{\frac{1}{2}} \right\} \quad (12)$$

For infinitesimally small increments of mass,  $1/N \rightarrow 0$ ; and therefore this equation is approximately:

$$E_0(\infty) = E_m(\infty) \left( \frac{\Delta N}{\Delta N + 1} \right)^{\frac{1}{2}} \quad (13)$$

Thus, the efficiency of converting matter into radiation is... 87 per cent, 82 per cent, 71 per cent, 0, for  $\Delta N = \dots 3, 2, 1, 0$ . When the last free particle is added (that is,  $\Delta N = 0$ ), the mass becomes critical and the conversion ratio  $E_0(\infty)/E_m(\infty)$  drops to zero because the final radiated particle is unable to escape in a finite time. On differentiating equation (12) it is found that the conversion ratio has a maximum value of:

$$E_0(\infty)/E_m(\infty) = 1 - \frac{3}{2} \left( \frac{\beta^2 \nu}{\alpha^2 N} \right)^{\frac{1}{2}} \quad (14)$$

and occurs at:

$$n = N - \left( \frac{\alpha^2 N}{\beta^2 \nu} \right)^{\frac{1}{2}} \quad (15)$$

If a critical mass equal to a solar mass is built up from free particles of nucleonic mass, and if  $\alpha$ ,  $\beta$ , and  $\nu$  are approximately unity, then the conversion ratio has a maximum value of  $1 - 10^{-19}$ , which occurs at  $\Delta N \sim 10^{19}$  when the mass is  $10^{-5}$  g short of its critical value.

The model outlined here is interesting from the point of view of the conservation of energy principle. If, for example, the initial energy is expressed as the sum of two terms:

$$E_m(\infty) = E_0(\infty) + \beta m (1 - 2M/R)^{\frac{1}{2}}$$

where the first term on the right-hand side is the final radiation energy and the second term is the energy lost

to  $M$ , it follows from equation (5) that  $M = M'$ , and therefore  $\beta$  must be equal to zero. Evidently, the above equation does not provide a proper energy balance and omits a term for the binding energy. The original free particle divides on impact with  $M$  into two particles, and on separating these two particles exert a gravitational effect on each other. Conservation of energy therefore requires that:

$$E_m(\infty) = E_0(\infty) + \beta m(1 - 2M/R)^{\frac{1}{2}} + \Delta E \quad (16)$$

where  $\Delta E$  is the binding energy of the two particles. By comparing (5) and (16) it follows that:

$$\begin{aligned} \Delta E &= E_m(\infty) \left\{ (1 - 2M/R)^{-\frac{1}{2}} - \beta/\alpha \right\} \left\{ (1 - 2M/R)^{\frac{1}{2}} - (1 - 2M'/R)^{\frac{1}{2}} \right\} \\ &= E_m(\infty) \left\{ 1 - \frac{\beta}{\alpha} \left( \frac{\Delta N + 1}{N} \right)^{\frac{1}{2}} \right\} \left\{ 1 - \left( \frac{\Delta N}{\Delta N + 1} \right)^{\frac{1}{2}} \right\} \quad (17) \end{aligned}$$

For  $\Delta N \gg 1$ , it is seen that  $\Delta E$  is negligibly small. However, the binding energy is no longer negligible when  $\Delta N$  becomes small, and for  $\Delta N = 0$  one has  $\Delta E = E_m(\infty)$ . The dependence of the binding energy on  $M$  is an example of the non-linear superposition of gravitational fields.

In a gravitational field the rest mass of a particle is modified. Thus, at the surface of  $M$  it is:

$$m(R) = m(1 - 2M/R)^{\frac{1}{2}} \quad (18)$$

where  $m$  is the rest mass at infinity. It has been conjectured<sup>1</sup> that an infinite number of free particles must be added before  $M$  attains its critical value. It is argued that as  $2M \rightarrow R$  each free particle makes a vanishingly small contribution of  $m(R)$  to  $M$ , and therefore an infinite number of particles must be used in the process. Neither McCrea's results nor the present results support this conclusion. The rest mass  $m$ , as observed at infinity, is an invariant proper mass for all local observers. The mass  $m(R)$  is the rest mass at  $R$  as deduced by an observer at infinity, and it is not the rest mass for an observer at  $R$ . The contribution made to  $M$ , as deduced by a local observer, is the proper mass  $m$ . Hence, only a finite number of particles are necessary for  $M$  to reach its critical value.

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### Uniqueness of the Mass-surface Relative to Iron-56

In practice, the mass of a neutral atom is expressed in terms of a particular nuclidic mass which is assumed to have an integral value and is termed the primary standard. With  $^{12}\text{C}$  as the primary standard ( $^{12}_6\text{M} = 12u$ ) nuclidic masses are found to be close to integral values and the difference between the measured mass ( $^{A}_Z\text{M}$ ) and the integral value  $Au$  is called the 'mass excess'<sup>1</sup>. Alternatively, the mass of a nuclide ( $A, Z$ ) relative to  $^{12}\text{C}$  could be expressed in the form:

$$^{A}_Z\text{M} = [A + z(\Delta)^{A}_{12}] u \quad (1)$$

where  $z(\Delta)^{A}_{12}$  is a small proper fraction ( $\pm$ ) which will be referred to as the 'mass fraction'. It should be noted that the 'mass fraction' is a dimensionless quantity whereas the 'mass excess' equals  $z(\Delta)^{A}_{12} u$ .

It is evident from a comparison<sup>2</sup> of recent nuclidic masses (relative to  $^{12}\text{C}$ ) with earlier values (relative to  $^{16}\text{O}$ ) that certain characteristics of the mass surface  $z(\Delta)^A = \Phi(A, Z)$  are dependent on the choice of primary

standard. In particular,  $^{90}\text{Zr}$  has the least value of  $(\Delta)_{16}$  whereas  $^{118}\text{Sn}$  has the least value of  $(\Delta)_{12}$ ; in other words, the location ( $A, Z$ ) of the minimum of the mass surface is dependent on the choice of primary standard. This suggests that it might be possible to choose the primary standard so that it corresponds to the minimum of the mass surface; such a primary standard would render all mass excesses positive. It is therefore of interest to examine the nature of the dependence of the mass fraction on the choice of primary standard and thereby establish a criterion for the nuclide that would have the least mass fraction when employed as the primary standard.

Let us consider changing to a new scale where the new unit of mass equals  $(1 + \phi)u$ , then the mass of the nuclide ( $A, Z$ ) on the new scale is given by the equation:

$$^{A}_Z\text{M} = [A + z(\Delta)^{A}_{12}]/(1 + \phi) \text{ new mass units} \quad (2a)$$

Assuming that the change of unit does not alter the 'mass number' we can write:

$$^{A}_Z\text{M} = [A + z(\Delta)^{A}_n] \text{ new mass units} \quad (2b)$$

where  $z(\Delta)^{A}_n$  is the new mass fraction. A comparison of equations (2a) and (2b) yields:

$$z(\Delta)^{A}_n = [z(\Delta)^{A}_{12} - \phi A]/(1 + \phi) \quad (3)$$

The convention of assigning an integral mass to the primary standard requires that  $z(\Delta)^{A}_n$  for the new standard be equal to zero. Thus equation (3) shows that  $\phi$  equals the value of  $z(\Delta)^{A}_{12}/A$  for the nuclide that is chosen as the new standard. The quantity  $z(\Delta)^{A}_{12}/A$  is the equivalent of Aston's so-called 'packing fraction' and will be represented by the symbol  $zf^{A}_{12}$ . Accordingly, possible values of  $\phi$  are given by the packing-fraction surface ( $zf^{A}_{12}$  versus  $A$  and  $Z$ ).

The dependence of the packing-fraction surface on the choice of primary standard is given by the equation:

$$zf^{A}_n = (zf^{A}_{12} - \phi)/(1 + \phi) \quad (4)$$

which is obtained by dividing equation (3) by  $A$ . A consideration of primary standards by Mattauch<sup>3</sup> includes an approximate form of equation (4). Equation (4) shows that, with a change in the primary standard, each packing fraction is changed by the same amount, except for the scale factor  $(1 + \phi)$ ; the effect can be regarded as a displacement of the packing-fraction surface parallel to the  $f$  axis.

It is well known that the packing-fraction surface has a minimum; we designate the nuclide with the least value of  $zf^{A}_{12}$  as  $(A_m, Z_m)$  and note that the same nuclide would have the least value of  $zf^{A}_n$ . It follows that if the nuclide  $(A_m, Z_m)$  were taken as the primary standard, its packing fraction would be zero and all other values of  $zf^{A}_n$  would be positive. Consequently all mass fractions relative to  $(A_m, Z_m)$  would be positive and the nuclide  $(A_m, Z_m)$  would have the least mass fraction, which would be zero. Therefore the nuclide  $(A_m, Z_m)$  constitutes the required primary standard.

Empirically it is found that for  $A$  equal to a constant, the most-stable isobar has the smallest mass fraction and therefore the smallest packing fraction. Thus, to identify the nuclide  $(A_m, Z_m)$ , it is sufficient to consider packing-fractions for most-stable isobars. The minimum of the packing-fraction surface is in the region  $50 < A < 70$ ; the packing fractions, and their errors, for nuclides with the least mass excess for each mass number from 50 to 68 are given in Table 1. Examination of the values reveals that the packing fraction for the nuclide  $^{56}\text{Fe}$  is uniquely the smallest in the group and consequently is the least value of  $zf^{A}_{12}$  for all values of  $A$  and  $Z$ . Accordingly the nuclide  $(A_m, Z_m)$  is identified as  $^{56}\text{Fe}$ .

The use of the nuclide  $^{56}\text{Fe}$  as the primary standard would retain the conventional mass numbers; the condition



Table 1. PACKING FRACTIONS AND THEIR ERRORS FOR NUCLIDES WITH THE LEAST MASS EXCESS\* FOR EACH MASS NUMBER FROM 50 TO 68

Nuclide	Packing fraction $\times 10^4$
<sup>50</sup> Ti	-11.042 $\pm$ 0.0010
<sup>51</sup> V	-10.985 $\pm$ 0.0008
<sup>52</sup> Cr	-11.440 $\pm$ 0.0007
<sup>53</sup> Cr	-11.198 $\pm$ 0.0007
<sup>54</sup> Cr	-11.819 $\pm$ 0.0009
<sup>55</sup> Mn	-11.268 $\pm$ 0.0008
<sup>56</sup> Fe	-11.619 $\pm$ 0.0011
<sup>57</sup> Fe	-11.334 $\pm$ 0.0011
<sup>58</sup> Fe	-11.505 $\pm$ 0.0012
<sup>59</sup> Co	-11.324 $\pm$ 0.0008
<sup>60</sup> Ni	-11.536 $\pm$ 0.0010
<sup>61</sup> Ni	-11.303 $\pm$ 0.0015
<sup>62</sup> Ni	-11.557 $\pm$ 0.0011
<sup>63</sup> Cu	-11.176 $\pm$ 0.0010
<sup>64</sup> Ni	-11.256 $\pm$ 0.0009
<sup>65</sup> Cu	-11.110 $\pm$ 0.0009
<sup>66</sup> Zn	-11.205 $\pm$ 0.0015
<sup>67</sup> Zn	-10.873 $\pm$ 0.0016
<sup>68</sup> Zn	-11.049 $\pm$ 0.0013

\* Values from Everling, König, Mattauch and Wapstra<sup>2</sup>.

assumed for equation (2b) would therefore be satisfied. Furthermore, <sup>56</sup>Fe is the only 'primary standard' nuclide that would render all mass excesses positive; in this respect it would therefore constitute a unique primary standard.

Recalling that  $\phi$  equals the value of  $z f^{A}_{12}$  for the nuclide that is chosen as the new primary standard, it follows that if <sup>56</sup>Fe were the primary standard,  $\phi$  would have the least possible value; likewise, the new unit of atomic mass  $(1 + \phi)u$  would have the least possible value. Furthermore, it is apparent from equation (3) that each mass fraction relative to <sup>56</sup>Fe would have the greatest possible value. Accordingly, the mass of a nuclide ( $A, Z$ ) relative to <sup>56</sup>Fe could be expressed in the significant form:

$$\frac{A}{Z}M = [A + z(\Delta)A_{\max}] U_{\min} \quad (5)$$

Thus the mass surface  $z(\Delta)A_{\max} = \Phi(A, Z)$  is unique not only because all the values are positive but also because each value is a maximum.

Although the mass excess depends on the primary standard, the difference (in MeV) between the mass excesses for two isobars is independent of the primary standard. Thus in the investigation of isobaric transitions, for example,  $\beta$ -decay, a mass-excess diagram ( $\Delta$  versus  $Z$ ) serves as an energy diagram. On the other hand, when there is a

change in the mass number, for example,  $\alpha$ -decay, the difference (in MeV) between two mass excesses is dependent on the primary standard. However, the difference between two mass excesses relative to <sup>56</sup>Fe is a maximum and therefore corresponds to a unique energy difference. This suggests that the mass surface relative to <sup>56</sup>Fe would be appropriate for the investigation of the energetics of nuclidic transformations in general. In this regard, it is of interest to note that the mass excess relative to <sup>56</sup>Fe is potentially the largest portion of the nuclidic mass that can be transformed into energy. Thus the mass surface relative to <sup>56</sup>Fe has a special significance and as a potential-energy surface is unique, inasmuch as the lowest point on the surface corresponds to zero potential energy.

Conversion from the <sup>12</sup>C scale to the <sup>56</sup>Fe scale<sup>1</sup> is given by the relation:

$$\frac{A}{Z}M = [A + z(\Delta)A_{12}]/(1 + z f^{56}_{12}) U_{\min} \quad (6)$$

Thus, using the heaviest stable nuclide (<sup>209</sup>Bi) as an example:

$$\begin{aligned} \frac{209}{83}M &= (209 - 0.01958)/(1 - 0.0011619) U_{\min} \\ &= (208.98042)/(0.9988381) U_{\min} \\ &= 209.22352 U_{\min} \end{aligned} \quad (7)$$

A graphical representation of mass fractions, relative to <sup>56</sup>Fe, for most stable isobars is given in Fig. 1. For odd values of  $A$  they are shown as energy-levels whereas for even values of  $A$  they depict what might be termed a mass-fraction curve; the latter is a profile of the mass surface. It is noteworthy that all nuclidic masses relative to <sup>56</sup>Fe are given directly in terms of the mass number  $A$  and the mass fraction  $z(\Delta)A_{\max}$ .

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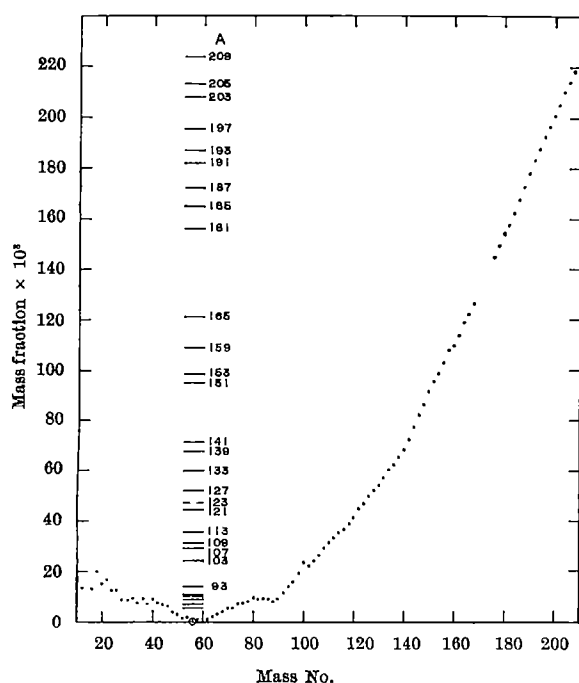


Fig. 1. Mass fractions, relative to <sup>56</sup>Fe, for most-stable isobars corresponding to two types of nuclides: even  $A$ , even  $Z$  (dots), and odd  $A$ , odd  $Z$  (lines).

## Chlorine-38 and Sulphur-38 produced by Cosmic Radiation

DURING the past few years several cosmic-ray spallation products of atmospheric argon have been found in rain water and aerosols. The first observed argon spallation product was <sup>32</sup>P (ref. 1), and was reported to be present in rain water in 1955. During the next two years the presence of <sup>36</sup>Cl (ref. 2), <sup>35</sup>S (ref. 3), <sup>33</sup>P (ref. 4) and <sup>22</sup>Na (ref. 5) were reported. In December of 1963 an additional radionuclide, <sup>24</sup>Na (ref. 6), was reported to be present in rain water. The radionuclides <sup>38</sup>Cl and <sup>38</sup>S have now been detected in rain water for the first time.

Our interest in radionuclides produced by cosmic rays in the atmosphere is concerned with the fall-out rates and mechanisms involved in the deposition of air-borne radionuclides. The ratio of those cosmic-ray produced radionuclides in rain water which have half-lives of the order of that required for a rain cloud to form should provide valuable information about both cloud formation and the washing of the atmosphere by rain.

Estimates of the expected production-rates of the possible spallation products of argon using both the methods of Rudstam<sup>7</sup> and of Baranovskii and Murin<sup>8</sup> showed that <sup>38</sup>Cl (half-life, 37.3 min) should be present in the atmosphere at concentrations comparable with <sup>36</sup>Cl. The calculations also indicated that <sup>38</sup>S (half-life, 2.9 h) should be present at a concentration of about an order of magnitude lower.

The decay schemes of these radionuclides permit direct measurement of their concentrations by coincidence-

Table 1. CONCENTRATIONS OF THE COSMIC-RAY-PRODUCED RADIONUCLIDES  $^{36}\text{Cl}$ ,  $^{35}\text{S}$  AND  $^{38}\text{Cl}$  IN RAIN WATER\*

Date	Volume (l.)	Collection time (min)	(d.p.m./l.) $^{36}\text{Cl}$	$^{35}\text{S}$	$^{38}\text{Cl}$
7/29/64	5.10	50	147 $\pm$ 15	13 $\pm$ 5	200 $\pm$ 17
7/30/64	0.64	15	$\sim$ 18	4 $\pm$ 2	$\sim$ 10
8/1/64	3.5	50	9 $\pm$ 5	†	15 $\pm$ 3
8/1/64	1.8	10	31 $\pm$ 12	†	28 $\pm$ 5
8/12/64	2.2	5	42 $\pm$ 13	$\sim$ 7	62 $\pm$ 12
8/18/64	10.0	40	55 $\pm$ 9	†	53 $\pm$ 3

\*  $\pm$  values are the S.D. for the measurements.

† Not measured.

counting techniques. The decay of  $^{38}\text{Cl}$  involves emission of a 1.60- and a 2.16-MeV  $\gamma$ -ray in cascade. The radionuclide  $^{35}\text{S}$  decays to  $^{36}\text{Cl}$  and the cascade of  $^{36}\text{Cl}$  can therefore be used in its measurement. The decay of the radionuclide  $^{36}\text{Cl}$  involves emission of 0.246- and 1.226-MeV photon cascades, and coincidence techniques are also applicable to its measurement.

All the measurements considered here were made using a  $\gamma$ -ray spectrometer of the type described by R. W. Perkins<sup>9</sup>. The sample was placed between the two anti-coincidence shielded NaI(Tl) detectors (4 in. thick  $\times$  6 in. diam.) which fed their respective signals to two analogue to digital converters of a 4096 channel multiparameter analyser. Total absorption events which occurred simultaneously in the two crystals were stored in the energy-energy plane of the memory at a point unique to these two energies. A thin lead absorber, covering the area between the crystals not occupied by the sample, eliminated backscatter interference at the  $^{36}\text{Cl}$  coincidence energy from the naturally occurring  $^{208}\text{Tl}$  in air samples.

The counting efficiencies (counts per disintegration) for  $^{36}\text{Cl}$  and  $^{38}\text{Cl}$  were only 0.010 and 0.067 respectively; however, the background counting-rates are extremely low, 0.008 c.p.m. and 0.12 c.p.m., respectively. Also, the Compton interference at the  $^{36}\text{Cl}$  coincidence peak is negligible and at the  $^{38}\text{Cl}$  coincidence peak it is very small.

By rapid evaporation (30 min) of a 4-l. sample of rain water to a small volume it was possible to make a direct measurement of the  $^{36}\text{Cl}$ , and, following its decay, of the  $^{38}\text{Cl}$  in the presence of the relatively large amounts of the natural and artificial radionuclides which are normally present. However, a more satisfactory method was to perform a carrier precipitation of the  $^{36}\text{Cl}$  and  $^{38}\text{Cl}$  as  $\text{AgCl}$  from 0.1 N nitric acid at 50°–60° C in the presence of lead and bismuth carriers. The precipitate is filtered through a membrane filter, washed with 100 ml. hot water then dissolved with 100 ml. 5 N ammonium hydroxide. The ammoniacal solution is acidified by addition of 100 ml. 8 N nitric acid and the chlorides reprecipitated by adding an excess of silver nitrate. The precipitate is filtered through a second membrane filter which is counted directly for a simultaneous  $^{36}\text{Cl}$  and  $^{38}\text{Cl}$  measurement. The residues are evaporated, mixed with the first filter, and placed on a standard counting dish for direct measurement of  $^{35}\text{S}$ , and certain other radionuclides of interest in the sample. The observed concentrations of  $^{35}\text{S}$ ,  $^{36}\text{Cl}$  and of  $^{38}\text{Cl}$  for six rain samples are tabulated in Table 1. The samples were collected by placing a 100–200-ft.<sup>2</sup> plastic sheet on the ground during the light summer showers. The first sample contained the most activity and the measurements, as indicated, are more precise than on the other five samples; however, the latter measurements do give the approximate concentrations and indicate the variations which exist, both in concentration and radionuclide ratios. The rain collection periods ranged from 5 to 50 min, and the concentrations shown were calculated to be those present at the time the rain struck the ground by assuming that the deposition rate of the radionuclides was constant during the sampling period. It is expected that the collection of samples 10 times larger will permit sufficiently precise measurements of these radionuclides and of  $^{23}\text{Na}$  and  $^{24}\text{Na}$  (which can also be measured on this size of sample<sup>10</sup>) to allow cloud formation rates and subsequent wash-out rates by rain to be investigated.

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## GEOLOGY

### Changes of Sea-level in Southern Australia

RECENT geomorphic investigations in relation to the history of sedimentation and to soil development in the Adelaide, Yorke Peninsula, and Gippsland areas of southern Australia have shown that the varied landforms of these areas are related to a sequence of stranded shorelines which extend from modern sea-level up to a height of several hundred feet. These shorelines are marked by former coastal features, including submarine platforms backed by abandoned sea cliffs, often with beach deposits at their base, coastal foredunes and bars, and notches of marine erosion. Their elevations above mean sea-level and other data relating to them are listed in Table 1.

The successive alluvial terraces of the river systems in the areas investigated are each graded to particular shorelines of the indicated sequence. On Yorke Peninsula, however, drainage was never integrated and alluvial terraces are generally absent. Here valleys are short and the watercourses, now dry, terminate at the coastlines to which they were originally graded. Each successive stillstand in this district brought about anew the development of a few shallow valleys at the new coast, and little or no down-cutting of existing valleys attended each withdrawal of the sea.

Exposures of the alluvial sediments allow a distinction to be made between river terraces due to lateral planation, considered to reflect stillstand of the sea, and terraces due to aggradation, which indicate a relative rise of sea-level. By this means shorelines that record stages of a general recession can be distinguished from shorelines that represent transgressive maxima. In Gippsland, where coastal gradients are very gentle, transgressive shorelines are also recognized by the development of massive foredunes. These impound minor drainage lines and from place to place alternate with abandoned low cliffs.

Some intervals between successive high sea-level stands were characterized by the introduction of aeolian materials, including sief dunes, which mantled the contemporary landscape. These wind-deposited sediments continue at the modern coast to levels now below sea-level, demonstrating a clear correspondence of aeolian sedimentation with former stages of low sea-level, and confirming, for the intervals in which they occur, withdrawals of the sea implied by investigations of the riverine terraces.

The Adelaide and Yorke Peninsula areas lie on opposing sides of the tectonic graben of St. Vincent Gulf. Three major faults pass through the Adelaide area. The last

Table 1. ELEVATION OF FORMER SHORELINES IN SOUTHERN AUSTRALIA AND THEIR NORTHERN HEMISPHERE CORRELATIVES

Elevations of former shorelines in southern Australia (ft. above mean sea-level)			Levels in the northern hemisphere, with nomenclature and glacial-age correlation adopted by Zeuner (ref. 1) (ft. above mean sea-level)		
Adelaide	Yorke Peninsula	Gippsland			
600	695		685	Calabrian	
540	640		590		
	560		490		
	480		420		
R	420	420			
370	360	R	340	Siellian	
	255	360	260		
		260			
	185	R	183	Milazzian	Günz (= Nebraskan)
		170	R		
	130	R	130		Mindel (= Kansan)
105	95	130	98	Tyrrhenian	
R		100	R		
> 35	60	R	57	Main Monastirian	Riss = Illinoian
R, deposition of wind-borne clay		60	R		
26	26	R			
R	R, deposition of wind-borne marl then formation of first sief dunes	26	25	Late Monastirian	
	12	R	R		
10	R, formation of second sief dunes	11	11	Epimonastirian	Würm 1 } (= Wisconsin)
R, deposition of wind-borne sand		R, formation of blowouts in old coastal dunes	R		Würm 2, 3 }
8	8-3	7-5	9-8	Postglacial	
	4-3	4-5			
	2-9	3-0			
		2-2			
		1-8			

The letter *R* indicates a regression extending considerably below the level reached by the subsequent transgressive high sea-level. The 600-ft. shoreline of the Adelaide area marks the limit of a marine transgression. The elevations for coastlines above 26 ft. (60 ft. on Yorke Peninsula) are derived from photo-grammetrically contoured maps (contour interval no greater than 25 ft.). Lower coastlines have been surveyed with a dumpy level. The evidence for marine activity at all levels is very well preserved, and the elevations given for the shorelines have an error in the main no greater than  $\pm 2-3$  per cent.

movements on two of these are shown on the field evidence to ante-date the rise of sea-level which culminated in the development of the 600-ft. coastline. Some movements persisted on the third fault after the development of this, and of the later 370-ft. shoreline, but these movements were presumably small, for they failed measurably to dislocate former submarine platforms related to these levels. It is therefore concluded that the shorelines in this area essentially post-date the differential tectonic movements. A shallow graben which passes through the Yorke Peninsula area was invaded by the sea when it occupied the 480-, 420- and 360-ft. levels. These shorelines are nowhere dislocated or deformed. The Gippsland area lies in a separate tectonic province but no evidence has been found of movements within the period of shoreline development.

The repeated changes in level of the sea described here and their accordance from district to district deny hypotheses of land movement relative to a stable sea, and are best explained by supposing that the land has been stable and the sea has moved. This view receives strong support when the levels for southern Australia are compared with the sequence of former sea-levels recorded for the northern hemisphere (Table 1)<sup>1</sup>. This correlation suggests that marine transgressions in southern Australia are to be correlated with interglacials of the northern hemisphere. The marine regressions that have been identified correlate mostly with the known glacio-eustatic depressions of ocean-level. The period of deep erosion, to levels below modern sea-level, which intervened between the development of the 600-ft. and 370-ft. shorelines and which is well attested in the Adelaide area appears to be too great in amplitude to be wholly glacio-eustatic in origin.

Detailed reports on the Adelaide area, which was surveyed by W. T. Ward, and the Yorke Peninsula area, surveyed by R. W. Jessup, are to be published shortly. Work by W. T. Ward is still in progress in the Gippsland area.

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### Iron-deficient Low-temperature Pyrrhotite

IN a recent communication<sup>1</sup>, Sawkins, Dunham and Hirst discussed the occurrence of pyrrhotite in low-temperature veins in the northern Pennine orefield. They found that, on experimental inversion and application of the hexagonal pyrrhotite-pyrite solvus curve<sup>2</sup>, monoclinic pyrrhotite in these deposits yields temperatures of deposition markedly in excess of those indicated by fluid inclusion studies. Grønvold and Haraldsen<sup>3</sup>, however, have demonstrated that at temperatures below c. 300° C the stable phases in the iron-rich part of the FeS-S system include troilite, intermediate hexagonal pyrrhotite (Fe<sub>0.936-0.900</sub>S), and monoclinic pyrrhotite (Fe<sub>0.87-0.875</sub>S). These three phases have very restricted ranges of composition, although the compositions of the iron-deficient synthetic phases differ somewhat from those observed in nature<sup>4</sup>. In the light of these relations it seems unreasonable to expect that primary monoclinic pyrrhotite of low-temperature origin co-existing with pyrite will show an iron-deficiency related to temperature of formation by the hexagonal pyrrhotite-pyrite solvus (which probably terminates at some temperature between 250° and 300° C). In the absence of very iron-deficient hexagonal pyrrhotite, the occurrence of troilite and intermediate pyrrhotite, with or without associated monoclinic pyrrhotite, in pyrite-bearing ores may be accepted as evidence that either the initial crystallization or the re-equilibration of the assemblage occurred at temperatures below c. 250° C. I have recently described<sup>5,6</sup> the pyrrhotite assemblages in the Ylöjärvi copper-tungsten deposit, Finland, and have suggested that cooling of hexagonal pyrrhotite from 500° to 565° C to below 300° C resulted in the inversion of much of the pyrrhotite to the monoclinic+hexagonal and monoclinic forms<sup>7</sup>, the formation of hexagonal pyrrhotites having compositions in the range Fe<sub>0.887-0.901</sub>S, and the development of troilite and intermediate hexagonal pyrrhotite (Fe<sub>0.912</sub>S). The last-mentioned two phases form characteristic lamellar intergrowths showing transitions to granular aggregates and monomineralic patches and veinlets. The formation of mackinawite<sup>8</sup> (tetragonal FeS), largely by replacement of magnetite and iron- and copper-sulphides, apparently postdated the crystallization of troilite. In this deposit, the monoclinic and monoclinic+hexagonal pyrrhotites have compositions in the narrow range, Fe<sub>0.869-0.873</sub>S. If inversion from the hexagonal form



was iso-chemical, these compositions would indicate temperatures of formation of 510°–515° C. Because natural monoclinic pyrrhotite shows little departure from the composition,  $\text{Fe}_{0.875}\text{S}$ , however, the close coincidence of the crystallization temperatures deduced from the compositions of the co-existing hexagonal and monoclinic pyrrhotites may be fortuitous<sup>9</sup>. In other sulphide deposits, low-temperature re-equilibration of hexagonal pyrrhotite has resulted in the formation of monoclinic and monoclinic+hexagonal-pyrrhotite, and hexagonal pyrrhotite having a composition in the approximate range  $\text{Fe}_{0.896-0.899}\text{S}$ . Intermediate pyrrhotite ( $\text{Fe}_{0.912}\text{S}$ ) and, especially, troilite may be subordinate or absent in such assemblages (unpublished work).

Table 1. X-RAY DATA FOR PYRRHOTITES FROM THE GREAT SULPHUR VEIN

Locality <sup>10</sup>	Assemblage	Monoclinic pyrrhotite		Intermediate hexagonal pyrrhotite	Troilite
		$d(202)$ (Å)	$d(202)$ (Å)	$d(102)$ (Å)	$d(102)$ (Å)
Aglionby Beck	Quartz, pyrrhotite, pyrite	2-0641	2-0525		
Cross Gill	Quartz, pyrrhotite, pyrite, sphalerite	2-0636	2-0530	2-0707	2-0932
St. John's Mine	Quartz, pyrrhotite, pyrite	2-0635	2-0514	2-0700	2-0936
St. John's Mine	Quartz, pyrrhotite, sphalerite, chalcopyrite	2-0621	2-0522	2-0703	
St. John's Mine	Quartz, pyrrhotite, pyrite	2-0630	2-0538	2-0703	

It should also be emphasized that pyrrhotite occurring, with or without associated pyrite, in low- to high-grade regionally metamorphosed rocks and in unmetamorphosed sediments is generally monoclinic and very iron-deficient. This feature prevents the estimation of the maximum temperatures of metamorphism on the basis of pyrrhotite compositions (unpublished work).

Examination of several pyrrhotite-bearing specimens from the Great Sulphur Vein<sup>10</sup> has shown that, as found by Sawkins *et al.*<sup>1</sup>, monoclinic pyrrhotite predominates over hexagonal, which is represented only by troilite and intermediate pyrrhotite (Table 1). The hexagonal phases have compositions identical to those observed in the Ylöjärvi deposit and at other localities. The ease with which they could be magnetically separated from each other suggests that the troilite and intermediate pyrrhotite occur as granular, rather than fine lamellar, intergrowths.

These few data are in fairly good agreement with those given by Sawkins *et al.*<sup>1</sup>, and generally support the low-temperature origin of the vein assemblages. Hexagonal pyrrhotite with a (102) spacing of 2-088 Å was not, however, detected by me. There are apparently no reliable records of homogeneous natural pyrrhotites having compositions between those of troilite and intermediate pyrrhotite ( $\text{Fe}_{0.912}\text{S}$ ), perhaps reflecting the presence of a solvus between these compositions at temperatures below c. 200° C (von Gehlen; personal communication). Cooling of iron-rich pyrrhotites from moderate temperatures (> 250° C) probably invariably results in the mutual exsolution of troilite and intermediate pyrrhotite, but primary crystallization at low temperatures might permit the formation of, presumably, metastable pyrrhotites within the proposed two-phase region.

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WE welcome the preceding communication by Dr. A. H. Clark, which does much to clarify the problem of low-temperature pyrrhotites. We wish to point out that our only intention was to question the use of experimentally inverted monoclinic pyrrhotite as a geological thermometer (Buseck<sup>1</sup>, Kullerud *et al.*<sup>2</sup>) and to show that in some natural occurrences the use of inverted monoclinic pyrrhotite produced results at variance with independent temperature data obtained from fluid inclusions.

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<sup>1</sup> Buseck, P. R., Annual Report of the Director of the Geophysical Laboratory, Carnegie Institution, Washington, *Year Book* **61**, 161 (1962).

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## GEOCHEMISTRY

### Fixation of Nitrogen by Aurora and its Contribution to the Nitrogen Balance of the Earth

CONSIDERABLE amounts of energy are dissipated in the upper atmosphere of the Earth by such geophysical phenomena as aurora, air glow and particulate bombardment. It is generally believed<sup>1</sup> that the dissipation of this energy leads to the formation of chemical species among which are oxides of nitrogen or their corresponding charge species. If these chemical species could reach the surface of the Earth they would contribute nitrate and nitrite to the biosphere. One of the aims of the Eighth Victoria University Antarctic Expedition was to estimate the magnitude of this effect by the chemical analysis of snow at the South Pole.

The South Pole was chosen because (a) thunderstorms, the usually accepted source of  $\text{NO}_2'$  and  $\text{NO}_3'$  in the atmosphere, are unknown; (b) if snow is collected from depths corresponding to times before human activities at the Pole, the chances of biological contamination are diminishingly small. The South Pole, being situated at 9,300 ft. on an ice plateau, is so remote that all normal sources of possible contamination can be eliminated; (c) the South Pole is surrounded by the auroral zone.

Freshly fallen snow (November 3, 1963) and snow from the year 1962–63 was collected one mile from South Pole Station. Samples of snow from 3 ft. inside the wall of the snow mine 44 ft. below the surface (approximately 100 years old) were analysed at the Base for  $\text{NO}_3' + \text{NO}_2'$  using the phenoldisulphonic acid method as described in ref. 2, except that a 500-ml. sample was used. All samples measured contained 0-005 p.p.m. N as  $\text{NO}_3' + \text{NO}_2'$ .

The nitrate plus nitrite content of South Polar snow was almost equal to that of the chloride content (0-027 p.p.m.). This is in marked contrast to analysis of snow from other areas less remote from the oceans<sup>3</sup>. Since the accumulation of snow at the South Pole is 8 g/cm<sup>2</sup>/yr., it follows that the yearly infall of nitrogen as  $\text{NO}_3'$  and  $\text{NO}_2'$  is  $5 \times 10^{-8}$  g/cm<sup>2</sup>/yr., which is 0-005 kg/hectare or 0-0045 lb./acre.

Another possible source of this material is extraterrestrial. Whipple<sup>4</sup> has reviewed the evidence for the infall of meteoric material and gives an accretion of 1,000 tons of meteoric material per day for the whole Earth, which is 0-007 kg/hectare. This is inadequate to provide the amounts of fixed nitrogen observed.

The conclusion from this work is that the annual infall of nitrogen as nitrate and nitrite at the South Pole due to geophysical phenomena in the upper atmosphere is less than, or equal to, 0-005 kg/hectare (0-0045 lb./acre).

This work was supported by the New Zealand University Grants Committee and the Victoria University of

Wellington Research Committee. We also thank the New Zealand Antarctic Division and personnel of the U.S. Navy, U.S.A.R.P., and Scott Base, Antarctica, for their help.

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<sup>1</sup> Nicolet, M., in *Physics and Medicine of the Atmosphere and Space*, edit. by Benson, O. U., and Strughold, H., 14 (John Wiley and Sons, New York, 1960).

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## METALLURGY

### Direct Evidence for Three Transformation Mechanisms for the $\beta \rightarrow \alpha$ Phase Change in Uranium - Chromium Alloys

PURE uranium exists in three allotropic forms:  $\alpha$ , which is orthorhombic and stable up to 667° C;  $\beta$ , which is tetragonal and stable between 667° and 775° C; and  $\gamma$ , which is body-centred cubic and is stable between 775° C and the melting point 1,132° C. In the pure metal the phase changes occur with great speed, and investigation of the mechanisms of the changes is extremely difficult. The addition of small concentrations ( $\sim 0.5$  atomic per cent) of certain solutes, of which chromium is one, retards the  $\beta \rightarrow \alpha$  change so effectively that  $\beta$  can be retained at moderate cooling rates to sub-critical temperatures and then allowed to proceed isothermally. White<sup>1</sup> and others<sup>2,3</sup>, using either dilatometric or metallographic techniques, showed that the time-temperature-transformation (TTT) diagram associated with isothermal  $\beta \rightarrow \alpha$  transformation in various dilute uranium alloys consisted of two separate C-curves. Metallographic and crystallographic investigations<sup>4-7</sup> have shown conclusively that the low-temperature C-curve (hereafter referred to as  $C_2$ ), which in U-0.5 per cent Cr alloy extends from room temperature to about 400° C, is associated with a martensitic mechanism. It was supposed that the upper C-curve, covering the range 400°-620° C in this alloy, was associated with transformation involving thermally activated atomic movement. However, metallographic observation showed that the micro-structure of the  $\alpha$  formed at temperatures between about 525° C and 620° C was quite different from that formed between 525° C and 400° C, suggesting that two formation mechanisms were possible, one in each temperature range. In order to explore this possibility further Dixon and Burke<sup>8</sup> redetermined the TTT diagram for a U-0.5 atomic per cent Cr alloy using an electrical resistivity method which was regarded as a more sensitive technique than those used in previous investigations. Evidence was found to suggest that the upper C-curve was composed of two overlapping C-curves, intersecting at about 520° C as required by the two-mechanism hypothesis.

Unfortunately, Dixon and Burke's evidence for the third C-curve was only indirect. The temperature coefficients of resistivity of the  $\alpha$ - and  $\beta$ -phases are quite different with the result that the magnitude of the change in resistance accompanying the phase change, which determines the sensitivity of the method, varies with the temperature of transformation, being about +4 per cent at 620° C, 2 per cent at 400° C, and zero at approximately 520° C. Thus the sensitivity was least in the critical temperature range. Since it is clearly desirable to get direct confirmatory evidence of the proposed third C-curve a detailed examination has been made of the phase change in other

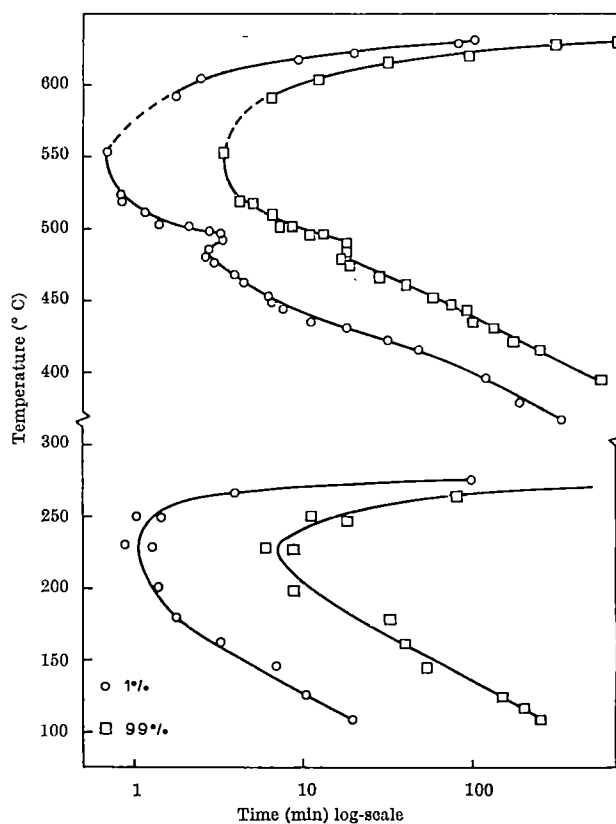


Fig. 1. The time-temperature-transformation diagram for the  $\beta \rightarrow \alpha$  phase change in U-0.85 per cent Cr alloy

uranium-chromium alloys. In the case of an alloy containing 0.85 atomic per cent Cr, it was found that the temperature of zero resistance change was raised relative to that in U-0.5 per cent Cr, enabling the division into separate C-curves to be firmly established for the first time.

The technique used was basically that described by Dixon and Burke<sup>8</sup>, although several modifications were made to improve the accuracy of the resistance measurements and to improve the quench speed. Wire specimens, 0.025 in. diam., were mounted in an evacuated silica tube and surrounded by a furnace controlled at a selected temperature in the  $\alpha$  range. The temperature of the specimen was then raised to 710° C by passing an electric current through it. After holding for 5 min, the specimen was quenched back to the furnace temperature by switching off the current and injecting argon over the specimen. With this technique quenching times of less than 5 sec were achieved. Changes in resistivity accompanying the  $\beta \rightarrow \alpha$  phase change were measured continuously using the potential drop method, the potential across the specimen being recorded on a potentiometric strip chart recorder.

The complete TTT diagram derived from the resistivity measurements is shown in Fig. 1. No results could be obtained between 560° and 585° C because the magnitude of the resistance change in this range was too small to give reasonable sensitivity. This is some 50° higher than the corresponding range in U-0.5 atomic per cent Cr. The upper C-curve contains a well-defined cusp in the range 470°-500° C. This cusp is formed by the overlapping of two C-curves as suggested by Dixon and Burke. It is convenient to refer to the higher temperature one as  $C_1$  and the lower one as  $C_2$ . Fig. 1 is considered to fully establish the existence of three C-curves in the TTT diagram for the  $\beta \rightarrow \alpha$  phase change in U-Cr alloys.

The microstructures of the transformation products were similar to those described by Dixon and Burke<sup>8</sup> and



Beaudier, Cabane and Mouturat<sup>3</sup>. In  $C_1$  the  $\alpha$  was equiaxed and the grain size decreased with temperature of formation; in  $C_2$  the  $\alpha$  had a fine acicular structure.

It follows from this work that the  $\beta$ -phase can transform to  $\alpha$  by three mechanisms. In  $C_1$  the insoluble chromium is precipitated, showing that long-range diffusion is involved. Electron microscope evidence indicates that this is a eutectoid type process<sup>4</sup>.  $C_2$  is clearly martensitic<sup>4-7</sup>. However, the evidence relating to  $C_2$  is scant. Dixon and Burke suggested that this curve corresponded to a reaction in which supersaturated  $\alpha$  is formed by shear of the  $\beta$  and the insoluble chromium is simultaneously rejected from the  $\alpha$  lattice in the form of a fine precipitate. However, it can be seen by comparison of Fig. 1 with the TTT diagram of Dixon and Burke that increasing the chromium content from 0.5 to 0.85 per cent depresses  $C_2$  much more than  $C_1$  and produces a marked separation between the  $C_2$  and  $C_3$ . It is therefore arguable that the closer proximity of the two upper  $C$ -curves indicates that the mechanism in  $C_2$  is more akin to the known diffusional reaction in  $C_1$  than to the martensitic reaction in  $C_3$ . This would suggest that the phase change in  $C_2$  involves diffusion as the predominant process.

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### Precipitation of Vanadium Carbide on Stacking Faults

THE precipitation of niobium carbide and titanium carbide in association with stacking faults in austenitic steels has been widely reported in the literature<sup>1-4</sup>. During the course of an examination of precipitation in high-alloy austenitic steels we have observed the precipitation of the vanadium carbide,  $V_4C_3$ , also in association with stacking faults.

The austenitic steel (25 per cent Ni, 20 per cent Cr, 5 per cent V, 2 per cent Si, and 0.15 per cent C) was solution treated at 1,200° C, water quenched and aged in the range 650° C–850° C. During the early stages of ageing,  $V_4C_3$  precipitated in association with stacking faults (Figs. 1 and 2). The precipitation phenomenon could be examined by both thin foil (Fig. 1) and carbon extraction replica (Fig. 2) techniques. The precipitation of  $V_4C_3$  in association with stacking faults brought about a substantial increase ( $\sim 50$  Vicker's pyramid number) in the hardness of the alloy. On further tempering the  $V_4C_3$  precipitates were replaced by  $\sigma$  phase, with a further increase in hardness of the alloy.

Details of the investigation will be given in a future publication. We would, however, like to point out some differences between our investigation and that of earlier work on the precipitation of NbC and TiC.

(1) In niobium- and titanium-containing alloys a solution temperature of 1,300°–1,350° C was necessary to induce this type of precipitation. In our work a solution temperature of 1,200° C was quite sufficient.

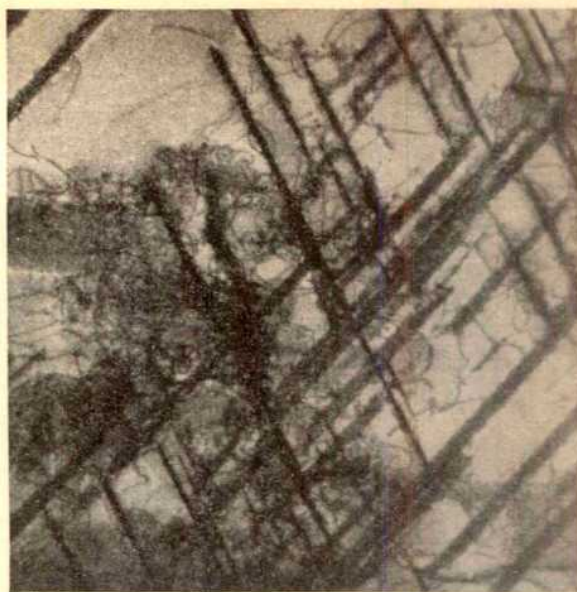


Fig. 1. Austenitic steel aged 17 h at 800° C (thin foil;  $\times 20,000$ )

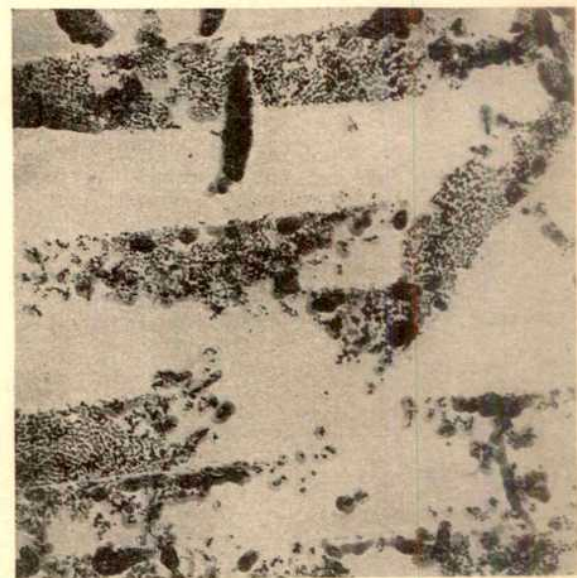


Fig. 2. Austenitic steel aged 50 h at 750° C (carbon extraction replica;  $\times 50,000$ )

(2) The range of temperature (650°–850° C) over which the carbide precipitated in association with stacking faults was much wider than that for niobium- and titanium-containing alloys.

(3) From the earliest stages of ageing the  $V_4C_3$  particles were much coarser ( $\sim 80$ – $120$  Å) than the niobium carbide and titanium carbide particles ( $\sim 50$  Å) reported in previous work. This is in agreement with the proposed mechanism of stacking fault precipitation<sup>4</sup>.

(4) At no stage (including the shortest periods at 650° C) were stacking fault 'fringes' observed in thin foils.

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## CHEMISTRY

## Sucrose Crystallization

PREVIOUS reports<sup>1,2</sup> described how static films of aqueous sucrose solution may be evaporated under controlled vapour pressure in humidators<sup>3</sup>, facilitating examination of nucleation and crystal growth throughout the whole range of supersaturation (SS). In these circumstances, when nucleation occurred at the higher concentrations, that is, more than 3.0 SS (ref. 2), spherulitic multicrystals were found to be the normal form. In many cases they closely resembled polymer crystal forms.

Nucleation may be much more speedily initiated by adequate movement within the film, either of some pre-existing sucrose crystal, or of some neutral body. In the latter case more violent movement is necessary<sup>4</sup>. In either case monocrystal nuclei result throughout the whole range of supersaturation, even in the near-glassy regions. By controlling the vigour of the movement the nuclei may be either well dispersed, or so crowded as to overgrow one another, and individual crystals 'assert themselves', developing with characteristic contour protruding from the formless mass. At sufficient dispersion an investigation of the component monocrystals is possible.

H. E. Buckley<sup>5</sup> and others have recorded a great deal of evidence of crystal habit modification by adsorption of certain impurities on one or more faces of a growing crystal. Experiments with sucrose reveal that extreme variations may result from the same solution of highly refined sucrose in distilled water by initiating nucleation at different concentrations. The evidence suggests that this is the logical result of variation in mechanism only, this being the more tenable hypothesis now that some variations in growth mechanisms are already known.

The monocrystals which result from movements in solutions at high SS appear to be remarkably uniform in size, rate of growth, and shape, appearing 'square' in contour. Every fresh movement results in a fresh genesis of similarly uniform monocrystals. Thus two or more 'generations' of crystals differing in size may be produced

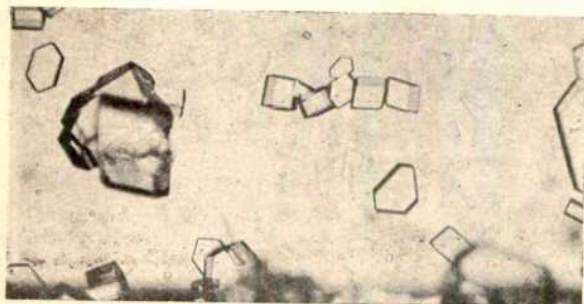


Fig. 1. 'Late developer' acicular sucrose. Axis ratios observed exceeding 100:1.

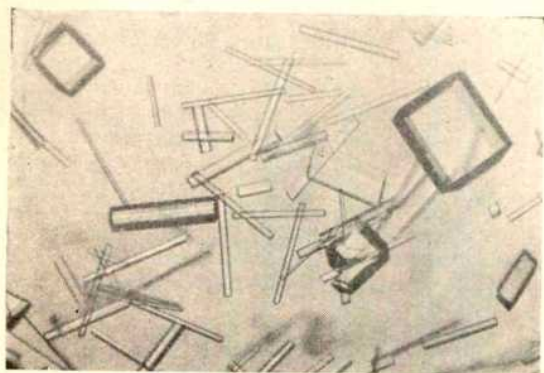


Fig. 2. 'Late developer' sucrose, both acicular and square type

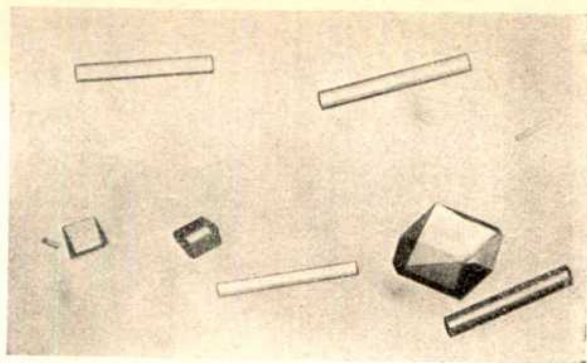


Fig. 3. Example of acicular crystals after extensive growth

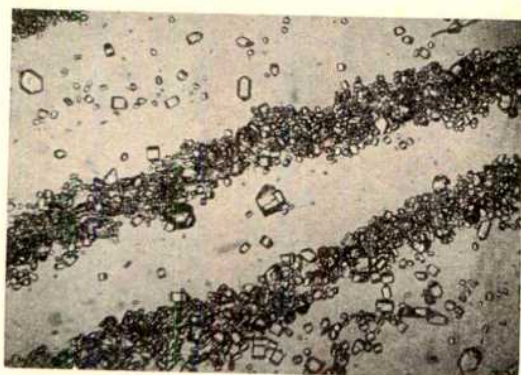


Fig. 4. One 'generation' of extreme size variation nucleated at 1.1 SS

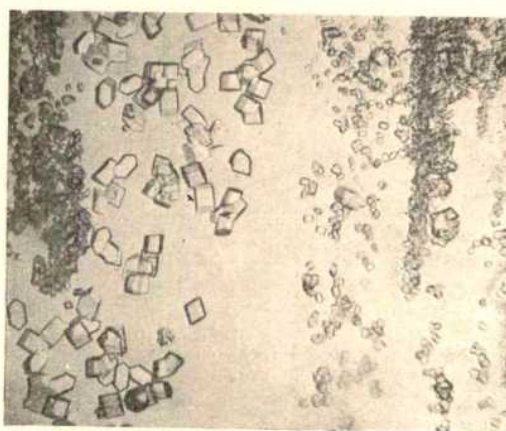


Fig. 5. Two 'generations' of uniform type

between two glasses under the microscope (Fig. 1). Below 2.0 SS this regularity becomes increasingly less evident.

At SS in the region of 1.6-1.3 the nuclei produced by crystal movement show considerable difference in form and uniformity of growth. In place of the 'square' contour the more familiar, somewhat rectangular, contour normally develops. (The usual sucrose crystal habit is  $100 \geq 110 \geq 001 > 1\bar{1}0 > 011$ ; H. E. Buckley, private communication.) More strikingly, acicular or needle-shaped forms appear—'late developers' (Figs. 2-4)—in the sequence of crystals growing to a size visible under the microscope. These needles are almost invariably the last to appear, occasionally accompanied by small groups of the square contour type. They sometimes resemble whiskers both in dimensions and mode of growth, and may indicate the existence of different nucleation mechanisms. By using a tall column<sup>4</sup>, with preferential subsidence of the more rapidly-growing crystals, the acicular form may be obtained almost exclusively.



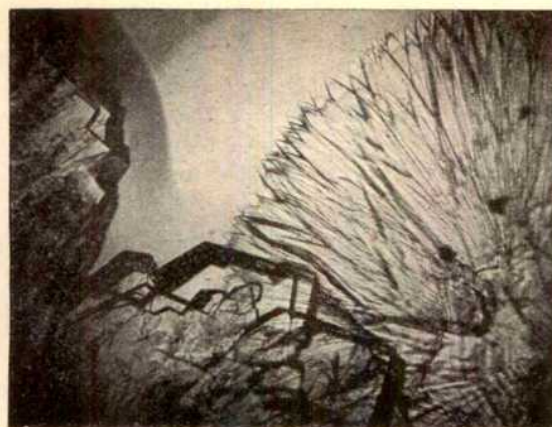


Fig. 6. Two types of spherulite, one of which shows a visible sleeve of exhaustion

At SS in the region of 1.2–1.05 the nuclei initiated by crystal movement show really striking differences in rate of development, and hence in size<sup>2</sup> (Fig. 5). The investigations now reported were designed to provide further evidence on this subject. It would appear probable that a differential solubility effect becomes insignificant at supersaturations above 2.0, also that genesis of nuclei then operates almost exclusively by one mechanism, and that growth mechanism is similarly uniform. In the region of 1.2–1.05 SS, not only do crystals show great variation in rate of development in the same medium, but also time lapse cinemicrography shows fairly numerous cases of a sudden burst of growth in single crystals, sometimes mainly in one direction. This supports the suggestion that varied growth mechanisms may operate.

Another predictable effect of differential solubility has been successfully recorded by time lapse cinemicrography. Every crystal growing in a static supersaturated solution soon creates a zone or sleeve of lower supersaturation as crystallization proceeds<sup>4</sup>. In the case of a crystal growing in a solution of about 1.1 SS, a sufficiently small crystal entering this zone may be dissolved. This would not happen in a solution of high supersaturation since even the sleeve would still be well above the critical concentration. In fact, if the supersaturation of the medium were sufficiently high, the small crystal might be expected to grow more rapidly, since at sufficiently high supersaturation the rate of crystal growth decreases with increasing concentration. Actual instances of these phenomena have been filmed.

The sleeve of exhaustion may even be seen, under appropriate conditions, by the unaided eye<sup>6</sup>, but far more effectively by means of special optical techniques. Occasionally ordinary photomicrography is able to record it, as in the example here reproduced (Fig. 6). There are many types of spherulites and in the illustration two strongly contrasted types are seen growing in a film of highly supersaturated solution. The pointed type grows much more slowly, and the impurities (mainly water) are able effectively to disperse, but with the flat-fronted type a sleeve of exhaustion is clearly visible moving ahead of the crystal front. I regard the former as a true spherulite, the latter as a pseudo-spherulite which arose from the ultimate development of an original group of adjacent monocrystal nuclei. Growth on the surface of the true spherulite would be slower owing to the relative perfection of the lateral lattice.

Finally, in earlier reports details were given of methods whereby thin films of sucrose solution could be evaporated under completely undisturbed conditions, without nucleation, even to the glassy state. Random nucleation appeared to peak at about 2.5 SS, and it did not appear likely that grosser depths of solution could be so evaporated without the incidence of nucleation. It has since

been found that under conditions in which aerial contamination by potential nuclei is eliminated much thicker films can be obtained. Films 1–2 mm thick are relatively easily obtained, and several of 10–15 mm have been successfully carried to a near glassy state. This is an effective reminder that air-borne potential crystal nuclei may be of the order of  $10^{-3}$  of the diameter of air-borne organisms, and hence even more difficult to exclude completely.

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<sup>2</sup> *Nature*, **196**, 58 (1962).

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### Identification of Carbonic Anhydrase in an Atmospheric Aerosol

BASED on the fact that the measured values of carbon dioxide of atmospheric air samples never could be determined as specific (using Pettenkofer's titration method  $\text{CO}_2$  values of 0.025–0.037 per cent/vol. were measured) a series of tests have been performed over a period of years to explain these irregularities. While some years ago the influence of light as opposed to darkness and the influence of temperature on the  $\text{CO}_2$  values could be proved<sup>1–3</sup>, the existence of a substance which acts as a catalyst and which seems to play an essential part in the deviations of the measured  $\text{CO}_2$  values was determined by these last tests.

As to the effects of daylight, darkness and temperature, the following facts are obvious: (1) Defined volumes of atmospheric air samples, which previously were stored in complete darkness for 3+ h and which also were titrated in darkness, in 90 per cent of all tests showed higher  $\text{CO}_2$  values ('dark values') than the corresponding  $\text{CO}_2$  values ('daylight values') achieved with air samples which had been exposed to daylight only. The remaining 10 per cent were equal to the  $\text{CO}_2$  values compared but the 'dark values' were never less than the 'daylight values'. The air samples stored in complete darkness nevertheless showed reversible character, that is to say, after a further irradiation in daylight those  $\text{CO}_2$  values were almost equal to the daylight values measured at the same time; (2) Atmospheric air samples, which were heated at different temperatures, showed no effects if the temperature was kept below 50° C. As soon, however, as the temperature exceeded the critical value of 50° C a rise in the  $\text{CO}_2$  values was observed in strict analogy with the  $\text{CO}_2$  values measured in air samples which had been placed in absolute darkness for 3+ h. The reversibility, however, was lost and the  $\text{CO}_2$  values represented an unchangeable value which could neither be raised nor lowered through the influence of light, darkness or temperature.

While it seemed obvious that the deviations may be caused by a photochemical effect in considering the results of the influence of light and darkness on the  $\text{CO}_2$  values, the tests with different temperatures suggested that the atmospheric aerosol contains a substance which becomes ineffective at temperatures higher than 50° C.

The latter suggestion was supported essentially by the results obtained when samples of air were sucked through asbestos filters (certain types of Schleicher and Schüll filters were used in these tests). In fact the air behind the filters proved to be absolutely inert and neither daylight, darkness nor temperature had further influence. Therefore the  $\text{CO}_2$  values of the samples were equal to the dark values or to the  $\text{CO}_2$  values obtained after having heated the air samples higher than 50° C.



Interpreting the fact that the air samples became insensitive to light and darkness after having been filtered, the postulation of a pure photochemical process has to be abandoned. It is suggested rather that any photochemical effect is only effective in the presence of a substance acting as a catalyst and will disappear as soon as this substance is removed from the atmospheric air samples by filtering off. Furthermore, the effect of temperature defined at 50° C gave a valuable hint for the identification of the substance itself and of its efficiency and stability as well. Considering the fact that among the innumerable organic compounds and complexes 50° C is a limiting temperature especially for the efficiency of enzymes, it was obvious, on consideration, that such an enzyme might exist in the atmospheric aerosol. Carbonic anhydrase is known as an enzyme, which can displace the equilibrium between  $\text{H}_2\text{CO}_3$  and  $\text{CO}_2$  according to the equation:



and the possibility that this enzyme might be existent in the atmospheric aerosol led to a further series of tests.

For these tests some cubic metres atmospheric air were pumped over glass frits into glass vessels, which were filled with  $\text{CH}_3\text{COOH}$  and  $\text{CHCl}_3$  and the extracts of these solvents were tested for amino-acids and for zinc (the central atom of carbonic anhydrase) with paper chromatography, after the solvents had been hydrolysed with hydrochloric acid. As blank test carbonic anhydrase (Light and Co., Great Britain) was used. Taking the nearly 100 chromatograms performed during these tests as a basis, the conformity between the test substance and the substance we removed from the atmospheric air was proved beyond doubt. At the same time another series of tests was performed whereby carbonic anhydrase was added to samples of air which first were made inert by filtering. The results were surprising, for samples of air which showed no deviations in their  $\text{CO}_2$  values except the high  $\text{CO}_2$  content, now after the enzyme had been added became sensitive to light, darkness and also temperature. Thus, the deviations of the measured  $\text{CO}_2$  values of atmospheric air samples appear to be solved since through the enzyme as catalyst and probably simultaneous influence of light the equilibrium will be displaced more to the left side of the equation, that is to say, strongly in  $\text{H}$  and  $\text{HCO}_3$  dissociating  $\text{H}_2\text{CO}_3$ . Consequently (the determination of the  $\text{CO}_2$  content according to Pettenkofer proceeds in  $\text{Ba}(\text{OH})_2$ ) the  $\text{CO}_2$  values appear to be less since two  $\text{HCO}_3$ -groups instead of one  $\text{CO}_3$ -group are bound to one barium ion. If the substance is missing very little of the  $\text{H}_2\text{CO}_3$  capable of dissociation remains and the atmospheric carbon dioxide in fact will be measured as pure  $\text{BaCO}_3$  in case the minimum dissociation of  $\text{CO}_2$  is neglected.

Concerning the concentration of the enzyme, certain tests induce us to believe that the concentration eventually depends on weather conditions.

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## BIOCHEMISTRY

### Graphical Analysis of Enzyme Kinetic Data

THE velocity of an enzyme reaction,  $v$ , is usually related to the substrate concentration,  $s$ , by the Michaelis equation:

$$v = \frac{V}{\frac{Km}{s} + 1}$$

where  $V$  and  $Km$  are the maximum velocity and the Michaelis constant, respectively. To obtain values for these constants from experimental results, it is usual to use one of several algebraic transformations of the Michaelis equation, to obtain a linear plot. The one probably most widely used is that commonly attributed to Lineweaver and Burk<sup>1</sup>, namely:

$$\frac{1}{v} = \frac{Km}{V} \cdot \frac{1}{s} + \frac{1}{V}$$

where, by plotting  $1/v$  against  $1/s$ , a straight line is obtained intercepting the  $1/v$  axis at  $1/V$  and of slope  $Km/V$ . (Dixon<sup>2</sup> has also pointed out that  $Km$  may be obtained from the intercept on the  $1/s$  axis, which is  $-1/Km$ .)

A second transformation is that which has been attributed<sup>3</sup> to Eadie<sup>4</sup>; but it appears that both plots were devised by Woolf<sup>5</sup>. Here:

$$v = V - Km \frac{v}{s}$$

then by plotting  $v$  against  $v/s$  a straight line intercepting the  $v$  axis at  $V$  and of slope  $-Km$  is obtained.

Dixon and Webb have recently<sup>6</sup> reiterated their preference<sup>8</sup> for the Lineweaver and Burk plot. They have expressed the opinion that both plots are satisfactory with respect to the accuracy of the constants determined, but they object to the Eadie plot because: (a) it is difficult to identify the points with respect to their substrate concentrations; (b) more calculation is involved to obtain  $v/s$  for plotting.

Hofstee<sup>7</sup> has pointed out that in the Eadie plot all points for a particular substrate concentration are limited to a straight line, of slope  $s$ , which passes through the origin. It is very simple to construct such lines for each substrate concentration merely by choosing a convenient arbitrary value of  $v$ , which is a simple multiple of the value of  $s$ . The value of  $v/s$  can then be calculated (mentally) and plotted against  $v$ . When one such point has been marked for each of the required substrate concentrations, a series of lines are drawn from the origin through each of these points; and each line can be marked with the appropriate substrate concentration. Obviously the values of  $v$  should be chosen such that the points lie as close to the perimeter of the graph as possible. To plot the values of  $v$  against  $v/s$  it is then only necessary to mark the values of  $v$  on the appropriate substrate concentration lines. This method is illustrated for theoretical values of  $V=100$  and  $Km=0.0004$  M for substrate concentrations from 0.00005 M up to 0.0032 M, increasing by a factor of 2 for each successive member of the series (Fig. 1). This is the ideal situation<sup>7</sup>, where the concentrations increase geometrically, and the value of the middle concentration is the same as that of  $Km$  (ref. 8).

This application of the Eadie plot meets both the objections of Dixon and Webb. These authors have also

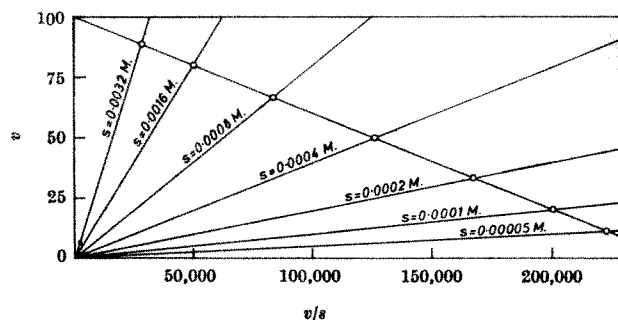


Fig. 1. Eadie plot for theoretical data,  $V=100$ ,  $Km=0.0004$  M.



### Specific Assays of some Phosphatases in Subcellular Fractions of Small Intestinal Mucosa

IN the mucosa of the small intestine, alkaline phosphatase (orthophosphoric monoester phosphohydrolase; EC 3.1.3.1) is known to be mainly localized in the brush border region<sup>1,2</sup> while glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase; EC 3.1.3.9) is a predominantly microsomal enzyme<sup>3</sup>. Thus these two enzymes may be used as markers for the assessment of the purity of subcellular fractions obtained in fractionation studies. However, as pointed out by Ginsburg and Hers<sup>4</sup> and Triantaphyllopoulos and Tuba<sup>5</sup>, the assay of glucose-6-phosphatase is complicated by the fact that glucose-6-phosphate may be hydrolysed by more than one enzyme. Similarly, acid phosphatase (orthophosphoric monoester phosphohydrolase; EC 3.1.3.2) and alkaline phosphatase of the intestinal mucosa have a rather broad pH optimum. These two enzymes are therefore difficult to assay accurately by merely choosing buffers of different pH values. These difficulties can be overcome by the use of specific inhibitors. In the following, procedures are described for the assay of these three phosphatases which will give a more accurate determination of their respective activities than was possible with the assay systems so far known.

Clark and Porteous<sup>6</sup> reported on the metal requirement of the alkaline phosphatase from small intestinal mucosa. An inhibition study with ethylenediamine tetraacetic acid (EDTA) showed that the alkaline phosphatase of cat small intestinal mucosa was almost completely inhibited in the presence of 4 mM EDTA (see Fig. 1). The particulate preparation used in this experiment was prepared in the absence of EDTA. The addition of 1–8 mM Mg<sup>++</sup> ions to the assay system did not change the reaction rate. When adding increasing amounts of Zn<sup>++</sup> ions to the assay system a correspondingly increasing inhibition was observed. A 50 per cent inhibition was obtained at 0.15 mM zinc sulphate. Fig. 1 B shows the inhibition of acid phosphatase by potassium fluoride. There was an almost complete inhibition at 1 mM potassium fluoride. In the assay of acid phosphatase, the potassium fluoride-induced inhibition was not reversed by 4 mM EDTA. Likewise, the EDTA-induced inhibition of alkaline phosphatase was not influenced by 1–2 mM potassium fluoride. However, the addition of EDTA increased the reaction rate of the acid phosphatase in the absence of potassium fluoride (see Fig. 1 B). The stimulation brought about by 3–5 mM EDTA varied between 10 and 15 per cent.

In conclusion, acid phosphatase may be assayed by determining the potassium fluoride-sensitive hydrolysis of  $\beta$ -glycerophosphate at pH 5.4 and alkaline phosphatase by determining the EDTA-sensitive hydrolysis of  $\beta$ -glycerophosphate at pH 9.5. Under the assay conditions described for both enzymes in Fig. 1, the reaction proceeded linearly with time up to 20 min of incubation at 37°. Also, the reaction proceeded linearly with protein concentration when up to 2.5  $\mu$ moles of inorganic phosphate were liberated. The control experiments should contain the same additions as the test, except that 2 mM potassium fluoride or 4 mM EDTA is added. Subtraction of the amount of inorganic phosphate liberated in the presence of the inhibitors from that obtained in the tests will give the potassium fluoride or EDTA-sensitive hydrolysis of  $\beta$ -glycerophosphate. Thus this method does not require the usual enzyme or substrate controls. Because of the stimulation caused by EDTA, acid phosphatase should be assayed by adding 4 mM EDTA to both test and control experiment. It is essential that in the assay of the acid as well as the alkaline phosphatase the reaction is started by adding the substrate, since there is a time lag before the action of the inhibitors becomes fully effective.

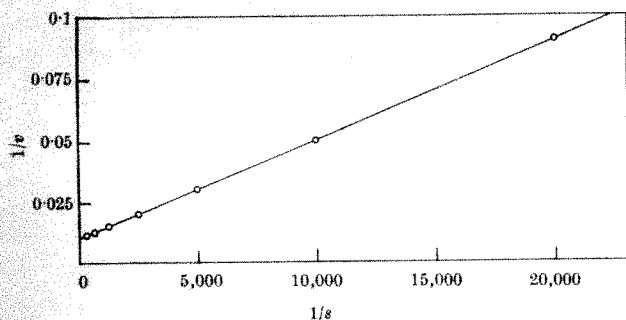


Fig. 2. Lineweaver and Burk plot for same data as Fig. 1

discounted<sup>8</sup> the advantage claimed for the Eadie method<sup>3,7</sup> of a more even spread of points, where the substrate concentrations increase arithmetically.

They argue that a geometrical increase in substrate concentration is to be preferred, when they say the Lineweaver and Burk gives a more uniform spread of points. However, inspection of a Lineweaver and Burk plot of the same data used above (Fig. 2) shows that this is not the case. Moreover, Dixon and Webb also argue<sup>6</sup> that the position of a straight line is most precisely determined by the points at both ends. It will be seen that such crowding as there is with the Eadie plot occurs towards the two ends, and so this argument favours the Eadie method. The values of the substrate concentration  $s_1, s_2, \dots, s_n$ , which give equal increments ( $\Delta v$ ) of the velocity, starting from the intercept on the  $v/s$  axis, are given by:

$$s_n = \frac{Km}{\frac{V}{n \cdot \Delta v} - 1}$$

The saving in time of plotting enzyme data by the Eadie method outlined here is appreciable for a single set of results. Where a series of measurements is made at various temperatures, for example, and for the same series of substrate concentrations at each temperature, then the saving of time is very great indeed, since all the results can be plotted together. The same considerations also apply to a series of inhibitors measured against the same set of substrate concentrations<sup>9</sup>. A further advantage of this method is that it reduces the tendency to over-emphasize the significance of the values for the lowest substrate concentration, which occurs with the Lineweaver and Burk plot. This is particularly acute in the case of enzymes showing substrate inhibition, where measurements have perforce to be made at very low substrate concentrations. Here one may be working near the limits of the assay methods available, and the percentage error of the lowest velocities may be much greater than those at higher concentrations.

The Eadie plot also offers less temptation to draw a straight line, when there is departure from linearity at the higher substrate concentrations.

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The effect of EDTA on glucose-6-phosphatase activity is shown in Fig. 2. There was a reduced rate of hydrolysis of glucose-6-phosphate when 4 mM EDTA was added to the assay system, but, in the presence of 7 mM EDTA, the rate of hydrolysis was not further decreased. This would be expected if part of the hydrolysis observed in the absence of EDTA were due to the action of alkaline phosphatase. When increasing amounts of potassium fluoride were added to assay systems already containing 4 mM EDTA, there was only slight, if any, decrease of the reaction rate, indicating that the hydrolysis of glucose-6-phosphate due to the action of acid phosphatase is very small indeed. A specific assay system for glucose-6-phosphatase should, therefore, include EDTA and potassium fluoride to inhibit alkaline and acid phosphatase respectively and thus allow the determination of the true glucose-6-phosphatase activity.

Under the conditions indicated in the legend of Fig. 2, the hydrolysis of glucose-6-phosphate proceeded linearly with time (up to 20 min of incubation at 37°) and protein

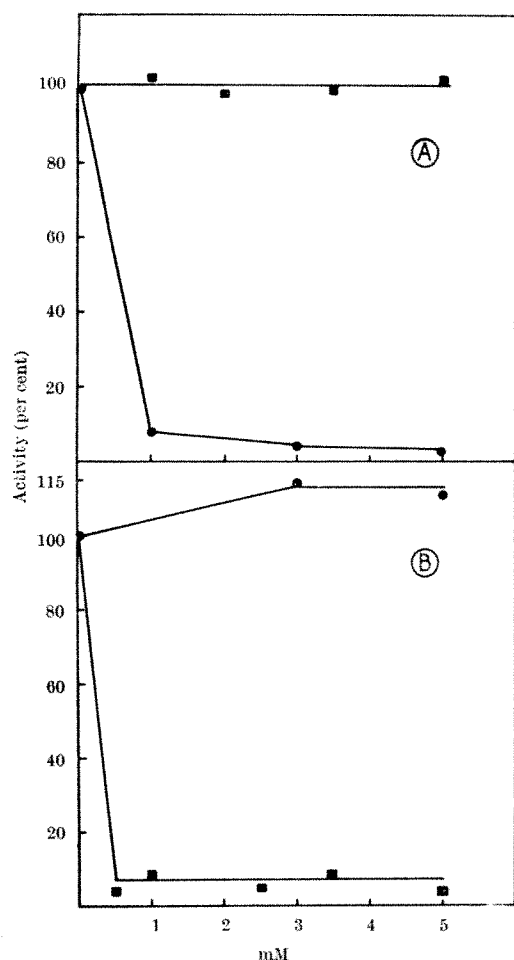


Fig. 1. The effect of EDTA and KF on alkaline (A) and acid (B) phosphatases. Alkaline phosphatase was assayed as follows: in a total volume of 1 ml., 40 mM ethanolamine-HCl buffer, pH 9.5; 40 mM  $\beta$ -glycerophosphate, pH 9.5; and 0.2 mg protein of a mixed brush border and nuclei fraction from cat small intestinal mucosa were incubated for 15 min at 37°. The reaction was stopped by adding 1 ml. of 10 per cent trichloroacetic acid (TCA) and 2 ml. of 5 per cent TCA containing 12.5 g of 'Norit A' per 100 ml. After filtering, an aliquot of the filtrate was assayed for inorganic phosphate<sup>1</sup>. Acid phosphatase was assayed by incubating, in a final volume of 2 ml., 50 mM acetate buffer, pH 5.4; 15 mM  $\beta$ -glycerophosphate, pH 5.4; and 0.8 mg of protein for 15 min at 37°. A mitochondrial fraction from cat small intestinal mucosa suspended in 0.3 M sucrose was used as source of enzyme. Just prior to the assay, the preparation was diluted 1:10 with deionized water and homogenized in a Waring blender for 4 min. The reaction was stopped by adding 0.2 ml. of 50 per cent TCA and 1.8 ml. of 5 per cent TCA containing 12.5 g of 'Norit A' per 100 ml. The determination of inorganic phosphate was carried out as indicated above. EDTA (●—●—●) and KF (■—■—■) were added as indicated. The values quoted were corrected for enzyme and substrate controls

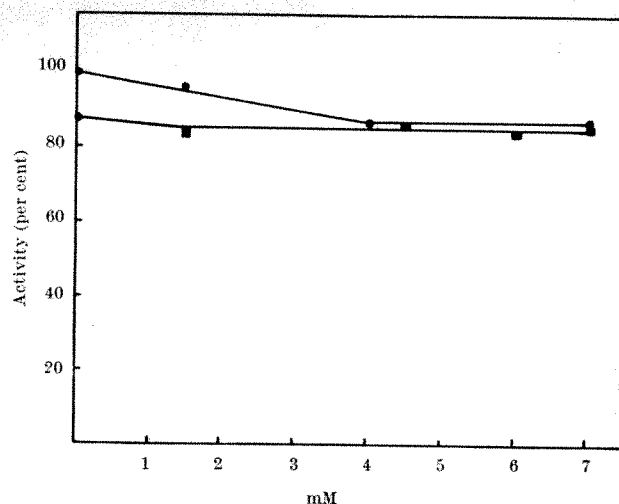


Fig. 2. The effect of EDTA and KF on glucose-6-phosphatase activity. The assay system contained, in a final volume of 0.7 ml., 43 mM maleate buffer, pH 6.0; 28.5 mM glucose-6-phosphate, pH 6.0; and 1.8 mg protein of a microsomal fraction from cat small intestinal mucosa. After incubation at 37° for 15 min, the reaction was stopped by adding 1.3 ml. of 10 per cent TCA and 2 ml. of 5 per cent TCA containing 12.5 g of 'Norit A' per 100 ml. Inorganic phosphate was determined as indicated in Fig. 1. EDTA (●—●—●) was added as indicated and KF (■—■—■) was added as indicated in the presence of 4 mM EDTA. The values quoted were corrected for enzyme and substrate controls

concentration (up to 2  $\mu$ moles of inorganic phosphate liberated per 20 min). In the assay of glucose-6-phosphatase, three tubes should be set up all containing 4 mM EDTA and 2 mM potassium fluoride. The amounts of inorganic phosphate liberated in the absence of substrate and enzyme respectively should be subtracted from that obtained with the complete system.

The experiments quoted so far were all done with particulate preparations from cat small intestinal mucosa but preparations from rabbit or guinea-pig small intestinal mucosa, although not so extensively studied, gave similar results.

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### Kinetic Evidence for a Nucleic Acid which regulates RNA Biosynthesis

THE rate of synthesis of nucleic acids in *Escherichia coli* strain B<sub>u</sub> has been examined in a variety of situations by measuring the incorporation into TCA insoluble material of 20-sec pulses of <sup>14</sup>C-8-guanine. In most cases the 20-sec pulses are long enough to give useful amounts of incorporations. However, extensive washing of the 'Millipore' filters must be carried out and frequently long counting times must be used. There is a short lag in the utilization of <sup>14</sup>C-8-guanine, but under most conditions 20 sec gives about half the radioactivity which would have been estimated from the rate of incorporation at later times, that is, there is about 10-13 sec worth of materials in pools already present in the cell, or made before feed-back inhibition becomes effective. Incorporation of radioactivity is the same at 0.1 the external concentration of <sup>14</sup>C-8-guanine as that standardly employed

(5  $\mu$ M), implying that feed-back inhibition is under both conditions maximal and rapid.

When a culture growing slowly ( $\sim 300$ -min doubling time) in 0.2 per cent DL-alanine as carbon source is 'shifted up' by the addition of 0.2 per cent glucose and 0.1 per cent casamino-acids, the rate of RNA synthesis starts to increase in less than 5 sec. The acceleration is constant until the rate achieves that characteristic of the new medium ( $\sim 31$ -min doubling time). This has been observed when vitamins in addition are included, when tryptophan is present throughout, and when chloramphenicol (50  $\mu$ g/ml.) is present in the shift-up medium (Fig. 1). In every case the acceleration is essentially instantaneous and is constant with values ranging from  $46$  to  $51 \times 10^{-13}$  moles  $^{14}\text{C}$ -8-guanine/mg dry weight  $\cdot 20$ -sec pulse  $\cdot$  sec. The rate characteristic of the new medium is achieved in 95–110 sec. From this it follows that new protein synthesis is not required to support the higher rate of RNA synthesis, because of the well-known 3-min lag in the production of new protein and because it occurs in the presence of chloramphenicol. Therefore, there are adequate amounts of RNA polymerase and the enzymes of nucleic acid intermediary metabolism in the slowly growing cells to support the forty-fold increase in the rate of RNA synthesis. In addition, these findings make the new suggestion that no special protein controlling the rate of RNA synthesis need be newly synthesized to increase the rate of RNA synthesis. Although there is doubt about the quantitative relationships, it is clear that messenger, transfer, and ribosomal RNA synthesis contribute significantly to the pulse incorporation in both media. Thus, a good deal of both messenger and ribosomal RNA synthesis (and probably transfer RNA) is controlled in the same rapid fashion, which would suggest a common regulation.

The strain of bacteria used requires uracil, which is normally supplied at 3  $\mu$ g/ml. When the culture is shifted by membrane filtration into medium devoid of uracil, the rate of RNA synthesis progressively decays (Fig. 2). If uracil is included in the radioactive pulse medium (open circles), 20–40 per cent greater rates of incorporation are observed than if uracil is omitted (solid circles). The stimulation of uracil in the pulse medium is a constant percentage increase no matter how long the starvation is protracted, or if the uracil concentration is increased and supplemented with uridine and cytidine. The half-life for the decay of the rate of RNA synthesis is 3.5 min in the experiment shown. Other experiments have shown

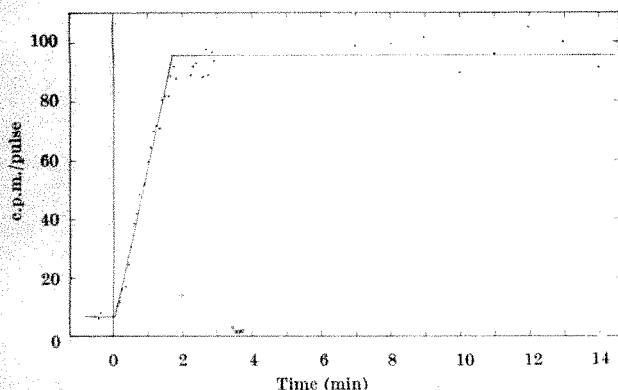


Fig. 1. To a culture growing with a doubling time of 320 min in M-9 at  $37^\circ$  with aeration with 0.2 per cent DL-alanine as carbon source at time zero, 0.2 per cent glucose, 0.1 per cent casamino-acids, 1 per cent by volume of a vitamin preparation (MEM) of Microbiological Associates, and 50  $\mu$ g/ml. chloramphenicol were added. The bacterial concentration was  $0.052$  mg/ml. At the times indicated, 1.0 ml. aliquots were added with an automatic syringe to 1.0-ml. medium of the final composition of the culture, except that  $^{14}\text{C}$ -8-guanine (10 mc./mmole) was present. 2.0 ml. of 10 per cent TCA were squirted in after precisely 20 sec. The open circles are controls in which the TCA was added to the radioactive medium before the addition of the bacteria. Note that the pre-shift values do not indicate the rate of RNA synthesis in that medium because of the additional nutrients in the pulse medium.

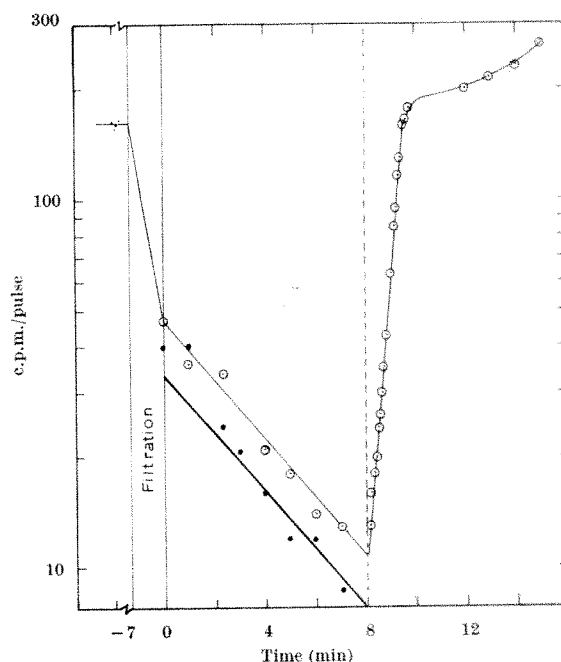


Fig. 2. A culture (doubling time 35 min) was filtered and resuspended in the pre-warmed rich medium of the same composition, but lacking uracil. Filtration commenced at  $-3$  min. Uracil was added (3  $\mu$ g/ml.) at 8 min. Pulse measurements in medium containing 3  $\mu$ g/ml. uracil (open circles), pulse measurements in medium lacking uracil (closed circles).

that it is not constant but about 1.5 min in the first 2 min after uracil removal, and that it is much slower than 3.5 min after the first 8 min. On the addition of uracil, after the eighth min (Fig. 2), RNA synthesis is accelerated exponentially with a 20-sec doubling time. This exponential type of acceleration has been observed several times including when the cells have been starved of uracil for 1 h. In this case, the doubling time was 30 sec. Because of the decreased counting rates, 1-min pulses were used to get measurable radioactivities.

Necessary controls showed that even with much larger amounts of uracil, together with uridine and cytidine, no faster recovery of RNA synthesis was obtained. Other controls showed that the 20-sec pulse measurements, although approximately proportional to the steady-state rate of guanine incorporation, are not exactly proportional. In fact, the guanine pools are altered by the uracil starvation, but this can only account for a factor of less than two-fold in the exponential acceleration that spans more than a decade (more than two decades in the 1 h uracil starvation experiment). A sham filtration with uracil in the wash and resuspension medium showed that RNA synthesis is not altered by the manipulations employed.

It thus seems necessary to postulate a type of RNA that is rapidly turning over in the cell and the function of which is to stimulate nucleic acid synthesis in general and, specifically, its own synthesis. It could do so by interaction with the polymerase, or by competing with the DNA for the various kinds of repressor molecules. In addition, the synthesis of this special type of RNA would be controlled by the general nutritional level of the cell and we would presume it to be the product of RC locus<sup>1</sup>.

When nutrients are removed (the 'shift down') ribosomal RNA synthesis abruptly ceases, but at least some messenger synthesis continues longer. A similar situation should obtain when an analogue of an amino-acid is added which is a non-utilizable feed-back inhibitor of the normal amino-acid, such as 5-methyl tryptophan.

When  $10^{-5}$ – $10^{-4}$  M DL-5-methyl tryptophan is added to a culture growing in glucose, casein hydrolysate, vitamins, and uracil (doubling time 30 min), the growth abruptly slows



to a doubling time of about 330 min. The rate of RNA synthesis in less than 5 sec commences to decay exponentially with a half-life ranging (in five experiments) from 30 to 38 sec. The same decay constant is observed when the amino-acids and vitamins are removed by membrane filtration. The experiments in this case are technically much more difficult. When large amounts of tryptophan (or the vitamins and amino-acids) are added back after 3–10 min, an induction phase is noted which is consistent with a 20–30 sec doubling time for an autocatalytic process, and it may take 10 min to recover full synthetic rate.

Absolute control of all RNA synthesis cannot be exercised by the autocatalytic control loop and the induction of the *RC* locus above. Neidhardt<sup>2</sup> has shown that relaxed mutants still control their RNA synthesis to give the ribosomal content consonant with growth supporting capabilities of the medium. Similarly, the postulated controlling RNA is not limiting under the conditions of slow growth on alanine as indicated in Fig. 1. As well, a culture starved of its carbon source (uracil present) for 3 h immediately without lag synthesizes RNA at a linearly accelerating rate, similar to the conditions of Fig. 1, when the amino-acids, vitamins, and glucose are added. Thus, it is necessary to postulate an additional control which obviates the influence of the special RNA on its own synthesis. This, of course, requires further investigation, but we have an example (addition of tryptophan to a rich culture treated with 5-DL-methyl tryptophan for 0.5–1 h) which shows an exponential followed by a linear recovery phase.

The only alternative explanation which we have been able to imagine is that RNA synthesis is generally limited by some small molecular weight compound, say a uracil derivative, of which the rate of synthesis is under physiological and genetic control. The exponential build-up shown in Fig. 2 would then simply be indicative of a number of steps from exogenous uracil to this derivative. This appears to us less likely, since the autocatalytic law seems to be obeyed over a large factor and, secondly, because uracil replacement should have the same absolute restoring action on RNA synthesis no matter how long the starvation was protracted. Further, the investigations with 5-methyl tryptophan would require an entirely different explanation.

It also does not appear possible to explain these results on the hypotheses that uncharged transfer RNA molecules act as inhibitors of RNA synthesis generally<sup>3</sup> or as co-repressors for the majority of operons in the cell<sup>4</sup>.

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### Isolation of an Unknown Component from Hydrolysates of Elastin

PARTRIDGE *et al.*<sup>1</sup> have described two new polyfunctional amino-acids obtained as products of an acid hydrolysis of bovine elastin. Compounds of identical nature were also obtained by us<sup>2</sup>. These substances, designated desmosine and isodesmosine by Partridge *et al.*, have been shown by these investigators to contain a pyridine nucleus with amino-acid substituents on both carbon and nitrogen atoms of the ring<sup>3</sup>. The special significance of desmosine

and isodesmosine is that they appear to be involved in the cross-linking of polypeptide chains of elastin<sup>1</sup>.

To gain more information about the nature of cross-linking in elastin, we have attempted to isolate peptides from enzymatic hydrolysates of elastin, in which desmosine and isodesmosine are present in large amount. In the course of this work we observed that such peptides indeed can be obtained but that they contain yet another hitherto undescribed ninhydrin-positive substance, designated by us as  $X_4$ .

For the isolation of peptides, 100 mg of elastin suspended in 10 ml. of 0.1 M potassium carbonate-hydrochloric acid buffer, pH 8.9, was digested by addition of 1 mg of either elastase, nagarse or papain. Following overnight incubation at 38° C, the digests were acidified with hydrochloric acid and placed on columns of 'Dowex 50-X2'. Elution was started with 0.2 N citrate buffer, pH 3.1, followed by 1.1 N citrate buffer and 2.1 N citrate buffer, both at pH 5.1. Then, 500 ml. of water was passed through the column and final elution was carried out with 0.05 N sodium hydroxide. By this procedure, the peptides enriched in desmosine, isodesmosine and  $X_4$  were eluted with 0.05 N sodium hydroxide. All the enzymatic digestions investigated yielded a similar fraction. This fraction accounted for 2–4 per cent of the ninhydrin-positive material and approximately 35–48 per cent of the 275 mμ absorbing material placed originally on the column.

The amino-acid composition<sup>4</sup> (Technicon analyser) of the desmosine-isodesmosine rich fraction obtained after digestion with either elastase, papain or nagarse is given in Table 1. The amino-acid analysis of bovine elastin before treatment with enzyme is also given in Table 1. Isodesmosine and desmosine, in these analyses, appear in eluate fractions 18 ml. and 28 ml. respectively after those containing ammonia.  $X_4$  appears in fractions 13 ml. before those containing lysine and, if ornithine is also present, between ornithine and lysine. It should be emphasized that residues of desmosine and isodesmosine are calculated using a ninhydrin colour equivalent equal to four times that of leucine. On the other hand, in view of our lack of knowledge of the structure of  $X_4$ , residues of this substance are calculated using a colour equivalent equal to that of leucine. It may be seen that the peptide fraction relatively rich in desmosine and isodesmosine is also rich in  $X_4$ .

Table 1. AMINO-ACID COMPOSITION OF PEPTIDE FRACTIONS RICH IN DESMOSINE, ISODESMOSINE AND  $X_4$ \*  
(Expressed as residues per 1,000 residues)

Amino-acid	Bovine elastin	Elastase	Papain	Nagarse
Cystic acid	3.2	13.7	12.2	13.8
Hydroxyproline	7.1	7.7	6.1	Trace
Aspartic acid	7.0	7.3	10.3	12.3
Threonine	10.1	9.7	9.7	9.6
Serine	9.9	17.1	11.8	23.1
Glutamic acid	17.4	11.5	18.9	17.3
Proline	125.4	123.5	120.4	141.9
Glycine	316.2	275.0	239.5	236.9
Alanine	213.3	269.3	283.6	243.1
Valine	134.0	66.5	91.0	60.8
Methionine	—	—	0.4	—
Isoleucine	26.6	12.4	22.1	21.9
Leucine	64.7	48.6	51.0	40.8
Tyrosine	6.1	8.1	13.7	21.2
Phenylalanine	33.6	36.8	41.2	47.7
Lysine	3.6	9.1	10.5	11.5
Histidine	0.5	1.7	2.1	3.1
Arginine	6.6	7.7	12.0	11.9
Isodesmosine	1.1	6.3	3.4	8.3
Desmosine	1.7	9.1	5.1	11.1
$X_4$	1.8	6.1	4.8	7.7

\* Values are uncorrected for slight losses during hydrolysis.

Desmosine, isodesmosine and  $X_4$  were isolated in the following manner. 15.6 g of elastin of bovine ligamentum nuchae was hydrolysed in 6.0 N hydrochloric acid under reflux for 24 h. The hydrolysate was evaporated to dryness, redissolved in water and placed on a 'Dowex 50-X8' column of dimensions 4.5 cm × 17 cm. Elution was carried out with 1 l. of 1.5 N hydrochloric acid, followed with 5 l. of 2.5 N hydrochloric acid, and finally with 6 l. of 4.0 N hydrochloric acid. The resulting chromatogram is

shown in Fig. 1. Fractions 320–350 were pooled, and the combined fraction was evaporated to dryness. Most of the reactivity of this fraction with ninhydrin was due to the presence of  $X_4$ . The latter was further purified by dividing the eluant stream coming from the column on the Technicon amino-acid analyser. The fractions containing  $X_4$ , obtained in this manner, were pooled, acidified to pH 2 with hydrochloric acid, and desalted on a 'Dowex 50' column utilizing 0.25 N ammonium hydroxide as the eluting solvent. The  $X_4$  fractions obtained by this procedure were evaporated to dryness, washed with ethanol and finally with ether. Re-chromatography of the purified material resulted in emergence of only one peak; this corresponded to  $X_4$ . Paper chromatography in *n*-butanol-acetic acid-water (4:1:5) revealed only one ninhydrin-positive spot with an  $R_F$  of 0.13.

$X_4$  does not exhibit absorption of visible light nor, in contrast to the desmosines, of ultra-violet light. It has an infra-red spectrum characteristic of an amino-acid; the compound showed absorption maxima for ionized carboxyl at 1,600  $\text{cm}^{-1}$  (asymmetrical) and 1,415  $\text{cm}^{-1}$  (symmetrical) and amino-acid I and II bands (deformation frequencies) at 1,635  $\text{cm}^{-1}$  and 1,510  $\text{cm}^{-1}$ . The hydrochloride of  $X_4$  revealed an absorption maximum for unionized carboxyl at 1,740  $\text{cm}^{-1}$ . The spectrum also suggests the presence of a hydroxyl group, but the evidence is not conclusive. There is no indication of the presence of ketone, aldehyde or lactone functions. Elemental analysis, kindly performed by the F. and M. Scientific Co., Avondale, Pa., using their Model 180 carbon-hydrogen-nitrogen analyser, showed: C, 44.7; H, 7.1; N, 10.4 per cent. This corresponds to an empirical formula of  $\text{C}_6\text{H}_{10}\text{NO}_3$ . High-voltage electrophoresis of  $X_4$  at either pH 6.4 or pH 3.4, using pyridine-acetate buffers, revealed only one ninhydrin-positive component. In 30 min at 3,500 V,  $X_4$  migrated 7.0 cm towards the negative pole at pH 3.4 and 6.2 cm towards the negative pole at pH 6.4.

Without special prejudice, the authors consider that any of four possible relationships of  $X_4$  to the original elastin may account for its ultimate appearance in hydrolysates of that protein prepared with acid. These possibilities are: (1)  $X_4$  is a hitherto undescribed amino-acid present in the polypeptide backbone *per se* of elastin molecules. (2)  $X_4$  is a discrete amino-acid involved in the cross-linking of several polypeptide chains of elastin. (3)  $X_4$ , together with desmosine and isodesmosine, are degradation products of the true cross-linking substance present in elastin before hydrolysis with acid. (4)  $X_4$  is a

degradation product of another substance unrelated to the desmosines.

At present, investigations are under way to determine which of these possibilities actually applies, and to determine the precise structure of  $X_4$ .

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### Relation between Serum and Colostrum Antibody in the Goat

It has been suggested that antibody synthesis can occur in mammary tissue<sup>1</sup>. It has also been suggested that the observed differences in  $\gamma$ -globulins and in specific antibodies between serum and milk or colostrum are due to selectivity or to active transport mechanisms<sup>2,3</sup>. On the other hand, Hanson<sup>4</sup> was unable to identify a milk protein antigenetically identical to human serum 7S  $\gamma$ -globulin, and Chodirker *et al.*<sup>5</sup> were able to demonstrate little or no  $\gamma_2$ -globulin in milk. Much of this work was based on the measurement of antibody activity without actual isolation of the antibody, or on  $\gamma$ -globulin determination by some means unrelated to the presence of specific antibody. Using labelling techniques, Askonas *et al.*<sup>6</sup> showed that in the rabbit, and probably in the goat also, specific antibodies in the milk appeared to be derived directly from serum antibody. The work recorded here was undertaken to compare the electrophoretic mobility in starch gel of a particular antibody from the serum of the goat with that from colostrum, both of which were free of non-specific protein. The electrophoretic patterns of the papain digests of the respective antigen-antibody precipitates were also compared.

A normal female goat was immunized with bovine serum albumin (Armour) in complete Freund's adjuvant by weekly subcutaneous injections. Blood was obtained from the jugular vein and colostrum by usual milking techniques. A quantitative precipitin curve was obtained using the 40 per cent ammonium sulphate fraction of the goat serum, and the proportions at equivalence were determined. Freshly obtained colostrum was centrifuged at 4,500 r.p.m., 2° C for 60 min. The fatty layer was removed and the supernatant poured off. The pH of the supernatant was lowered to 4.5 by the addition of concentrated HCl with constant mixing; the precipitate which formed was centrifuged off, and the pH of the supernatant restored to 6.5 with NaOH<sup>7</sup>. Precipitin tests were then performed both with this material directly and with the 40 per cent ammonium sulphate fraction.

The immune precipitates formed at equivalence from serum and colostrum were washed four times with 0.9 per cent NaCl at 2° C, then dissociated by treatment with glycine-sulphuric acid buffer<sup>8</sup>, pH 2.5, or digested by crystallized papain<sup>9</sup> in 0.1 M sodium phosphate buffer containing 0.01 M

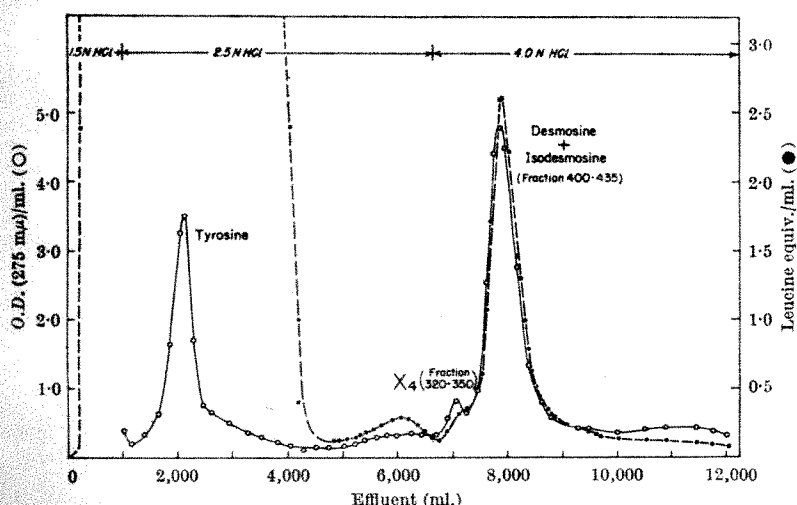


Fig. 1. Chromatogram of an acid hydrolysate of elastin from bovine ligamentum nuchae eluted from 'Dowex-50'. The flow rate of elution was approximately 2 ml./min, and fractions of 18–20 ml. each were collected. The ultra-violet absorption spectrum and content of amino groups (ninhydrin) were determined on every third fraction.



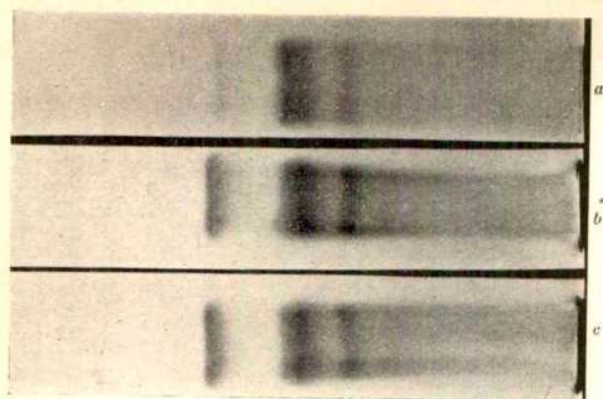


Fig. 1. Starch-gel electrophoresis in glycine-sulphuric acid buffer, pH 2.5. Anode situated on the right and direction of migration from right to left. (a) Goat serum, ammonium sulphate fraction; (b) goat serum, dissociated antigen-antibody precipitate; (c) goat colostrum, dissociated antigen-antibody precipitate. The most rapidly moving band was a goat albumin contaminant in (a), and bovine serum albumin in (b) and (c). The middle band was 7S  $\gamma$ -globulin in (a), and antibody in (b) and (c). The slow band in all three was not identified.

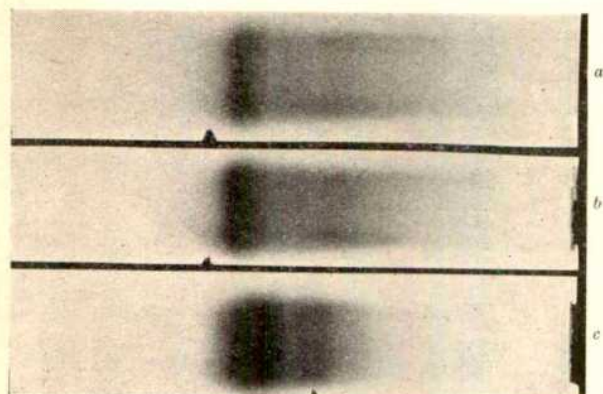


Fig. 2. Starch-gel electrophoresis of papain-digested antigen-antibody precipitates derived from: (a) goat serum; (b) goat colostrum; (c) rabbit serum. Procedure as in Fig. 1. The notches in the side of the gels indicate the location of the fluorescein-labelled antigen or its derivative.

EDTA and 0.01 M cysteine for 2 h. The digests were then dialysed against water and lyophilized.

Two mg of each immune precipitate in glycine-sulphuric acid buffer, ionic strength 0.25, were electrophoresed in starch gel<sup>10</sup> using the same buffer in the electrode compartments and an eight-fold dilution for the gel. A constant current of 40 or 50 m.amp was applied for 16–20 h at 4° C, following which the gel was cut horizontally, one half stained with amido-black and decolorized in the usual manner. The bands representing antibody coincided exactly with each other and with goat serum  $\gamma_2$ -globulin (Fig. 1). The papain digests of the respective antigen-antibody precipitates were electrophoresed under the same conditions and the resulting bands also corresponded exactly (Fig. 2). For comparison, papain digests of antigen-antibody precipitates derived from rabbit anti-bovine serum albumin are also shown. The pattern of the latter is entirely different.

Fluorescein isothiocyanate (General Biochemicals) was coupled to bovine serum albumin<sup>11</sup> and the unbound dye removed by passage of the mixture over a 'Sephadex G 25' column equilibrated with 0.9 per cent NaCl. No significant reduction in precipitation with antibody was noted. Papain digests of the antigen-antibody precipitates were prepared from fluorescein-labelled antigen and antibody derived from both serum and colostrum. After electrophoresis in starch gel, the unstained halves of the gel were examined with long-wave ultra-violet light. The fluorescent bands were observed to coincide and their location was indicated by notches made on the stained

portions of the gels (Fig. 2). With digested rabbit precipitate the fluorescent band occupied a different position. Further investigations on the relation between rabbit and goat antibody will be presented elsewhere.

'Sephadex G 100' chromatography in 0.9 per cent NaCl of the papain digests of each of the serum and colostrum goat antigen-antibody precipitates showed two well-separated peaks in the same position in each case. When the fluoresceinated antigen was used the fluorescence was confined to the first peak in both instances. In addition, lines of identity were observed in agar gel between bovine serum albumin on one hand and serum and colostrum on the other.

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## PHYSIOLOGY

### Fasting Levels of Growth Hormone in Men and Women

RECENT methodological improvements in radio-immunoassays for growth hormone<sup>1,2</sup> have led to increasing use of these techniques in clinical and physiological research. We have found a large and apparently previously unreported difference in the fasting growth hormone level of normal males and females.

Fifteen healthy, non-obese male volunteers, averaging 32 years of age (19–54 years), and 8 similarly healthy, non-obese, non-pregnant females, averaging 41 years of age (30–62 years), were assembled. All had a normal glucose tolerance by the criteria of Fajans and Conn<sup>3</sup>, and gave a negative family history of diabetes. After a 3-day high carbohydrate diet and an overnight fast, a fasting blood specimen was obtained and assayed for growth hormone by the radio-immunoassay technique of Roth, Glick, Yalow and Berson<sup>2</sup>. All specimens were assayed in duplicate. Neither the duration of the overnight fast nor the amount of physical exercise before the test was measured.

The mean growth hormone concentration of the females greatly exceeded that of males, averaging 4.91  $\mu\text{g}/\text{ml}$ . ( $S.D. \pm 3.32$ ) with a range of from 0.9 to 10.6  $\mu\text{g}/\text{ml}$ . The mean level in men was 0.27  $\mu\text{g}/\text{ml}$ . ( $S.D. \pm 0.14$ ) with a range of from 0.1–0.7  $\mu\text{g}/\text{ml}$ . This difference is statistically significant ( $P < 0.05$ ). Fig. 1 shows the mean pattern of growth hormone concentration noted before and during a 100-g oral glucose tolerance test in the two groups, and reveals the persistence of the inter-group difference until at least 40 min after glucose ingestion.

The mean fasting plasma glucose concentration, as determined by the method of Hoffman<sup>4</sup> on the Technicon Autoanalyser, was 94 mg per cent (89–104) in the female



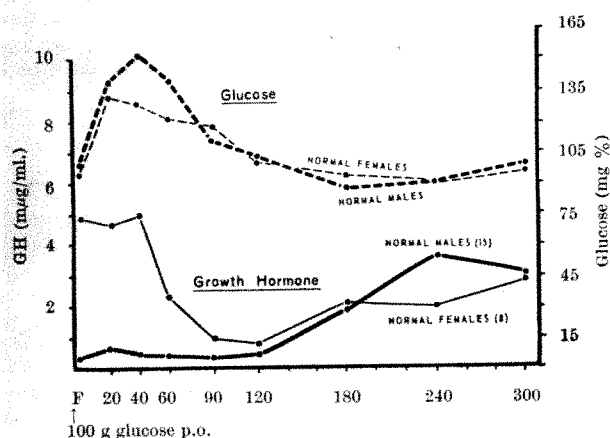


Fig. 1. Growth hormone concentrations before and during a 100 g oral glucose tolerance test in 8 normal non-obese females and 15 comparable males. The higher fasting growth hormone level of the female group persists until at least 40 min after glucose ingestion. No significant inter-group differences are noted after the 60-min point.

group and 99 mg per cent (72–111) in the male group. Neither this difference nor the age difference between groups adequately explains the difference in growth hormone levels, since their elimination by regrouping of subjects fails to diminish the difference in growth hormone.

Examination of some earlier data tends to support these findings. Hunter and Greenwood<sup>5</sup> measured fasting growth hormone levels in 6 men and 4 women, 3 of whom were obese; despite this dissimilarity in their subjects, qualitatively similar sex differences in growth hormone concentration can be calculated from their data.

Although the physiological implications of this sex difference remain to be determined, it is apparent that it should be taken into consideration in investigations of human growth hormone concentrations.

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## A Charge-transfer Complex between Procaine and Ribonucleic Acid

An understanding of the mechanism of action of local anaesthetics is of great importance for the molecular physiology of nerve. An intense colour reaction between the local anaesthetic procaine (*p*-aminobenzoyldiethylaminoethanol) and ribonucleic acid (RNA) has been discovered. The active nucleic acid components is apparently ribose. The pentose sugars xylose and arabinose are also active. There is no reaction with deoxyribose or with DNA. Hydrolysates of RNA produced by the hydrochloric acid or perchloric acid method are both active.

The method of obtaining the reaction with RNA is very simple and is as follows: 200 mg of commercial

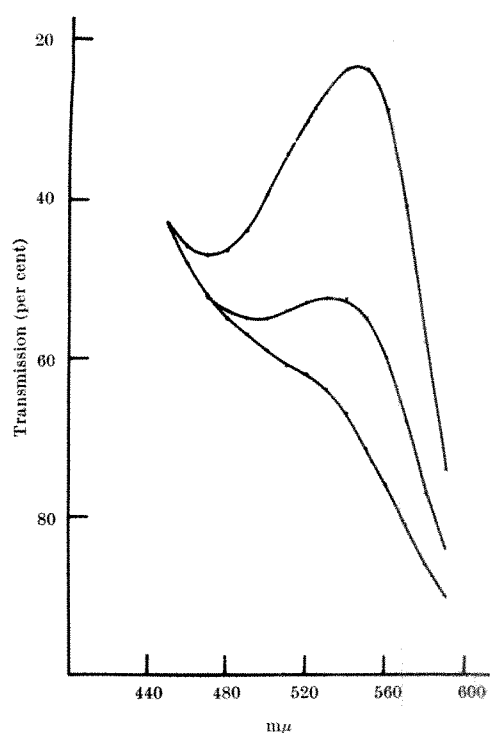


Fig. 1. Charge-transfer bands of procaine-RNA complex. RNA hydrochloric acid hydrolysate. Absorption spectra recorded with an automatic recording spectrophotometer at 1-min intervals: upper curve, at 1 min; middle, at 2 min; lower, at 3 min. Ambient temperature 25° C.

yeast RNA and 200 mg of procaine hydrochloride are placed in a dry test-tube with 3 ml. of distilled water and the test-tube is shaken gently. Within 1–2 min the RNA mass turns a brilliant magenta colour ( $\lambda_{\text{max}} = 550 \text{ m}\mu$ ). At room temperature this colour is transient and rapidly begins to fade and to be replaced by browning probably due to furfural formation. However, if the magenta complex is frozen at 0° C the absorption band at 550 mμ may remain stable for several months.

A similar sequence of events occurs with yeast RNA hydrochloric acid hydrolysate (Fig. 1) after clarification by passage through a 0.22-μ 'Millipore' filter. In contrast with RNA and RNA hydrolysate, the reaction with D-ribose requires several hours and evidently does not occur above pH 3–4. There is no reaction with any of the nucleic acid bases nor with phosphoric acid.

In a series of reports<sup>1–3</sup> Eckert has attempted to demonstrate that procaine can enter into a charge-transfer complex with various electron-acceptor compounds, including thiamine, and he has suggested that it is a  $\pi$ -electron or charge-transfer complex with thiamine which is responsible for the anaesthetic action of the drug. Two recent authoritative reviews<sup>4,5</sup> have directed attention to these experiments and to the intriguing possibility that a charge-transfer reaction is involved in certain classes of anaesthetic action. I have repeated Eckert's experiments with procaine. His 'charge-transfer' bands are actually artefacts produced by his method of spectrophotometric analysis. They are difference bands and unrelated to his conclusions.

The essential result of a charge-transfer complex is the appearance of a new intense absorption band, usually at a longer wave-length, where neither compound has any significant absorption. Such a complex belongs to the class of weak interactions and has a negative temperature coefficient. Many  $\pi$ -electron complexes can therefore be produced only at dry-ice temperatures and their stability is extremely sensitive to any thermal changes. The molecular structure of each member of the complex must be such as to allow for a degree of electron delocalization.

If this requirement is satisfied, then the appearance of a new temperature sensitive absorption band is sufficient evidence for the existence of charge-transfer.

The magenta complex between procaine and certain pentose sugars may be an intermediate stage in Schiff base formation. Procaine is a primary aromatic amine and these sugars possess a free aldehyde group. Such a reaction is the basis for several nucleic acid colour tests. The magenta complex reported here, however, is obtained at room temperature and is transient. It suggests that cell-surface mucopolysaccharides may play an important part in the action of certain anaesthetics, and that a detailed examination of the interaction of polysaccharides with neurotropic primary aromatic amines may prove rewarding.

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### Urinary $\beta$ -Amino Isobutyric Acid in the Mouse: Elimination after Intravenous Administration of Deoxyribonucleic Acid

THE initial catabolism of DNA injected intravenously into higher mammals is under the control of the neutral DNases of blood plasma<sup>1</sup>.

This communication concerns the final steps of DNA catabolism followed by the urinary elimination of  $\beta$ -amino-isobutyric acid (BAIBA), an essential catabolite of thymine<sup>2</sup>, after intravenous administration of isologous tritiated DNA. Mice are very useful for such an investigation, since the plasma DNase activity varies in a narrow range for animals in each strain, but notably differs from one strain to another<sup>3</sup>.

Male and female mice, weighing 20–21 g (*C<sub>3</sub>H*) or 17–18 g (XLII) and 3–4 months old, were used. Urine was collected either with the aid of a metabolism cage containing 10 mice or by direct sample-taking. The extraction and the quantitative determination of the BAIBA in the urine were carried out according to a technique based on that of Gerber *et al.*<sup>4</sup>; the determination of the DNase activity of the plasma (pH = 7.5) was carried out according to the technique of Kurnick<sup>5</sup>, applied as a micromethod to mixtures of plasma from the various groups of animals and expressed as Kurnick Units (KU). The sodium in the urine was quantitatively determined by flame spectrophotometry.

The radioactive DNA was extracted from the intestine of the *C<sub>3</sub>H* and XLII mice, using the method of Kay *et al.*<sup>6</sup>, 3 h after intravenous injection of 20  $\mu$ c. of tritiated thymidine labelled in the 5-methyl group. These DNA samples showed the usual optical (spectrum and hyperchromicity in ultra-violet light), physical (light diffusion, viscosity, CsCl gradient density) and chemical (guanine, cytosine, RNA, protein and water contents) characteristics; their specific activities were very similar—of the order of  $2.5 \times 10^5$  d.p.m./mg DNA—as measured in a Packard scintillation counter, all the corrections of quenching being carried out after addition of standard labelled thymidine; DNA was quantitatively determined by the method of Burton; paper chromatography of the bases obtained after perchloric hydrolysis showed that all radioactivity was localized in the thymine; no radioactive contaminant was present (CsCl gradient and successive re-precipitations in alcohol).

In the initial series of experiments, the labelled isologous DNA was injected into one of the tail veins by a perfusion lasting 30 min (0.5 ml. of 0.14 M NaCl containing 1.7–2.0 mg/ml.). In a second series of experiments, a solution of DNA from *C<sub>3</sub>H* and XLII mice was mixed in equal parts and hydrolysed by pancreatic DNase (Worthington) at 37° for 1 h with 0.02 M MgSO<sub>4</sub>.

The values of physiological urinary excretion of endogenous BAIBA were twice as high in the XLII mice as in the *C<sub>3</sub>H* mice, while the volumes of the urine were of the same order (Table 1); this ratio was always found, whether the excretion was expressed in terms of concentration ( $\mu$ moles/ml.) or in absolute value ( $\mu$ moles/24 h). Although the urine samples were taken at random times, the possibility of large fluctuations in diuresis is excluded by the constant urinary sodium values found during the experiments ( $166 \pm 50$  m.equiv. per thousand in *C<sub>3</sub>H* mice;  $170 \pm 37$  m.equiv. per thousand in XLII mice).

Table 1. LEVELS OF THE ENDOGENOUS URINARY BAIBA IN *C<sub>3</sub>H* AND XLII MICE

	Random times urine <i>C<sub>3</sub>H</i>	Random times urine XLII	24-h urine <i>C<sub>3</sub>H</i>	24-h urine XLII
No. of experiments*	25	20	15	7
BAIBA ( $\mu$ mole/ml.)	0.40 $\pm$ 0.14	1.03 $\pm$ 0.35	0.53 $\pm$ 0.17	1.25 $\pm$ 0.57
Mean urinary volume (ml. per 24 h)			5.7	5.3
BAIBA ( $\mu$ mole/24 h)				
Extrapolated	2.28	5.45		
Measured			2.51 $\pm$ 0.65	5.39 $\pm$ 1.56

\* All the determinations have been carried out with a mixture of the urine from a group of ten animals.

After intravenous administration of comparable quantities of isologous labelled DNA, the rate of urinary excretion of labelled BAIBA resulting from the catabolism of this DNA also showed a two-fold difference between the *C<sub>3</sub>H* and the XLII mice (Fig. 1A). Furthermore, after injection of a quantity of hydrolysed labelled DNA corresponding to the dose of DNA used earlier, the rate of excretion of labelled BAIBA was increased in the two strains of mice; the quantities of labelled amino-acids excreted with the urine were always in a proportion of about 1/2 (Fig. 1B).

Since the same ratio (1/2) is found between the plasma DNase activities in *C<sub>3</sub>H* (0.14–0.23 KU) and XLII mice (0.28–0.39 KU) enzymatic activity and BAIBA elimination were determined on the same animals.

A direct correlation between plasma enzyme-level and endogenous urinary BAIBA excretion was found between the two strains (Fig. 2A); moreover, such a correlation holds in each strain between the plasma DNase activity and the speed of tritiated BAIBA elimination (Fig. 2B). These findings are statistically significant at the 1 per cent (*C<sub>3</sub>H*) and 5 per cent (XLII) level.

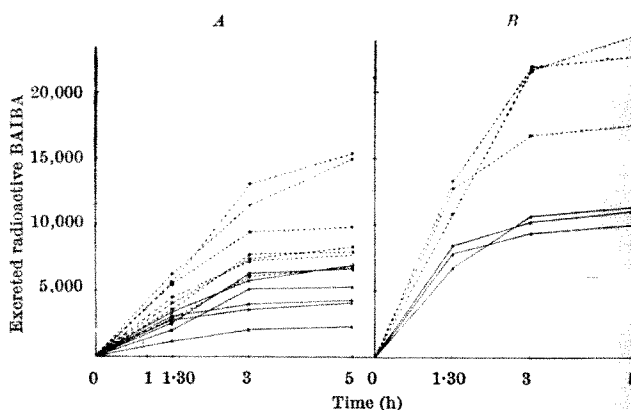


Fig. 1. Tritiated BAIBA urinary excretion after intravenous injection of isologous tritiated DNA. A, polymerized tritiated DNA; B, hydrolysed tritiated DNA. Each curve records five mice. ---, XLII; —, *C<sub>3</sub>H*.

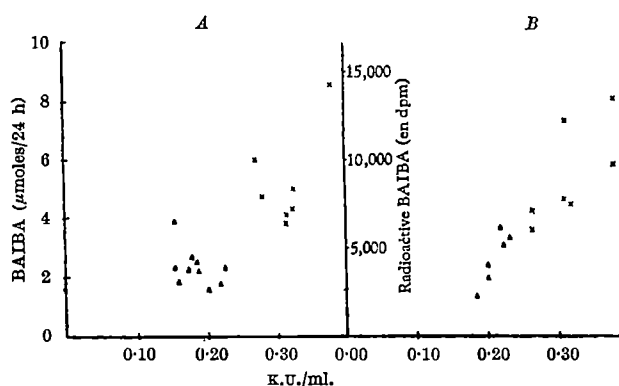


Fig. 2. Relationship between neutral plasma DNase activity and BAIBA urinary excretion. A, endogenous BAIBA (10 mice for each point); B, radioactive BAIBA 3 h after intravenous injection of tritiated DNA (5 mice for each point). x, XLII;  $\Delta$ , C<sub>3</sub>H

These results lead to three main conclusions:

(1) BAIBA is a catabolite of DNA thymine; nevertheless it can only account for a small fraction of it (5–10 per cent). Thymine may take other metabolic pathways or perhaps BAIBA itself may undergo catabolic degradation.

(2) Though the DNase activity of plasma regulates the initial degradation of intravenously injected DNA (ref. 1), it does not determine all the transformations from DNA thymine to BAIBA. These transformations are controlled by many other enzymes the activities of which are regulated at levels near those of neutral plasma DNases, but differing from one strain to the other.

(3) The rate of BAIBA elimination in urine depends on the intensity of exogenous DNA catabolism and affords a possible means of expressing it.

On the other hand, these results suggest that this conclusion might be extended to normal elimination of BAIBA which could be an index of the intensity of cell DNA degradation, and so would give a criterion for an easy biochemical evaluation of cellular turnover. This elimination is probably, as in man<sup>3</sup>, under genetic control, and its variations are not due to renal influences, since preliminary results show that the plasma-level of BAIBA is higher in XLII than in C<sub>3</sub>H strain mice.

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### Dilution of the Diet and Feed Intake in the Mouse

Mice were fed from 3 to 6 weeks of age (weaning to mating) on diets made up by diluting a normal diet with different levels of an indigestible filler (cellulose). A greater range of diets, and younger animals, were used than in earlier work<sup>1</sup>. The error (and labour) associated with separating waste feed and faeces when feeding pelleted food was overcome by feeding ground diets, so that waste feed could be sieved from the faeces.

The animals used in the present experiments were from a random bred strain previously fed normally. The

change to each experimental diet was made abruptly after normal weaning at 3 weeks of age. The diets used are denoted by the percentage dilution with cellulose of the normal diet.

**Experiment 1.** The mice used in this experiment were litter mates from litters adjusted to 4 of each sex at birth. Only those of one sex from each litter were used on each diet. Data were from 4 litter mates per cage and 3 cages of each sex per diet (Table 1).

Table 1. WEIGHT GAIN AND FEED INTAKE FROM 3 TO 6 WEEKS

Sex	Diet dilution (%)	No.	Gain/head/day (g)	Dry matter intake/head/day (g)	Dry matter intake (g)/gain (g)	Gut wt. (g)/body wt. (g)
M	—	12	0.67	3.82	5.70	0.16
	30	12	0.73	5.64	7.73	0.19
	50	12	0.66	7.70	11.67	0.23
	70	8	0.06	5.94	99.01	0.31
F	—	12	0.44	3.76	8.55	0.16
	30	12	0.48	5.35	11.15	0.19
	50	12	0.51	6.98	13.69	0.24
	70	—	—	—	—	—

The mice on the 70 per cent diluted diet had difficulty in dealing with this diet in the ground form. The same diet in the pelleted form was fed successfully to similar mice in the laboratory. All the female mice on this diet died and the 8 male survivors gained so little weight that the intake per g of gain was exaggerated.

Mice were killed at 6 weeks of age and the fresh weight of the intestinal tract was recorded. This clearly reflected the compensatory 'gut-fill' of the animals on the highly diluted diets.

The dry-matter intake per head per day was doubled on the 50 per cent diluted diet, and then dropped in the males at the higher level of dilution.

**Experiment 2.** In this experiment males only were used. Litters which had been adjusted to 4 mice of each sex at birth were bulked at weaning, and then randomized to each treatment. This was simpler, but gave results as reliable as the method in exp. 1. Data were from 4 mice per cage with 6 cages per diet (Table 2).

Table 2. WEIGHT GAIN AND FEED INTAKE FROM 3 TO 6 WEEKS

Sex	Diet dilution (%)	No.	Gain/head/day (g)	Dry matter intake/head/day (g)	Dry matter intake (g)/gain (g)	Gut wt. (g)/body wt. (g)
M	—	24	0.81	3.95	4.88	0.16
	30	24	0.68	4.99	7.34	0.18
	50	24	0.60	6.34	10.57	0.22
	70	16	0.48	4.16	8.67	0.30

As in exp. 1, dry matter intake per head per day increased up to a dilution of 50 per cent and then declined. This is similarly reflected in intake per g of gain, and the response to dilution is also shown in the 'gut-fill' of the animals on each treatment.

It is apparent that the compensation mechanism of the animal breaks down after about 50 per cent dilution. The 70 per cent diluted diet again caused the death of some animals. Up to a dilution of 50 per cent the mice appeared normal, but at 70 per cent dilution they were under stress.

It can be concluded that dilution of the diet brings about a definite compensatory response by the animal, increasing the total dry-matter intake. This takes place immediately after weaning and breaks down at levels of dilution about 50 per cent. Further work is required to investigate the efficiency of utilization of the digested nutrients of the diets, to measure the true effect of the increased intake on the animal.

Before dietary dilution can be used in animals as a means of controlling nutrient intake, the compensation response of the animal, and the effect which increased intake has on digestibility of the diet, must be known over a wide range of dilutions.

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# Changes in Sino-atrial Node Transmembrane Potentials on Vagal Stimulation of the Isolated Rabbit Atrium

Hutter and Trautwein<sup>1</sup> reported that vagal stimulation results in hyperpolarization, complete disappearance of action potentials, and a shortening of duration of the post-stimulation pacemaker potentials of the frog sinus venosus. No report has appeared regarding the effects of stimulation of external vagal fibres on sino-atrial (S-A) node action potentials of mammalian heart. In the experiments described here, we set out to test the responses of the various types of S-A node fibres<sup>2</sup> to vagal stimulation of isolated rabbit atria.

Albino rabbits, weighing 1.5–2.0 kg, were used for making the isolated vagus-atrial preparation following the technique described by Toda *et al.*<sup>3</sup>. Ringer-Locke solution of the following composition in mmole/l. was used: NaCl, 147; KCl, 5.4; NaHCO<sub>3</sub>, 14.9; CaCl<sub>2</sub>, 2.2; and dextrose, 5.6. The solution was gassed with 95 per cent oxygen–5 per cent carbon dioxide. The temperature of the muscle chamber was maintained at 30° C. Transmembrane action potentials were recorded with the aid of the floating glass microelectrode<sup>4</sup>. An external bipolar electrode was used to record simultaneously from the atrial roof. Both vagi were brought above the surface of the solution for stimulation by a train of rectangular pulses 5–10 V in strength, 1.0 msec in duration, applied at 20/sec.

In the normal solution, vagal stimulation produced bradycardia but not asystole. Marked hyperpolarization, depression of action potential amplitude and shortening of duration in some of the S-A node action potentials occurred concomitantly with the effect on the rate. In other recordings, only slight or no change in action potential configuration occurred despite the appearance of bradycardia. Thus, the S-A node was divided into 4 parts as suggested by the pattern of response to vagal stimulation. Table 1 characterizes the types of action

Table 1. PARAMETERS OF S-A NODE ACTION POTENTIAL CONFIGURATION AND THE CHANGES INDUCED BY VAGAL STIMULATION

Parameter	Characteristics of action potential types			
	Type 1	Type 2	Type 3	Type 4
<i>N</i>	9	24	34	26
<i>MDP</i> (mV)	61.1 ± 1.62	56.3 ± 1.18	55.3 ± 0.66	65.0 ± 0.88
<i>APA</i> (mV)	71.1 ± 1.82	59.7 ± 1.47	58.6 ± 0.81	73.7 ± 1.25
<i>OS</i> (mV)	8.3 ± 0.84	3.9 ± 0.86	4.5 ± 0.48	9.3 ± 0.58
<i>SD</i>	Slight or none	Moderate	Marked	Slight
<i>SD to AP</i>	Sharp	Smooth or sharp	Smooth	Sharp
Changes on vagal stimulation				
Depression of <i>APA</i>	Marked	Marked	Slight	Slight
Hyperpolarization (mV)	7.9 ± 1.07	6.8 ± 0.62	3.0 ± 0.39	1.5 ± 0.35
Reduction of <i>APD</i>	Marked	None*	Slight	Slight or none
Change in <i>SD</i>	—	Decreased	Decreased	No change

The data presented represent mean values ± S.E. of the mean.

\* See text and Fig. 1B.

*MDP*, maximal diastolic potential; *APA*, action potential amplitude; *OS*, overshoot; *SD*, slow depolarization; *SD to AP*, transition from slow depolarization to action potential; *N*, number of experiments.

potentials and the changes in configuration resulting from vagal stimulation.

Type 1 action potentials were recorded from the septal branch of the S-A ring bundle and close to the superior vena cava. The configuration of these fibres was markedly altered by vagal stimulation as shown in Fig. 1A. After termination of the vagal stimulation, the changes in membrane polarization and in repolarization velocity reversed earlier than the change in action potential amplitude.

Type 2 action potentials were found in the S-A node near the superior vena cava, near the area from which type 3 potentials were recorded, as described below. Type 2 action potentials (Fig. 1B) closely resembled the configuration of the so-called true pacemaker potentials, but the action potentials were sometimes preceded by the simultaneously recorded atrial activity. In response to vagal stimulation, type 2 potentials were markedly depressed

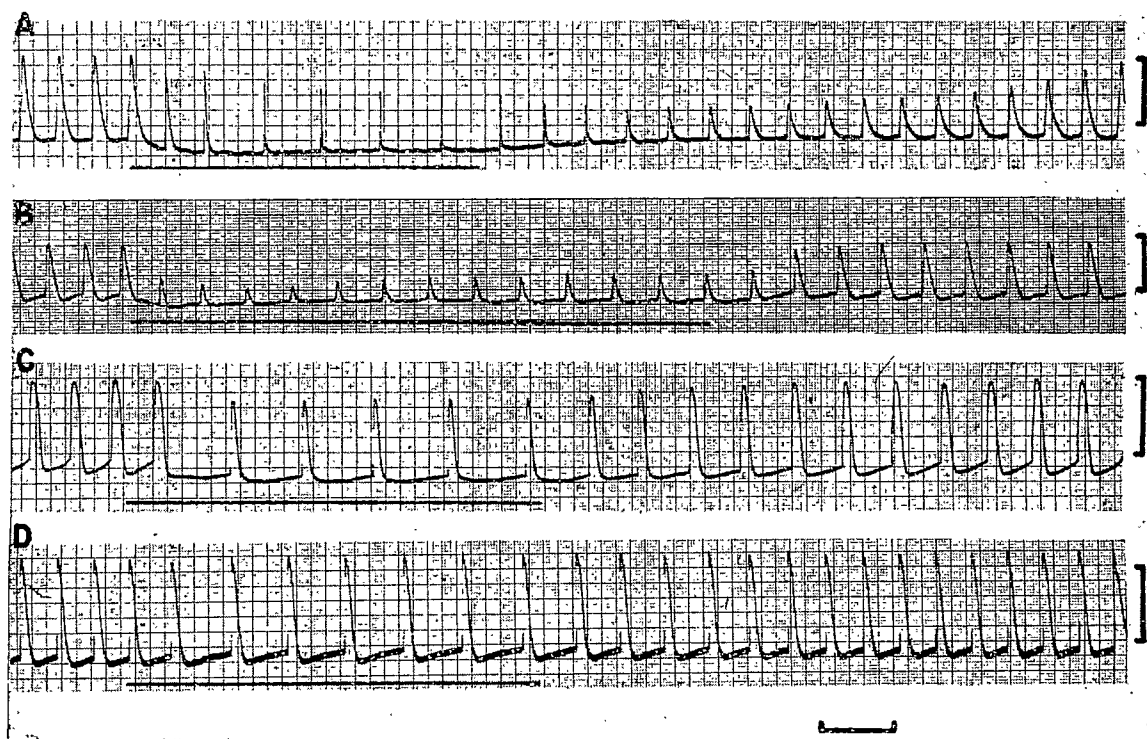


Fig. 1. Variations in response of S-A node action potentials to vagal stimulation. A, B, C and D in the figure represent Type 1, 2, 3 and 4 action potential respectively and responsiveness to vagal stimulation described in the text. Horizontal lines just below each tracing illustrate the period of vagal stimulation. Vertical lines in the right side show the 50 mV calibration and 0 level. Time scale at the bottom: 1 sec

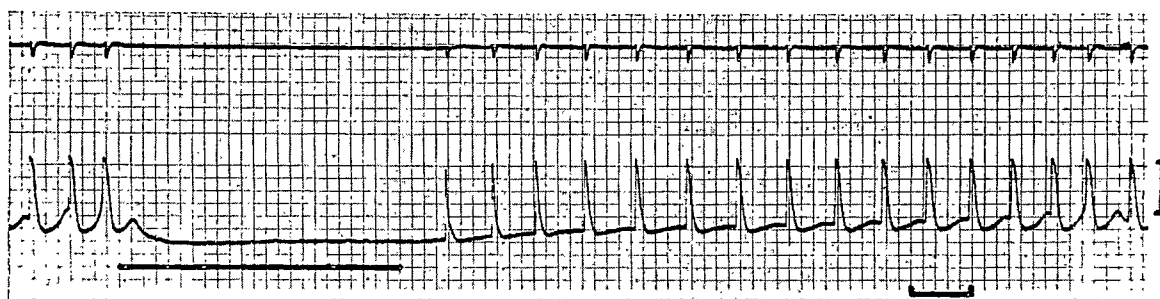


Fig. 2. Effects of vagal stimulation in a solution containing 74 mM Na<sup>+</sup>. Upper tracing represents the external recording of the atrial activity. Lower tracing shows transmembrane potentials of a Type 3 fibre. Horizontal line just below the lower tracing shows the vagal stimulation. Voltage calibration and time scale represent 50 mV and 1 sec, respectively

without a concomitant change in repolarization velocity, in contrast to type 1 potentials. In type 2 fibres, a step on the upstroke and a split on the peak of the action potential were often observed during recovery from vagal stimulation.

Type 3 action potentials were recorded from a part of the S-A node near the crista terminalis. Type 3 potentials always preceded the simultaneously recorded atrial excitation. Vagal stimulation decreased the slope of slow depolarization, resulting in a sharp transition from slow depolarization to upstroke; slight depression of the amplitude of these action potentials with little or no hyperpolarization accompanied the bradycardia (Fig. 1C). Type 4 action potentials were recorded from fibres of the S-A ring bundle close to the crista terminalis and appeared to be transitional fibres.

Type 4 potentials responded to vagal stimulation with a slight decrease in action potential amplitude and duration without change in slow depolarization slope (Fig. 1D). In contrast, atrial fibres responded to vagal stimulation with a slight hyperpolarization (5 mV at most), depression of amplitude (10–20 mV) and marked acceleration of repolarization velocity.

In each preparation, the most prominent configurational change in response to vagal stimulation occurred in parts of the S-A node near the superior vena cava and the interatrial septum. This may be due to the different distribution of vagal innervation in each part of the S-A node or the difference of the permeability increase to potassium ions in the presence of acetylcholine released from vagal terminals.

When the concentration of external potassium was increased (up to 16.2 mmole/l.) the maximal diastolic potential in all S-A node cells was reduced. Bradycardia resulting from vagal stimulation was similar to that observed in the normal solution, but hyperpolarization, particularly in types 1 and 2 action potentials, was markedly reduced in the presence of increased potassium concentration.

In the presence of reduced external potassium concentration (1.35 mmole/l.), the effects of vagal stimulation were augmented, both in the S-A node and in the right atrium. Hyperpolarization in S-A node fibres in response to vagal stimulation was much more pronounced than in the normal solution.

In some experiments, sodium chloride was reduced to 74 mmole/l. with the change in osmolarity balanced by sucrose. The effects of vagal stimulation were greatly augmented in the presence of diminished sodium concentration. Fig. 2 illustrates the marked vagal effects on a type 3 action potential in 74 mmole/l. sodium solution. Marked hyperpolarization (15 mV) and complete disappearance of action potentials occurred during the period of stimulation. In the normal solution, this type of pacemaker fibre responded by slight hyperpolarization (5 mV), decrease in action potential amplitude and duration, and moderate bradycardia during vagal stimulation.

While marked hyperpolarization and shortening in duration were observed in the pacemaker potentials of frog heart<sup>1</sup> in response to vagal stimulation, very little or no change in polarization and the configuration of pacemaker potential (type 3) occurred in the rabbit heart in the normal solution. Because the type 3 pacemaker potential appears to represent the activity of functionally 'true pacemaker' fibres, our results suggest that the cholinergic effect on the slope of diastolic depolarization is of primary significance to atrial rate in the rabbit; hyperpolarization and action potential depression are of lesser significance. On the other hand, in low sodium solution, changes in polarization and in duration similar to those in frog heart were recorded following stimulation of vagi to rabbit heart.

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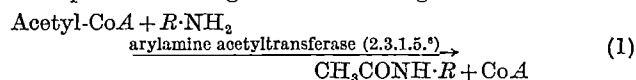
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### Metabolic Acetylation by the Mucosal Membrane of the Gastro-intestinal Tract

THE mucosal membrane of the gastro-intestinal tract has been shown to possess considerable capacity for detoxication reactions<sup>1-5</sup>.

On the basis of earlier observations concerning the metabolic acetylation which occurs in the liver and kidney, we have conducted investigations to ascertain whether acetylation occurs also in the different parts of the gastro-intestinal tract. Acetylation is known to take place according to the following reaction:



The foregoing reaction has been found to occur in the liver and kidney of most mammals and birds. The liver especially has proved to be an organ with a very great acetylation capacity. Conditions with high ATP- and coenzyme A-concentrations have also proved favourable in the liver.

The *in vitro* technique was used to examine acetylation in the gastro-intestinal tract. The tissues to be examined were removed from the animal and placed in cooled Krebs-Ringer phosphate solution (pH 7.4). Next, the tissues were cut into pieces about 3 mm in diameter and

transferred to substrate solution of about  $+4^{\circ}\text{C}$ . The composition of the substrate solution was as follows: phosphate buffer 0.1 M, pH 7.4, 5 ml.; Krebs-Ringer phosphate solution, 5 ml.; ATP 0.05 M solution, 2 ml.; sodium acetate 1 M solution, 2 ml.; coenzyme A 70–75 per cent (Sigma 5 mg/50 ml.), 1 ml.; sulphanilamide 0.02 M, 0.02 M  $\text{K}_2\text{CO}_3$  in solution, 0.15 ml.

The experimental animals were white female rats of Wistar strain, and male guinea-pigs and dogs. The animals were starved for some 30 h before the experiment. The following tissues and organs were removed from the animals: glandular stomach, duodenum about 15 cm downwards from the pylorus, about 15-cm length of the ileum, and about 3 g fresh weight of liver and kidney was taken for control purposes. From the kidney only cortex was taken. The pieces of tissue were placed in 50-ml. Erlenmeyer flasks which were stoppered with corks through which 2 glass tubes permitted a continuous flow of oxygen, and the flasks were placed in a Warburg apparatus of  $+37^{\circ}\text{C}$ . After 4 h of incubation, 2-ml. samples were taken and 0.5 ml. of 5 per cent trichloroacetic acid solution was added to them in order to stop the reaction and precipitate the proteins.

Table 1. ACETYLATION OF SULPHANILAMIDE BY VARIOUS TISSUES IN THE RAT

	Percentage of sulphanilamide acetylated	Tissue protein mg/ml. of substrate
Control	0.0	—
Liver	22.8	1.47
Stomach	10.0	1.27
Duodenum	26.0	1.50
Ileum	14.9	2.07
Kidney	31.6	1.15
Skeletal muscle	0.0	1.28

Table 2. ACETYLATION OF SULPHANILAMIDE BY VARIOUS TISSUES IN THE GUINEA-PIG

	Percentage of sulphanilamide acetylated	Tissue fresh weight (g)
Control	—	—
Liver	13.6–58.8	2–3
Stomach	9.0–30.8	2
Duodenum	23.7	2
Ileum	19.1–32.0	2
Kidney	10.5–22.2	2
Skeletal muscle	—	2

The experiments sought to establish the acetylation of sulphanilamide in the gastro-intestinal tract in addition to that occurring in the liver and kidney. The method described by Bratton and Marshall<sup>7</sup> was used in the determination of sulphanilamide. The analytical method differed from the original procedure, which was evolved for sulphanilamide determination from blood; here, after diazotizing, the test series were allowed to stand overnight in darkness—before the colorimetric measuring of colour intensity—and this produced a considerable levelling out in the parallel determinations.

To control the protein content of the tissues in the different experimental series, nitrogen determination was performed by the micro-Kjeldahl method. The results (Table 1) showed that acetylation occurs on a relatively large scale also in organs and tissues other than the liver and kidney.

The sulphanilamide concentration used in the experiments corresponds to 34.4  $\mu\text{g/ml}$ .

The results of the experiments with dog were completely negative for all the tissues, as has already been reported earlier for the liver<sup>8–10</sup>. However, the dog has been found to possess an enzyme system for the acetylation of amines other than glucosamine<sup>9</sup>.

In addition, both acetone-dry tissue powders and tissue homogenates were experimented with, but they failed in these experimental conditions to give a positive result in a single animal.

The results show that the gastro-intestinal tract also possesses a fairly distinct capacity for acetylation in addition to its other detoxication reactions.

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### Dissociation of Facilitatory Mechanisms in the Midpontile Decerebrate Cat

VAGOTOMIZED, midpontile decerebrate animals have been reported to exhibit decerebrate rigidity, apneustic breathing, tachycardia and arterial hypertension<sup>1,2</sup>. These manifestations of hyperactivity in the somatic postural, respiratory, and cardiovascular systems are abolished by transections of the central neuraxis at or caudal to the level of the pontomedullary junction<sup>1</sup>. These parallelisms suggest the hypothesis that interdependent if not identical facilitatory mechanisms in the lower pons are responsible for these hyperactive states. This hypothesis has been experimentally tested here.

Thirty-six adult cats were anaesthetized with ether and were subjected to midmesencephalic decerebration and vagotomy. The vagi were sectioned at the level of the fifth or sixth cervical vertebra. Afterwards, the brain stem was transected serially until midpontile decerebration was achieved.

The decerebrations were performed by a combination of ischaemic<sup>3,4</sup> and transection procedures. The common carotid arteries and their major branches were ligated. The basilar artery was exposed and then occluded at variable positions along its length by the application of silver clips. In alternating sequence with the placement of the basilar clips, the brain stem was transected at progressively more caudal levels, but always at planes rostral to the most caudal basilar clip. This alternation of basilar artery occlusions and brain stem transections normally minimized the problem of haemorrhage.

The critical level of decerebration in these experiments was the midpontile plane. During the course of an experiment, the physiological manifestations of decerebrate rigidity, apneustic breathing, tachycardia, and arterial hypertension provided very valuable criteria for judging this desired functional level of decerebration. Post-mortem examination of the brain provided anatomical evidence for determining the level and the extent of the surgical transections. Midpontile decerebration was taken to be transection through or immediately above the rostralmost portions of the acoustic tubercles and the trapezoid bodies.

Twenty-six vagotomized, midpontile decerebrate cats were given intravenous injections of urethane. In cats this drug has an effective narcotic action without significantly affecting normal somatic postural, respiratory, or cardiovascular reflex activity<sup>5</sup>. Each injection, 200 mg of urethane, constituted less than 20 per cent of the dosage generally recommended for surgical levels of narcosis. Successive injections were administered at intervals of 15–60 min until the hyperactivities in the three physiological systems under investigation were abolished.



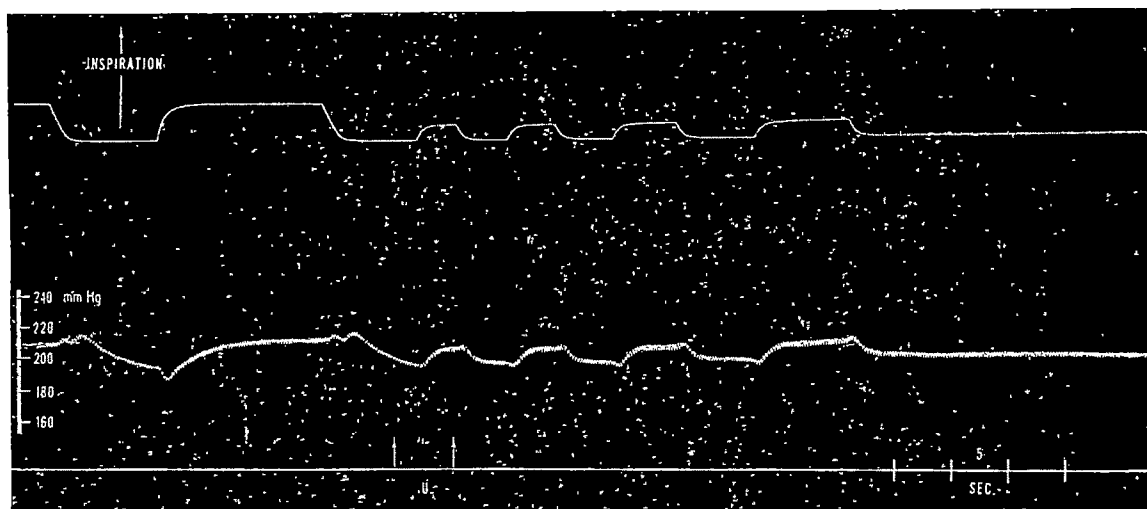


Fig. 1. Kymograph record of respiratory and cardiovascular responses to an intravenous injection of 200 mg of urethane (U) in a vagotomized, midpontile decerebrate cat. Respiration (recorded with a Krogh-type spirometer) changed from apneustic breathing to apnoea. Arterial blood pressure (recorded with a mercury manometer) reflected the changes in respiration but exhibited no significant depression in mean pressure level. Heart beat oscillations in the blood pressure tracing revealed no significant alteration in heart rate.

Significant differential effects on the hyperactive states in the somatic postural, respiratory, and cardiovascular systems were produced by the urethane. The somatic postural system was by far the most susceptible of the three systems to the depressant effects of the narcotic. Decerebrate rigidity consistently was markedly diminished, often abolished, by the initial injection of urethane even when no observable depression of the apneustic breathing or the cardiovascular hyperactivity occurred. The respiratory system was next in the order of susceptibility to urethane depression; the cardiovascular system was the least susceptible. In 17 of the 26 experiments in this series, apneustic breathing was markedly depressed or transiently abolished without significant concomitant depression in the cardiovascular system. In these 17 experiments, apneustic inspirations became shorter and more shallow in 9 animals, gasping respiration appeared in 4 animals, and apnoea occurred in 4 animals (Fig. 1). In the apnoeic group, when respiration reappeared a brief period of gasping respiration sometimes preceded the recurrence of apneustic breathing.

Ten vagotomized, midpontile decerebrate animals were observed throughout spontaneous deterioration to death. Their survival times after vagotomy and midpontile decerebration ranged between 4 and 8 h. The cardiovascular hyperactivity, particularly the arterial hypertension, was consistently the first to fade. Apneustic breathing and decerebrate rigidity tended to undergo progressive deterioration together; however, a moderate degree of spasticity often survived the last traces of apneustic breathing. It is noteworthy that the deterioration of the hyperactive states in this group of animals occurred in a reversed order from that obtained with urethane depression. The depressant effects of the urethane, therefore, cannot be attributed simply to progressive degeneration of the brain stem along its rostro-caudal axis. Rather, the results suggest that the urethane had a differential depressant effect on the brain stem facilitatory mechanisms responsible for the hyperactive states in the three systems.

The results obtained during this work reveal that the described hyperactive states in the somatic postural, respiratory and cardiovascular systems may each be dissociated from the other two. Thus these results do not support the hypothesis that interdependent or identical facilitatory mechanisms are responsible for these hyperactive conditions. On the contrary, the results indicate that decerebrate rigidity, apneustic breathing, and cardiovascular hyperactivity in vagotomized, midpontile decerebrate animals are each supported by a facilitatory

mechanism capable of functioning independently of the facilitatory mechanisms responsible for the hyperactivities in the other two systems. This conclusion does not deny the existence of co-ordinative interrelationships among the facilitatory mechanisms for the three systems, but merely asserts their capacities for independent action.

In view of the prominent position of the bulbar reticular facilitatory system of Magoun<sup>6</sup> in neurophysiological theory, it is pertinent to consider this facilitatory mechanism as it might relate to the phenomena described in this communication. The aggregate results of Bach and Magoun<sup>7</sup>, Bach<sup>8</sup>, and Domino<sup>9</sup> suggest that neither decerebrate rigidity, apnoeustic breathing, nor cardiovascular augmentation in midpontile decerebrate animals is primarily attributable to the bulbar reticular facilitatory system.

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## PHARMACOLOGY

### Interaction of the Analgesic Effects of Morphine and Codeine in Rats

In the experiments described here, the analgesic effects of 5.0 mg/kg of subcutaneous morphine and 60.0 mg/kg of subcutaneous codeine were determined individually at various intervals after the injections. Then, a dose of codeine too low to exert any analgesic effect by itself, when given simultaneously with morphine was demonstrated to increase the intensity and persistence of

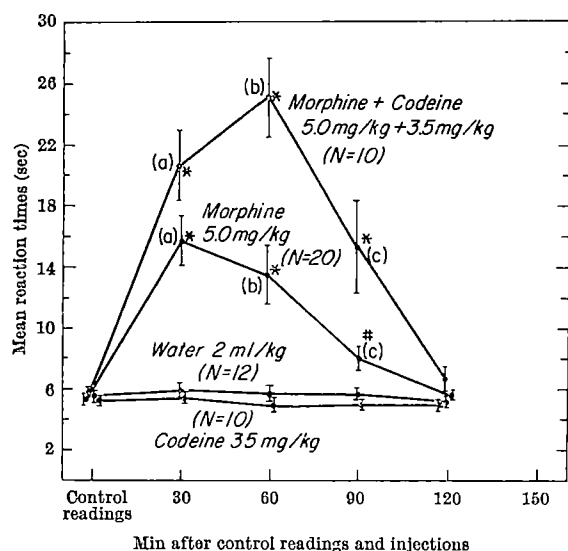


Fig. 1. Influence of a non-analgesic dose of codeine on morphine analgesia in rats. Drugs were given by subcutaneous injections and the analgesic effects determined by the hot plate method. Reaction times were read immediately before the injections (control readings) and then 30, 60, 90 and 120 min later (abscissa). The ordinate denotes the mean reaction times of rats in each group; vertical lines indicate the standard errors of the means. Morphine (5.0 mg/kg) was given to rats in one group and 3.5 mg/kg of codeine to rats in a second group, while rats in a third group received both drugs simultaneously. Rats in a fourth group received water only.  $N$  = number of animals in each group. Statistical analyses of mean values (' $t$ ' test): \* Statistically different ( $P' < 0.05$ ) from corresponding water controls. # Not statistically different ( $P' > 0.05$ ) from corresponding water control. (a), (a); (b), (b); (c), (c); statistically different ( $P' < 0.05$ ). # Scatter of results statistically different ( $P' < 0.05$ ) from that of corresponding water control (variance ratio test<sup>2</sup>).

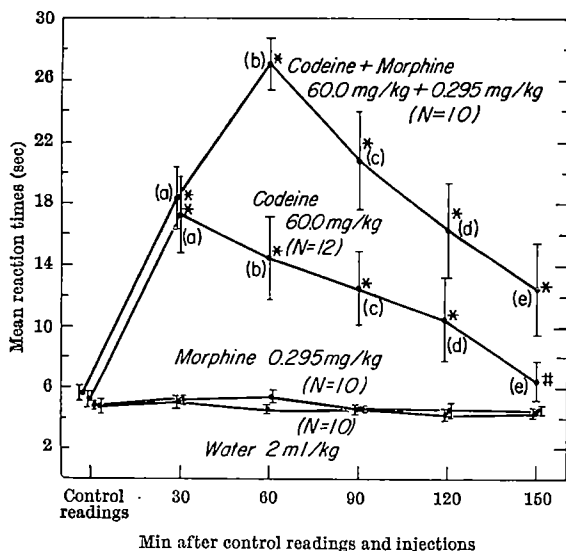


Fig. 2. Influence of a non-analgesic dose of morphine on codeine analgesia in rats. Morphine was given in amounts of 0.295 mg/kg and codeine in amounts of 60.0 mg/kg. Analgesia was determined at 30, 60, 90, 120 and 150 min after the injections (for explanations, see Fig. 1). Tests of significance (' $t$ ' test, variance ratio test<sup>2</sup>): \*, # as in Fig. 1. (a), (a); (d), (d); (e), (e); not statistically different ( $P' > 0.05$ ). (b), (b); (c), (c); statistically different ( $P' < 0.05$ ). Mean reaction times of rats given morphine (5.0 mg/kg) and codeine (60.0 mg/kg) were not statistically different at 30 or 60 min ( $P' > 0.05$ ), but at 90 min after the injections ( $P' < 0.05$ ) compare Figs. 1 and 2.

morphine analgesia. A similar experiment was performed with 60 mg/kg of codeine given with a dose of morphine too low to show analgesia.

The analgesic effect was determined by the modified hot plate method<sup>1</sup>. No rat was allowed to stay on the hot plate for longer than 30 sec; therefore, maximal analgesic effect obtainable was 30 sec. Morphine was always injected subcutaneously in the middle of the back

in the midline. When codeine was given alone subcutaneous injections were also made in the middle of the back. When both drugs were given to the animals, codeine was injected subcutaneously in the gluteal region immediately after morphine and from another syringe. Morphine sulphate and codeine phosphate were dissolved in distilled water. Injected volumes were 2 ml/kg and injections were given by means of tuberculin syringes, calibrated to deliver 0.01 ml. Doses refer to the drugs as bases. Male Holtzman rats (180–240 g) were used—each animal only once. The results are summarized in Figs. 1 and 2.

A significant and equianalgesic response was seen at 30 and 60 min after the high doses of morphine (5.0 mg/kg) and codeine (60.0 mg/kg). Mean reaction times of the water controls and the morphine rats did not differ significantly at 90 min. At 120 min the morphine rats were, with one exception, down at the pre-injection level (Fig. 1). The mean reaction time of the codeine rats was still significantly longer at 90 and 120 min than that of the control rats. Although the mean reaction times of the two groups did not differ significantly at 150 min, the significantly different scatter of the two sets of results around their means stressed the persistence of codeine analgesia (Fig. 2).

The low doses of morphine and codeine, being approximately 1/17 part of the higher doses, were, as is already mentioned, without analgesic action. However, when the low dose of codeine was given with the high dose of morphine, the mean reaction times were found significantly longer at 60 and 90 min than after the morphine alone (Fig. 1). A similar but more persistent effect was observed after codeine plus the low dose of morphine. It is of interest that the synergistic action was not significant at 30 min after the injections.

We have no ready explanation for the synergism of morphine and codeine shown here. The drugs were injected subcutaneously, but in different areas. A peripheral mechanism is therefore *a priori* rather unlikely. The greater persistence of codeine analgesia may be due to different rates of absorption of morphine and codeine from subcutaneous tissues<sup>1</sup>.

The reciprocal synergistic actions of morphine and codeine may be a phenomenon peculiar to the rat. Nevertheless, this phenomenon deserves further investigation, for example, in clinical pharmacological investigation in the human being.

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## PATHOLOGY

### Modification of Ultra-violet Erythema by Epidermal Stripping

Rottier<sup>1</sup> and Rottier and Mullink<sup>2</sup> reported that removal of the stratum corneum by stripping increases the erythema produced by 297 mμ light and decreases the erythema to 254 mμ. From this observation Rottier postulated that the erythema production mechanism was different at these two wave-lengths. Claesson, Juhlin and Wettermark<sup>3</sup> were unable to confirm these findings and reported increased erythema production at both wave-lengths. In the present investigation the effect of multiple wave-lengths of light on skin with and without stratum corneum was determined.

The ultra-violet light was provided by a xenon-arc monochromatic system described elsewhere<sup>4</sup>.

A 2 cm × 4 cm area on the forearms of ten young adults was stripped from eight to twelve times with 'Sello-tape' to remove stratum corneum. Both the stripped and adjacent unstripped control skin were irradiated with equal quantities of 254 and 297 mμ light. The erythema responses were graded 0-4+ after 8 h.

In five persons, one side of the back was stripped from eight to twelve times with 'Sello-tape'. Both the stripped and contralateral unstripped skin area were irradiated with varying amounts of energy at different wave-lengths of ultra-violet (250, 254, 260, 270, 280, 290, 297, 300 and 310 mμ). The minimal erythema dose (MED) was determined at each wave-length. The MED on the stripped side was then compared with the MED of intact skin.

In nine out of ten cases, forearm skin irradiated with 254 and 297 mμ light regularly developed greater erythema on skin stripped of stratum corneum (Table 1). A decrease in the MED of stripped skin was observed at nine different wave-lengths between 250 and 310 mμ (Table 2).

Table 1. CUTANEOUS ERYTHEMA AT 8 H

Patient	254 mμ		297 mμ	
	Stripped	Unstripped	Stripped	Unstripped
1	+++	+	+++	+
2	++	+	+++	++
3	++	+	+++	+
4	+++	++	+++	++
5	+++	++	+++	+
6	++	+	++	+
7	+++	++	+++	+++
8	++	++	+++	++
9	+++	+++	++	+
10	++	+	+++	+

Table 2. DECREASE IN MINIMAL ERYTHEMA ENERGY PRODUCED BY STRIPPING THE STRATUM CORNEUM

Wave-length (mμ)	Control MED minus stripped MED (Average 5 cases) (mW-sec/cm <sup>2</sup> )
250	3,736
254	3,414
260	3,181
270	3,445
280	1,351
290	3,551
297	6,071
300	4,921
310	18,500

The increase in the erythema resulting from irradiation after removal of the stratum corneum suggests that an important function of this epidermal layer is to protect the underlying tissues from harmful rays. Urocanic acid, a strong absorber of ultra-violet light, has been shown to be a regular constituent of the horn layer<sup>5,6</sup>. This compound may be the principal substance responsible for the observed protection conveyed by stratum corneum. These observations also suggest that the mechanism of erythema production is the same for each of the wave-lengths between 250 and 310 mμ and that the erythema-producing substance is produced or released at levels below the stratum corneum regardless of the wave-length of the eliciting light.

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## IMMUNOLOGY

### Reciprocal Relationship of Antigens 'I' and 'i' in Health and Disease

Giblett and Crookston<sup>1</sup> have recently described in thalassaemia and certain other diseases an increase in strength of the 'i' antigen in the red cells of adults without an apparent reciprocal decrease in 'I' antigen strength. McGinniss *et al.*<sup>2</sup> reported a decrease in 'I' antigen strength in the red cells of adults in leukaemia, but had not tested with anti-'i'. We have examined a case of leukaemia with weakened 'A' antigen in which the 'I' antigen was also depressed. Here, the 'i' antigen was stronger than in average adult cells so that the 'Ii' reciprocal relationship was maintained.

In the cases described by Giblett and Crookston, the estimation of the strength of the 'I' and 'i' antigens appears to be based entirely on agglutination titres. We suggest that a state of excessive erythrocyte sensibility to agglutination by anti-'I' and anti-'i' may exist in certain disease states, so that titration scores alone may not reflect the true antigen strength. Absorption and elution experiments would be useful in demonstrating the ability of the red cells to take up more than average amounts of the antibodies.

We have observed in an apparently healthy donor that both 'I' and 'i' were at the upper end of the normal distribution curve, which suggested the presence of a higher amount than normal of the precursor substance of 'i' prior to the development of 'i' in the red cells of this person.

It appears that in a few healthy adults and in certain disease states the usual 'Ii' reciprocal antigen strength is disturbed. Whether this is due to a genetically determined abundance of 'i' substance from which only a normal amount is utilized for conversion to 'I', or whether the 'i' antigen strength is stimulated by virtue of the disease is conjectural at present. We seek evidence of a change in the 'Ii' antigen strength during the development of a disease process. Such a change in 'Ii' status would support Giblett and Crookston's theory of "proliferative stress on early erythroid precursors".

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### Induction of an Immunological Response in Local Lymph Nodes by Chemical Carcinogens

AFTER a period during which intensive investigation of carcinogenesis continued along classical lines, there has recently developed a major shift in emphasis as it is becoming more apparent that certain carcinogens, even when applied locally, are capable of initiating significant changes in the genetic apparatus of the host cells. Some of the present developments have, to a large extent, been the outgrowth of investigations showing that some carcinogens arrest the synthesis of DNA dependent RNA, in a manner similar to actinomycin-D. Structurally related non-carcinogens, on the other hand, exert no such action. These results indicate the possibility that the initial event in carcinogenesis might be an impairment in the expression of genetic (DNA dependent RNA) information, as has been suggested recently by De Maeyer and



De Maeyer-Guignard<sup>1</sup>. Gelboin and Klein<sup>2</sup> have, moreover, shown that tumour formation by 7,12-dimethylbenz(a)anthracene can be markedly inhibited by the topical application of actinomycin-D before and after treatment. This would also support classical models for carcinogenesis such as that suggested by the work of Monod and Jacob on genetic systems in micro-organisms<sup>3</sup>.

In the course of immunological investigations on contact sensitivity with dinitrofluorobenzene (DNFB) and oxazolone (2-phenyl-4-ethoxymethylene oxazolone) in the guinea-pig<sup>4,5</sup> it was found that topical application of these agents was followed by the appearance of large pyroninophilic cells (LPC) in draining lymph nodes four days later, one day before the animal developed contact sensitivity; this reaction was immunologically specific<sup>4</sup>. Taken together with recent trends in cancer research and virology this has led us to consider the possibility that some carcinogens could be acting in a manner akin to compounds such as DNFB. If the stimulation of carcinogenesis by DMBA paralleled that of local lymph nodes by the cutaneous application of DNFB and oxazolone, one could expect that in mice sensitive to carcinogenesis the cutaneous application of a chemical carcinogen would provoke the appearance of LPC in local nodes; in contrast structurally similar non-carcinogenic components would be found relatively inactive.

Experiments were performed with several strains of mice and guinea-pigs with 3,4-benzpyrene (BP) and 7,12-dimethyl-1,2-benzanthracene (DMBA) as carcinogens, and anthracene as a structurally related non-carcinogen. It was found that LPC were indeed elicited by BP and DMBA at the appropriate time and to a higher degree in mice sensitive to carcinogenesis, whereas in the draining lymph nodes of control mice painted with the anthracene much smaller numbers of LPC were observed, in a range comparable with untreated mice. These results were compared with the reaction of lymph nodes of mice to the cutaneous application of oxazolone and DNFB.

Five strains of mice were examined: (1) Random bred albino mice (Tuck) obtained from Messrs. A. Tuck, Rayleigh, Essex. (2) Rudiger strain (*Ru*). This strain was obtained from Dr. K. H. Schmidt-Ruppin, Geigy A.-G., Basle, and has a heightened susceptibility to tumour transplants and cancer induction<sup>6,7</sup>. (3) *C57BL* from the Medical Research Council Rheumatism Research Unit, Taplow, Bucks. (4) *C<sub>3</sub>H*. (5) *DBA/2* from a stock held at the Forschungsinstitut, Davos. The guinea-pigs used were of the Hartley strain. The BP and DMBA were obtained from Fluka A.-G., Buchs, Switzerland, as also was the anthracene used as a control. DNFB was obtained from British Drug Houses, Ltd., and oxazolone was prepared according to Cornforth<sup>8</sup>. The carcinogens and DNFB were dissolved as a 5 per cent solution in an equal volume of acetone and olive oil; in some experiments the olive oil was replaced by croton oil. The oxazolone was dissolved as a 10 per cent solution in ethanol, and anthracene as a 5 per cent solution in equal volumes of olive oil and hot benzene. These solutions were painted on the left ears of both guinea-pigs and mice on one occasion only. The draining auricular lymph nodes were removed 4, 5, 9 and 10 days after application of the chemical agent to the ear, fixed in Carnoy's solution, and prepared for histological examination by conventional means. Sections were cut at 5 $\mu$  and stained with methyl-green pyronin.

The number of large pyroninophilic cells were counted in four adjacent microscopic fields of diameter 125 $\mu$  in the area previously defined as the paracortical area of the cortex<sup>9</sup>, where these cells were present at greatest concentration. Care was taken not to include those cells forming obvious germinal centres.

The significant increase in the number of large pyroninophilic cells present in the draining lymph nodes of mice and guinea-pigs on the fourth and fifth days after the application of a chemical carcinogen is shown by the data in Table 1; these findings contrasted with the

number of LPC present in normal nodes or in nodes on the fourth and fifth days after the application of anthracene or solvent alone. In Tuck albino mice and *C57BL* mice the number of cells found in local lymph nodes on the fourth day after the application of DNFB and oxazolone was somewhat higher than that following the application of BP or DMBA. The presence of croton oil did not increase the number of large pyroninophilic cells present in the cortex of *Ru* mice treated with DMBA; however, croton oil alone evoked a relatively high concentration of these cells.

Table 1. MEAN VALUE OF NUMBER OF LARGE PYRONINOPHILIC CELLS FOUND IN FOUR ADJACENT MICROSCOPIC FIELDS, EACH OF DIAMETER 125 $\mu$ , IN THE DRAINING LYMPH NODE

Treatment	(a) 4-5 days after application of carcinogen or solvent					
	Tuck	<i>Ru</i>	Mice <i>C57BL</i>	<i>C<sub>3</sub>H</i>	<i>DBA/2</i>	Guinea-pigs
Anthracene (in olive oil-benzene)	35 (6)	29 (6)	13 (6)	11 (4)	19 (6)	4 (4)
Benzene		38 (4)				
BP (acetone-olive oil)	62 (6)		76 (6)	44 (6)	46 (6)	96 (4)
Olive oil		33 (4)				
Acetone		33 (4)				
DMBA (acetone-olive oil)	99 (6)	96 (6)	77 (6)	49 (7)	46 (7)	93 (4)
DMBA-croton oil		93 (6)				
Croton oil		120 (4)				
Treatment	(b) 4 days after application of chemical sensitizing agent					
	Tuck	<i>C57BL</i>				
Unpainted (control)	33 (6)	20 (7)				
DNFB	162 (4)	96 (4)				
Oxazolone	159 (4)	95 (4)				

Figures in brackets indicate number of animals examined. All counts were of cells lying within the 'paracortical area' of the cortex of the lymph node. No cells within germinal centres were included. The areas examined were selected as those in which large pyroninophilic cells were present at highest concentration.

Lymph nodes were also examined 9 and 10 days after application of carcinogens or sensitizing agents. In all cases the proportion of large pyroninophilic cells had dropped to the level found in normal auricular lymph nodes. No increase in germinal centre development or plasma cell formation was found at any stage in the auricular nodes of mice. However, it should be noted that unstimulated auricular nodes of mice were far more active than those of guinea-pigs. This is probably due to a wider area of lymphatic drainage and this would also account for a high normal background of large pyroninophilic cells in the auricular lymph nodes of mice as compared with those from guinea-pigs.

Random bred albino mice have been shown to be more susceptible to skin carcinogenesis than *C57BL* mice and these in turn to be more susceptible than *DBA* mice<sup>9,10</sup>. Consequently, our findings would be consistent with a direct correlation between susceptibility to skin carcinogenesis in the mouse and the cellular changes in the local lymph node associated with the development of contact sensitivity. Indeed, *Ru* mice, a strain showing the highest susceptibility, was also one of the lines showing the strongest lymph node reactivity. It is of interest that the presence of croton oil, which also evoked the development of large pyroninophilic cells and has a marked enhancing effect on skin carcinogenesis<sup>11</sup>, did not alter the reactivity of the local lymph node to DMBA when croton oil was used as the solvent instead of olive oil.

Our results should be evaluated in relation to the work of Old *et al.*<sup>12</sup>, who investigated contact sensitivity to carcinogenic polycyclic hydrocarbons in the guinea-pig. They considered that the high degree of delayed sensitivity evoked by these carcinogens in this species might be of importance in understanding the relative resistance of guinea-pigs to chemical carcinogenesis. On the other hand, in mice, highly susceptible to chemical carcinogenesis, they were unable to induce the evidence of delayed sensitivity, so readily elicited in guinea-pigs. Mice have been reported by Crowle and Hu<sup>13</sup> to develop contact sensitivity to dinitrochlorobenzene, and similar observations have been made by G. L. Asherson (unpublished observation), though these reactions may often be

difficult to demonstrate. Moreover, mice show a normal rejection of homografts, a reaction generally believed to involve delayed-type hypersensitivity. It would therefore appear that poor contact sensitivity in mice is due to a failure of reaction in the periphery rather than a failure of the central immunological mechanisms which appear to be as well developed in the mouse as in the guinea-pig.

The large pyroninophilic cells in lymph nodes during the development of contact sensitivity have been shown to be in a premitotic state<sup>4,5</sup>, as have cells with a pyroninophilic cytoplasm in tissue cultures and cultures of leucocytes stimulated by antigens. To what extent these states are related is not known, but investigations of agents which are known to affect specific cell pathways, particularly of the genetic apparatus, might be rewarding.

In summary, this work suggests that some carcinogens can act as sensitizing agents in mice, evoking LPC in local lymph nodes. The ability of certain strains of mice to show changes in their lymph nodes associated with the development of contact sensitivity closely parallels their susceptibility to skin carcinogenesis.

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## RADIOBIOLOGY

### Pyromellitic, Benzenepentacarboxylic and Mellitic Acids as Radioprotectors

THE radioprotective action of pyromellitic acid in mice was reported by Barnes and Philpot<sup>1</sup>. The work recorded here includes the related acids of higher basicity, which were synthesized as follows:

**Benzenepentacarboxylic acid.** The route described by Fleischer and Retze<sup>2</sup>, starting from tetralin, was followed, with technical modifications. Purification of the product of the penultimate stage by recrystallization was unnecessary; the crude, substituted naphthindanedione, isolated by ether extraction, was distilled through a Vigreux column and collected within the range 211°–216° at 13 mm pressure (lit. b.p. 205°–210° at 12 mm) (found: C, 79.9, 80.1; H, 8.69, 8.58; calc. for C<sub>18</sub>H<sub>8</sub>O<sub>2</sub>: C, 80.2; H, 8.51 per cent). The gum solidified on standing, m.p. 37°–39°. Portions (2 g) were oxidized with fuming nitric acid (s.g. 1.5, 5 ml.) with added water (2 ml.) in 'Pyrex' tubes 60 cm long and about 22 mm in diameter, sealed by drawing out to a capillary. These were heated for 21 h in a thermostatically-controlled Carius furnace, first at

180°, then at 200° for the final 3 h. After allowing to cool, the high pressure was released by heating the capillary near the tip with a looped resistance wire. The clear liquid yielded crude benzenepentacarboxylic acid on chilling. A single recrystallization from concentrated nitric acid (s.g. 1.42) afforded pure acid, m.p. 235° decomposition (lit. m.p. 233° decomposition), after drying *in vacuo* over sodium hydroxide and phosphoric oxide (found: C, 44.2, 44.3; H, 2.17, 1.98; calc. for C<sub>11</sub>H<sub>6</sub>O<sub>10</sub>: C, 44.3; H, 2.03 per cent). Further pure material was obtainable by evaporation of mother-liquors and recrystallization. 16 g naphthindanedione gave a total of 10.2 g pure acid (61 per cent). The acid was readily soluble in water.

**Mellitic acid.** Permanganate oxidation of hexamethylbenzene, as described by Fishwick<sup>3</sup>, proved unsatisfactory owing to steam-volatilization of the starting-material. Oxidation of hexa(hydroxymethyl)benzene (Chaigneau<sup>4</sup>) failed to give a pure product. Heating hexamethylbenzene with fuming nitric acid in a sealed tube under the conditions given by Wibaut *et al.*<sup>5</sup> gave an incompletely oxidized product, m.p. 290° decomposition, with previous sintering, after precipitation from dioxan with benzene (found: C, 43.4, 43.6; H, 2.35, 2.66; calc. for C<sub>6</sub>(COOH)<sub>6</sub>: C, 42.1; H, 1.77. CH<sub>3</sub>C<sub>6</sub>(COOH)<sub>6</sub> requires: C, 46.15; H, 2.58 per cent). 0.171 g neutralized 27.5 ml. 0.1 N NaOH (theor.: 30 ml.) using methyl red as indicator. The infra-red absorption spectrum of this material (product A) was identical with that of an authentic specimen of mellitic acid. More vigorous oxidation conditions were used: hexamethylbenzene (2 g) fuming nitric acid (s.g. 1.5, 7 ml.) and water (2 ml.) were heated in a sealed tube (see above) at 120° for 3 h. The tube was allowed to cool, opened and further fuming nitric acid (3 ml.) added, then resealed and heated at 180° for 16 h. The contents of four tubes, a clear, green solution, on evaporation *in vacuo* gave 5.67 g crude mellitic acid. Recrystallization from nitric acid (65 per cent, 160 ml.) keeping overnight at 4°, gave pure acid (4.32 g, 26 per cent) m.p. 295°–300° decomposition, after drying *in vacuo* (found: C, 42.1, 41.9; H, 1.82, 1.94; calc. for C<sub>12</sub>H<sub>6</sub>O<sub>12</sub>: C, 42.1; H, 1.77 per cent). This neutralized the theoretical amount of NaOH.

The pattern of radioprotection and acute toxicity experiments, the conditions and the mice used were as previously described<sup>1,6</sup>. In all cases, acids were injected intraperitoneally as neutral, aqueous solutions of the sodium salts. Injections were given 10 min before irradiation.

Mellitic acid protected neither at its LD<sub>50</sub> (~0.5 m moles/kg) nor at lower doses, but the incompletely oxidized product A (see above), containing material of lower basicity, was less toxic and protected considerably (Table 1). As expected, benzenepentacarboxylic acid (BPCA) had favourable properties. The LD<sub>50</sub> is about 8 m moles/kg and no deaths were observed in mice given 5 m moles/kg or less. Highly significant survival (*P* < 0.01) was shown in groups of mice given 4 or 5 m moles/kg before a normally lethal dose (1,025 r.) of X-rays (Table 1). BPCA raised the LD<sub>50</sub> of X-rays for mice of *R* stock, normally 897 (± 6) r., to 1,106 (± 28) r.; it was therefore significantly less protective than AET, which gave a value of 1,337 (± 69) r. (Table 2).

Radioprotective doses of BPCA caused slight depression as in the case of pyromellitic acid, but with some evidence of hypocalcaemic tetany which increased with increasing dosage. The toxic effects were antagonized by calcium; while 5 mice given 10 m moles/kg BPCA died within a few minutes showing marked tetany, 5 given the stable solution containing the same dose in admixture with an equimolecular amount of calcium chloride were unaffected. The difference in survival is highly significant (*P* < 0.01). It may be noted that a dose of 10 mmoles/kg calcium chloride is not tolerated when given alone; the LD<sub>50</sub> is about 3 mmoles/kg. Such an interaction between the toxic effects of calcium and a radioprotector was demon-

Table 1. PROTECTION AGAINST 1,025 r. X-RAYS

Compound	Dose/kg body wt.	No. survivors out of 10 mice at 30 days
Product A	684 mg	7†
	513 mg	8†
BPCA	6 mmoles	4*, 4*†
	5 mmoles	9, 6†
	4 mmoles	8, 7†
	3 mmoles	2, 8†
	2 mmoles	3, 1

No controls survived.

\* Deaths occurred within 5 days, due to toxicity.

† Survival significant ( $P < 0.01$ ).

Table 2. PROTECTION BY BPCA AND AET

X-ray dose (r.)	No. of survivors at 30 days out of 5 mice given: BPCA (5 mmoles/kg)	AET (1 mmole/kg)
1,050	4	5
1,100	2	4
1,150	1	4
1,200	2	4
1,250	0	4
1,300	0	3
1,350	0	2

Table 3. EFFECT OF CALCIUM ON RADIOPROTECTION

Treatment	X-ray dose (r.)	No. of survivors at 30 days out of 20 mice
BPCA (5 mmoles/kg)	1,025	11
{ Alone		3*
{ + 1.0 Ca/mole		9
Cysteamine (2.5 mmoles/kg)	1,270	8†
{ Alone		7
{ + 0.5 Ca/mole		
{ + 0.25 Ca/mole		

\* Reduction in survival significant ( $P < 0.05$ ). Survival 1 out of 20 in untreated controls.

† Two toxicity deaths, within 5 days.

Table 4. EFFECT OF DOSE AND MOLARITY ON PROTECTION AGAINST 1,025 r.

Acid	Dose (mmoles/kg)	Molarity	No. of survivors at 30 days
			No. mice used
Pyromellitic	9	0.3	19/30
	12	0.3	10/30*
	9	0.3	13/19
	9	0.15	6/20*
	5	0.2	14/20
BPCA	5	0.1	16/20
	4	0.2	13/20
	4	0.1	19/20
	4	0.1	19/20

\* Reduction in survival significant ( $P < 0.05$ ).

strated for tetrametaphosphate and EDTA<sup>6</sup>, but it was not evident in the case of cysteamine. Radioprotection by BPCA was virtually abolished by adding calcium (Table 3). It is clear that for BPCA, as shown<sup>6</sup> for inorganic condensed phosphates and EDTA, both toxic effects and radioprotection in the mouse are due to a lowering of calcium-levels. Tetany is unlikely to be involved in protection by BPCA since its prevention<sup>6</sup> by mephenesin did not significantly affect protection: survival after 1,025 r. in mice given BPCA alone was 28/40 and 19/36 in mice given BPCA and mephenesin. Therefore, like certain condensed phosphates<sup>6</sup> BPCA probably protects through circulatory depression with consequent hypoxia.

In the case of pyromellitic acid, evidence on the mode of protection was less readily available. The possibility of an effect on calcium could not be tested directly because the calcium salt is insoluble; injection of mixtures would not have been informative. Conceivably, pyromellitic acid might affect calcium levels by precipitation. Tetany was not observed but might have been masked by an accompanying depressive action. In a self-contained experiment, confirming a previous result<sup>1</sup>, a dose of 9 mmoles/kg was significantly more protective ( $P < 0.05$ ) than one of 12 mmoles/kg (Table 4). It should be noted that, of mice given 12 mmoles/kg, none died within 5 days of irradiation, that is, the lower survival in this group was not accounted for by an additive toxic effect of radiation and the drug but by a higher radiosensitivity. The occurrence of this phenomenon, of an optimum dose below the maximum tolerated, suggests that for pyromellitic acid the mechanism of protection differs from that of toxicity. It also suggests that pyromellitic acid protects differently from agents not showing the phenomenon: among these are polyphosphates<sup>6</sup> and BPCA (Table 4), the protective and toxic effects of which are due to calcium depletion.

Both protection by and toxicity of pyromellitic acid, at a given dose, depended on the molarity of the solution injected. Thus a dose of 9 mmoles/kg was significantly<sup>1</sup> more protective ( $P < 0.05$ ) at 0.30 M than at 0.15 M (Table 4). A dose of 16.5 mmoles/kg at the higher concentration killed 9 mice out of 10, but at the lower, only 3; the difference is significant ( $P < 0.05$ ). An explanation might be that material injected at the higher concentration would more rapidly reach an effective level in the blood. Protection by pyro- or tripolyphosphate was unaffected by concentration<sup>6</sup>: the difference might be explained by assuming a greater ability of the smaller inorganic molecules to pass into the blood. However, protection by 4 mmoles/kg BPCA was reduced by increasing the concentration, while that by 5 mmoles/kg was unaffected (Table 4).

In view of the foregoing evidence, it seems unlikely that pyromellitic acid protects through an effect on calcium. The fact that the radioprotective dose is very high suggests the possibility of a physical process. Conceivably, the osmotic effect of a high concentration of a polyionic substance, of low diffusibility, in the peritoneal cavity could lead to a hypoxic condition as a result of haemoconcentration. The effect of concentration on protection lends support to this idea. It could be suggested that such a process may contribute to the effects of a number of radioprotectors which are effective only in high dosage.

Since the product of incomplete side-chain oxidation of hexamethylbenzene, product A, showed considerable radioprotective effect it is suggested that benzene tetra- or penta-carboxylic acids with inert groupings, for example, alkyl or halogen, in the unsubstituted ring positions would repay investigation as radioprotective agents. These compounds, however, present considerable problems in organic synthesis.

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## BIOLOGY

## Induction of Tension Wood by 2,3,5-Triiodobenzoic Acid

TENSION wood, the reaction wood of angiosperms, is usually formed as a morphogenetic response to geotropic and other orientation movements of stems and branches. In general it is formed on the upper side of leaning stems and its formation is thought to be a regulatory response, maintaining the stems and branches in specific orientations. Anatomically, tension wood is characterized by fibres with a conspicuously thickened inner layer of the cell wall, the gelatinous layer, which is unligified or only partially lignified. In addition there is a marked reduction in the size and number of vessels in comparison with adjacent normal wood<sup>1</sup>. An eccentricity of growth is frequently associated with tension wood formation, with wider growth rings evident on the tension wood side<sup>2</sup>.

The formation of tension wood is one of the few responses of plants to stimuli involving physiological processes which result in recognizable changes in anatomy.



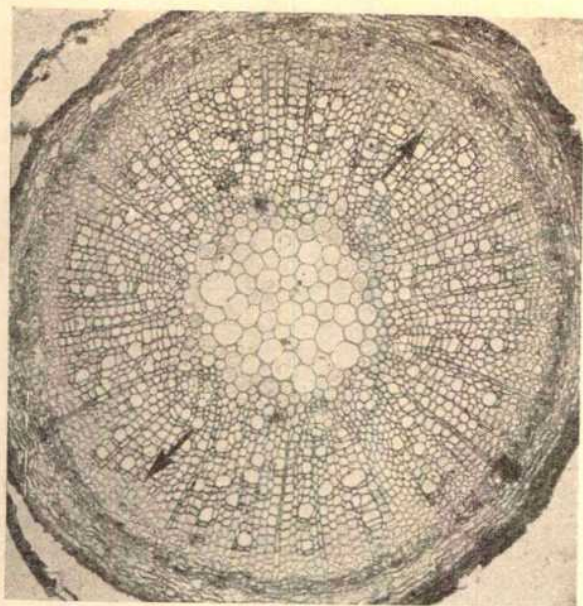


Fig. 1. *Acer rubrum*. Photomicrograph of a transverse section showing a ring of tension wood fibres (arrows) in a stem treated with 2,3,5-tri-iodobenzoic acid. Safranin light-green stained. ( $\times 75$ )

Our observations indicate a similar anatomical response following the application of the auxin-antagonist 2,3,5-tri-iodobenzoic acid (TIBA). That TIBA may have profound effects on wall differentiation was indicated some years ago by Gorter<sup>3</sup>, who observed that the germination of *Lepidium sativum* on porous plates soaked with a 0.02 per cent solution of TIBA is accompanied by the abnormal deposition of a mass of cell wall material at the tip of root hairs. Following the lateral application of TIBA to internodes of seedlings we have been able to induce the formation of tension wood similar in anatomy to natural tension wood. TIBA is a well-known auxin-antagonist the precise mode of action of which is still unknown; however, in general it is thought that its action is effected through an interference with the normal auxin system of the plant. As auxin has been shown to be involved in most stages of normal plant cell differentiation we have used TIBA with the expectation that changes in the differentiation of cambial initials could be induced. TIBA was applied in various concentrations of 0.25–2.0 per cent as a dispersion in lanolin. The lanolin paste was applied laterally as a ring around internodes of actively growing *Acer rubrum* seedlings.

A few days after the application the stem swelled above the TIBA rings and, at various times following the application, sections were cut from the regions above and below the ring. Fig. 1 is a cross-section of an 8-week-old seedling cut about 1/3 in. below a ring of 1.0 per cent TIBA in lanolin paste which had been applied 27 days previously. A band of fibres around the stem may be observed, which have a conspicuous thick inner layer of cell wall material. This inner layer stains green following normal safranin light green staining<sup>4</sup>. Very few vessels are evident in this ring. A transverse section of a similar specimen, showing part of the tension wood band and photographed with bright field, polarizing and fluorescent microscopes appears in Figs. 2, 3 and 4.

With bright field illumination (Fig. 2) the thickened inner layer of the secondary wall of the tension wood fibres is evident. It comprises the bulk of the secondary wall. Between crossed Nicols this inner layer is dark and only the outer region of the secondary wall appears bright, indicating that the thick inner layer is similar in organization to the 'gelatinous' layer of natural tension wood. In the fluorescence microscope, with ultra-violet illumination, the distribution of lignin can be observed in

the cell walls (Fig. 4). Lignin is absent from the thick inner layer and is concentrated in the outer region of the cell wall; again this is characteristic of tension wood fibres. The distribution of peroxidase has been determined in adjacent sections by the McJunkin method<sup>5</sup> using benzidine and peroxide. In the tension wood induced by TIBA intense peroxidase activity was observed in the unligified region of the fibre cell walls. Peroxidase activity was also evident in both pith and cambial regions.

The anatomy of the stem above the point of application of TIBA is also modified. Sometimes tension wood fibres occur in this region, but the main anatomical change we

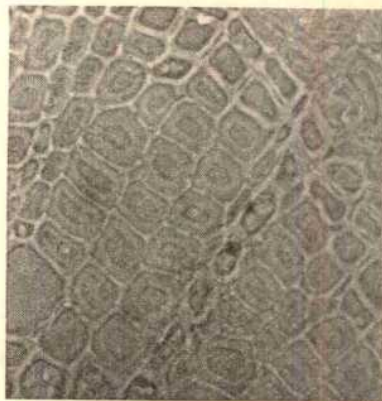


Fig. 2. *Acer rubrum*. Bright-field micrograph of an unstained transverse section of a similar specimen to that shown in Fig. 1 showing details of the tension wood. The fibres have a conspicuous thick inner layer. ( $\times 600$ )

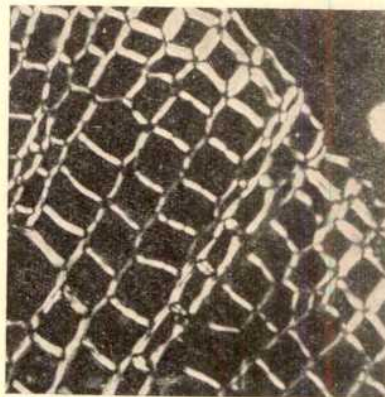


Fig. 3. *Acer rubrum*. The same specimen as in Fig. 2 between crossed Nicols. The thick inner layer is of low birefringence. ( $\times 600$ )

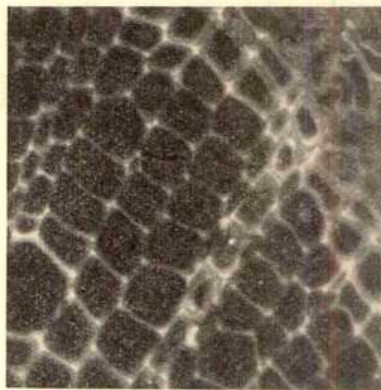


Fig. 4. *Acer rubrum*. The same specimen as in Figs. 2 and 3 photographed using a fluorescence microscope. The outer part of the cell wall shows intrinsic fluorescence, indicating that lignin is concentrated in that region. ( $\times 600$ )



observed was the formation of groups of vessels with a characteristic angular appearance.

These observations on *Acer rubrum* are of interest in that they give some insight into the causation of tension wood at the hormonal level. The participation of auxins in the development of tension wood has been postulated by Jaccard<sup>6</sup>, Onaka<sup>1</sup> and Nečesný<sup>7</sup>. Wardrop<sup>8</sup>, however, in a discussion of tension wood formation has suggested that auxin is only related to the peripheral distribution of cell division in the cambium. Thimann<sup>2</sup> has deduced that tension wood would be formed in a region where there is an indolyl-3-acetic acid (IAA) deficiency. He argues that tension wood forms on the upper side of stems, the side from which auxin tends to move. The auxin level would be further decreased by the presence of active peroxidase, known to be present in tension wood. Nečesný<sup>9</sup> has shown that when IAA is added to the upper side of stems which are actively forming tension wood, the formation of this tissue was retarded.

Our observations support the view that tension wood is formed under conditions of IAA deficiency. Tension wood is formed below the rings of TIBA, and it has been shown<sup>10</sup> that in petioles of sweet potato TIBA effectively blocks auxin transport, therefore a low auxin concentration is to be expected below the rings of TIBA. We have also observed in preliminary investigations that the induction of tension wood formation by TIBA can be prevented by IAA application.

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### Gelatinous Fibres in Ash (*Fraxinus excelsior* L.)

TENSION wood in dicotyledonous trees and shrubs is recognized by the presence of gelatinous fibres. These fibres have an inner layer, composed almost entirely of cellulose (the *G*-layer), which may replace either the *S*<sub>2</sub> or both the *S*<sub>2</sub> and *S*<sub>3</sub> layers of the secondary wall of the fibres. In some instances the *G*-layer may occur in addition to the *S*<sub>2</sub> and *S*<sub>3</sub> layers of the fibre wall. In transverse sections the *G*-layer may commonly be seen to have pulled away from the remainder of the wall on one side.

The difficulty of recognizing gelatinous fibres in ash suspected of containing tension wood has been remarked on by a number of authors<sup>1-4</sup>. The difficulty appears to be due to the lack of a distinct *G*-layer.

Recently a piece of fast-grown ash came into our possession. Sections which were taken from this specimen in order to investigate its lignification showed gelatinous fibres with well-developed *G*-layers. The log had been cut at approximately 3 m above the ground from the main stem of a tree growing in Pinner (Middlesex). There were 13 growth rings (Fig. 1), the widest being the ninth (1 cm). The log was approximately 17 cm in diameter.

Application of phloroglucinol and concentrated hydrochloric acid to the smoothed end-surface of the log showed the early wood of each growth ring to be well lignified, but the extensive late wood was poorly lignified. When transverse sections 20μ thick were treated with these

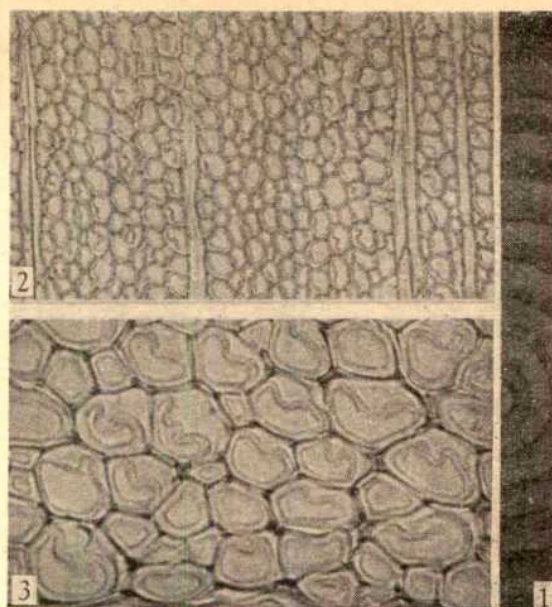


Fig. 1. Surface view of the log of fast grown ash used in this study ( $\times 0.5$ )

Fig. 2. Transverse section of ash stained with safranin and light green and showing gelatinous fibres in the early wood ( $\times 160$ )

Fig. 3. Gelatinous fibres in the early wood of ash ( $\times 560$ )

reagents the comparatively high degree of lignification in the early wood was seen to be confined to the vessels and parenchyma in that region. The fibres throughout the growth rings were uniformly poorly lignified except for the middle lamella which was well lignified.

Fibres with apparently typical, convoluted, gelatinous layers were widely distributed in the early wood (Figs. 2 and 3). These fibres were almost completely unlignified, with the inner (*G*) layer showing no coloration when tested with phloroglucinol and hydrochloric acid.

When sections were treated with chlor-zinc-iodine the secondary walls of the fibres in the early wood showed a consistent pale violet coloration; this colour was more deeply developed in the convoluted inner layer when this was present. Treatment of the sections with ruthenium red also revealed a distinct inner zone to the secondary wall of the fibres, although no such layer was seen in sections of ash that had been grown at a slower rate.

By the use of differential staining with safranin and light green the gelatinous layer was stained green, with an intensity depending on the degree of lignification. Using chlorazol black *E* and lignin pink<sup>5</sup> the gelatinous layer was stained grey/black with the remainder of the secondary wall pink.

Transverse sections viewed between crossed 'Polaroids' showed the inner convoluted layer of the fibres to be non-birefringent.

The presence of such well-developed gelatinous fibres in ash is of interest, and may well be accounted for by the extremely rapid rate of growth of this particular tree. It is known that the rate of growth affects the formation of reaction wood in conifers<sup>6</sup> and the same may well be true of dicotyledons, although there is no published evidence on this point.

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# Senescence of Daffodil (*Narcissus pseudonarcissus*) Cut Flowers treated with Benzyladenine and Auxin

ONE of the kinins, benzyladenine (BA), has been reported to retard the senescence of cut flowers of carnations<sup>1</sup> and King Alfred daffodils after the daffodils have been stored for two weeks at 0.5° C (ref. 2). Later it was found that BA was ineffective on freshly-cut daffodils. In the experiment described below, BA was combined with various concentrations of the sodium salt of 2,4-dichlorophenoxyacetic acid (2,4-D). Both 2,4-D and BA have been reported to be effective in delaying the senescence of cauliflower<sup>3</sup>.

Flowers of field-grown King Alfred daffodils were cut April 3, 1964, and floral parts only were dipped for 5 sec in combinations of 0 and  $5 \times 10^{-4}$  M BA and 0,  $10^{-5}$ ,  $10^{-4}$  and  $10^{-3}$  M 2,4-D. Each solution contained 0.1 per cent 'Tween 20' (sorbitan polyoxyethylene monolaurate). Each treatment was repeated four times and each repeat consisted of three flowers. The stems of these flowers were placed in tap water at a room temperature of 22° C. The replicates were arranged according to a completely randomized design<sup>4</sup>.

Fresh weights of the perianth segments were determined 5 and 6 days after treatment because a loss of fresh weight had previously been found to accompany senescence of cut King Alfred daffodil flowers. Dry weights were made 6 days after treatment to determine the relative importance of water loss. Differences between means were tested by Duncan's multiple range test<sup>5</sup>.

Freshly cut King Alfred daffodils may be preserved by a combination of  $5 \times 10^{-4}$  M BA and  $10^{-4}$  M 2,4-D, but not by BA or 2,4-D alone (Table 1). The preserved flowers were obviously alive and turgid. Flowers treated with  $10^{-3}$  M 2,4-D had badly twisted perianth segments, but flowers dipped in  $10^{-4}$  M 2,4-D did not begin to twist until 6 days after treatment. At this time, their commercial life was terminated despite the fact that they were turgid, with few markings. The successful BA-2,4-D treatments affected the dry weights of the perianth segments very little compared with their marked effect on fresh weights (Table 1). Senescence in daffodils is probably due, at least in part, to a dehydration of the flower. Flowers treated with  $5 \times 10^{-4}$  M BA and  $10^{-4}$  M or  $10^{-3}$  M 2,4-D contained more water than did the checks (Table 1). Water content was determined by subtracting dry weights from corresponding fresh weights.

Kaufman and Ringel found that treatment of cauliflower with 2,4-D retarded abscission and treatment with BA retarded yellowing<sup>6</sup>. The experiment reported here does not indicate whether each chemical has a separate function or not. There is definite indication that both BA and 2,4-D must be present to prevent a dehydra-

tion and ageing of perianth segments of freshly cut daffodils.

Previously, I found that BA preserved daffodil flowers if it was applied after the flowers had been stored at 0.5° C for two weeks<sup>2</sup>. BA alone is ineffective on freshly cut daffodils. The condition of the flower when treated may determine the effectiveness of hormones applied as senescence retardants.

The commercial use of BA and 2,4-D must depend on the cost of BA, as yet available only for experimental purposes, and on the ease of application. A flower dip is not as commercially applicable as a stem dip. However, the movement of BA through plant tissues is primarily basipetally polar<sup>6</sup>, and so cannot be used as a stem dip.

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## Effects of Nutritional Treatments of Seed-bearing Plants on the Performance of their Progeny

HARRINGTON<sup>1</sup> collected seed from carrot, lettuce and pepper plants showing severe deficiencies of several major nutrients and found that the deficiencies had little effect on the percentage germination of the seed. Szukalski<sup>2</sup> applied different amounts of a phosphorus fertilizer to flax and rape plants and showed that when seed from these plants was sown in phosphorus-deficient soil, high levels of phosphorus in the seed were advantageous for seedling growth. Results from Durrant's<sup>3</sup> experiments with flax indicated that nutritional treatments given to flax plants brought about apparently heritable changes. In the present experiments nutritional treatments were applied to watercress, pea and carrot plants and plants from their seed were raised to determine the effects of the seed-plant treatments on the progenies. This communication gives a report of the results obtained.

In experiments with watercress (*Rorippa nasturtium aquaticum* L., Hayek) seed was harvested from plants grown in sand cultures supplied with Hewitt's<sup>4</sup> nutrient solution modified to contain either 0.2, 1.0 or 4.0 m.equiv./l. phosphorus (as ortho-phosphate). Iron was supplied chelated with ethylenediamine tetraacetic acid. Seed from plants grown under the three culture treatments (called  $P_1$ ,  $P_2$  and  $P_3$  seed) had, respectively, 0.47, 0.84 and 0.95 per cent of their dry weight as phosphorus, while differences in their contents of nitrogen and potassium were slight. These seeds were all similar in size and, two months after harvest, all had a germination of more than 90 per cent. The three kinds of seed were then sown in cultures supplied with each of the three solutions, and plant weights were determined after 7-9 weeks of growth. In cultures with the lowest level of phosphorus, plants from  $P_1$ ,  $P_2$  and  $P_3$  seed had a mean dry weight of 0.095 g, 0.139 g and 0.181 g, respectively (*L.S.D.* 0.0252). At the higher levels of phosphorus supply, plant weights did not reflect differences in the phosphorus content of the seed. In further experiments it was found that none of the effects produced by treatments applied to a first

Table 1. FRESH WEIGHTS, DRY WEIGHTS AND WATER CONTENT IN GRAMS OF PERIANTH SEGMENTS OF KING ALFRED DAFFODILS TREATED WITH BENZYLADENINE (BA) AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

Concentration of BA (M)	0	Concentration of 2,4-D (M)	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>
Fresh weight, 5 days after treatment					
0	2.93* ab	3.20 ab	2.60 a	2.95 ab	
5 × 10 <sup>-4</sup>	2.87 ab	3.54 b	4.71 c	4.32 c	
not dipped	3.58 b				
Fresh weight, 6 days after treatment					
0	0.67 a	0.79 ab	1.56 bcd	1.94 cd	
5 × 10 <sup>-4</sup>	1.27 abc	2.16 d	3.93 e	3.74 e	
not dipped	1.71 cd				
Dry weight, 6 days after treatment					
0	0.23 a	0.24 a	0.28 abc	0.25 ab	
5 × 10 <sup>-4</sup>	0.24 a	0.23 a	0.33 c	0.31 bc	
not dipped	0.29 abc				
Water content, 6 days after treatment					
0	0.44 a	0.55 ab	1.28 bcd	1.69 cd	
5 × 10 <sup>-4</sup>	1.03 abc	1.93 d	3.60 e	3.43 e	
not dipped	1.42 cd				

\* Mean of 4 replications each consisting of perianth segments of three flowers. All means followed by the same letter are not significantly different at the 1 per cent level.



Table 1. WEIGHTS OF HAULM AND PEAS FROM SEED CONTAINING DIFFERENT CONCENTRATIONS OF PHOSPHORUS

(a) Glasshouse experiment				(b) Field experiment			
First generation		Second generation		First generation		Second generation	
Nutritional treatment	% P in seed produced	Nutritional treatment	Dry weight in g/plant Haulms Peas†	% P in seed sown	Fresh weight in g/plant Haulms Peas and pods		
Low P	0.24	Low P	0.558 1.62	0.32	58.3	81.6	
High P	0.58	Low P	0.722 2.17				
Low P	0.24	High P	0.903 4.10	0.58	73.0	96.2	
High P	0.58	High P	0.920 4.15				
			± 0.048* ± 0.168*		± 3.4*	± 5.9*	

\* S.E. of difference between 2 means with 51 and 68 d.f. respectively.

† Dried at room temperature.

Table 2. YIELDS OF CARROTS FROM SEED CONTAINING DIFFERENT CONCENTRATIONS OF PHOSPHORUS

Manurial treatments applied to seed-bearing plants		P in seed (%)	Yield of carrots (tons per acre)
N	P		
-	-	0.535	27.9
+	-	0.497	28.1
-	+	0.562	30.6
+	+	0.472	26.8
S.E. of difference between two means		0.011 (9 d.f.)	0.67 (28 d.f.)

generation of plants had measurably different effects on mean plant weight in the third generation, irrespective of the concentrations of phosphorus in which the second or third generation of plants were grown.

In a glasshouse experiment with a pure line of the pea variety 'Meteor', plants were grown in vermiculite cultures supplied with Hewitt's<sup>4</sup> nutrient solution modified to contain either 0.4 or 4.0 m.equiv./l. of ortho-phosphate in combination with either 1.0 or 10.0 m.equiv./l. of nitrogen as nitrate. Plants given low phosphorus or low nitrogen produced many fewer but only slightly smaller seeds than those given the high N, high P solutions.

The differences in nitrogen nutrition did not lead to different N contents of the seed but seed from phosphorus-deficient plants contained 0.24 per cent P while that from high P plants contained 0.58 per cent P. Seeds from plants which had received the four kinds of nutrient solution were then sown in vermiculite supplied with each of these solutions, giving sixteen treatments. It was found that differences in the nitrogen nutrition of the parent plants had no effect on the weights of the plants produced by their seeds. Plants produced from seeds with a high phosphorus content, however, were significantly larger than those from low phosphorus seeds when the seeds were grown in a low phosphorus medium (Table 1(a)). When plants from the 16 kinds of seed from this experiment were grown in each of the four nutrient solutions, none of the treatments given to the first generation produced measurable differences in plant weights in this, the third, generation.

Peas from a glasshouse experiment where the seed-plants had been grown in the four nutrient solutions described here were also sown in the field in soil of high fertility. The plants were harvested when most of the pods contained fully swollen peas. The mean fresh weights of the plants and of the peas and pods, given in Table 1(b), showed that the seed which was low in phosphorus gave plants that were smaller and had a lower weight of pods than those from the seed not deficient in phosphorus. Nitrogen deficiency in the parent plants had no effect on seed performance.

Further evidence of the effects of differences in seed phosphorus reserves was obtained from an experiment with carrots, using seed produced from plants grown on sixteen field plots receiving different manurial treatments (all combinations of nil and high rate application of mineral nitrogenous, phosphatic and potassic fertilizers and farmyard manure). The manurial treatments had no effect on the size or percentage germination of the seed. It was found, however, that the nitrogen and phosphorus treatments given to the seed-plants affected the phos-

phorus, and, to a smaller extent, the nitrogen content of the seed. The other treatments did not significantly affect its N, P or K content. The mean yields of carrots from field sowings of four lots of seed of differing phosphorus content are shown in Table 2, where it can be seen that there was a positive correlation between yield and the phosphorus content of the seeds.

These results indicate that parent plant nutrition can affect the concentration of phosphorus in seeds, which in turn may affect plant yields. The slight decreases in the nitrogen contents of the seeds which were correlated with their increased phosphorus content seem unlikely to have caused the differences in their performance. Similarly, although the uptake of molybdenum by plants may be affected by their phosphorus nutrition<sup>5</sup>, the molybdenum content of the pea and cress seeds were all above the levels associated with deficiency of this element in seeds and did not appear to be appreciably affected by the supply of phosphorus to the parent plants.

There was no evidence of heritable change of the kind reported by Durrant<sup>3</sup>, although it was observed that plants grown from seed produced by phosphorus-deficient plants were more variable in size than those from non-deficient plants. Further experiments to determine the nature of this effect are in progress.

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### Effect of X-irradiation on Changes of Cell Distribution Curve of Mouse Bone Marrow as detected by Electronic Particle Counting

THE Coulter counter has now been widely accepted as a blood counter in clinical laboratories. Favourable evaluations of the instrument for use as a cell-size analyser have been reported<sup>1-5</sup>. The principle, construction and operation of the counter have been described by Coulter in 1956 (ref. 6) and by Mattern *et al.* in 1957 (ref. 1). In brief, the counting and size analysing are based on electronic gating. Cells suspended in an electrically conductive medium are made to flow through a small aperture conducting an electric current between two electrodes. Each cell, being a poor conductor, displaces a certain amount of the conductive medium corresponding to the cell volume and consequently raises the impedance to the flow of electric current proportional to the amount of medium displaced. The resulting change in voltage across the aperture is recorded in threshold units by a decade counter and visualized on an oscilloscope screen.

With the aid of the Coulter counter we have examined the cell-size distribution of mouse femoral marrow from non-irradiated normal animals and the cell distribution changes in the marrow of irradiated animals.

LAF<sub>1</sub> male mice of 10-12 weeks of age purchased from Jackson Memorial Laboratory were used. The animals were divided into groups of five. Each group was given a different dose of whole-body X-irradiation (200 kVp., 20 m.amp, half-value layer, 1.35 mm copper), ranging from 10 r. to 800 r. (LD<sub>100</sub>). The animals were killed by an overdose of ether 24 h following irradiation. The femurs of each animal were excised. The two ends of the

femur were cut to expose the bone cavity in such a way that a minimum loss of marrow resulted. The marrow was blown out into a test-tube by injection of normal saline for intravenous injection from a 22 needle on a 10-ml. syringe. Ten femurs from five animals of the same experimental group were pooled together to make a total volume of 6 ml. cell suspension. The cells in clumps were freed by holding the thumb over the top of the tube and the index finger on the bottom and shaking 40 times in a strong, jerky action. The cells were then forced ten times through a 25-gauge needle on the same 10-ml. syringe. One ml. of the suspension was diluted with 99 ml. of normal intravenous saline. The diluted suspension was filtered through a layer of cotton of about 5 mm in thickness with a single ply gauze above and below the cotton. The filtered cell suspension was free of clumps under examination with a microscope. The process between the time of blowing the marrow out from the bone cavity and the time immediately before counting lasted about 15–20 min.

The Coulter counter model B was used for our experiments. Experimental setting of the counter were: amplification, 2; and aperture current, 0.707. The cells between 10 and 110 threshold units were counted in steps of five threshold units. The cells, mostly erythrocytes, between 5 and 10 threshold units were excluded from our experiments as they were mixed with a great deal of cell debris. Each count was repeated three times in order to minimize experimental errors. The mean of the three was used as the final count. Before each count, the aperture was cleaned with a brush and the suspension was stirred by bubbling air through a rubber tube into the suspension to prevent the precipitation of the cells. The results were calculated in percentage of cells for each step of five threshold units. The cell percentages were plotted against threshold units to form a cell distribution curve (Fig. 1). A comparison of the curves revealed that curves from animals exposed to the following radiation doses, 800, 600, 400, 200 and 100 r., differed from the standard curve of the non-irradiated animals in the 10–15 unit range; they had a much higher percentage of cells than that of the non-irradiated animals, and in the 30–70 unit range of the curve, the 30–70 unit section of the curve is shaped like a 'hump' in all the non-irradiated marrow. The 'hump' was greatly diminished in the marrow of the animals which were exposed to 100 r. or more. With the exception of the 10–15 unit section of the curve of 100 r., the remaining portions of the curves of the animals exposed to 100 r. or more differed very little from each other. The 10–15 unit section of the curve of 100 r. was half-way between the normal control and the 800 r. Marrow from animals exposed to 65, 50, 45, 40, 25 and 10 r. responded differently. Their cell distribution curves, including the 10–15 unit section and the 'hump', were similar to the curves of non-irradiated marrow. The diminution of the 'hump' at only doses of 100 r. or more, but not less, suggests that the response may be an 'all-or-none' phenomenon with a threshold somewhere around 100 r.

Since all the foregoing counting was done 24 h after irradiation, the possibility that the normal appearance of the curves of doses below 100 r. may have resulted from a fast recovery cannot be ruled out. For this reason, the marrow of animals exposed to 25, 40, 50 and 65 r. were also counted 1 h after irradiation. No change of the curve was observed. The 'hump' remained normal. These results suggest that after a critical dose of radiation the medium-sized cells suddenly diminish while the small cells increase.

These findings were rather surprising. As in our cellular dry mass examinations of bone marrow with non-irradiated animals and animals irradiated at various dose levels from non-lethal to supra-lethal, the results show that the cellular dry mass and the percentage of radiation-

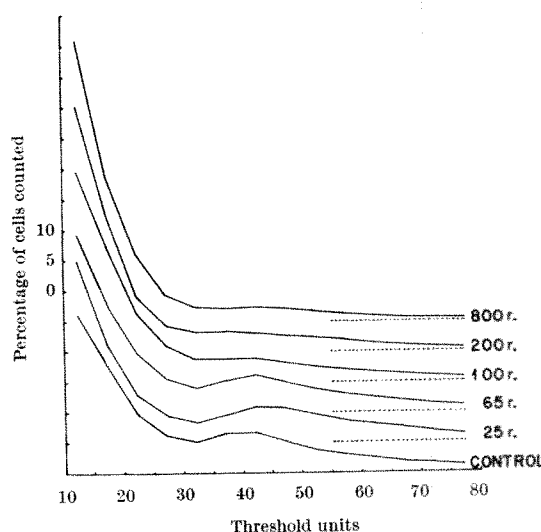


Fig. 1. Note that the 'hump' appears in the curves of the unirradiated control, 25 r. and 65 r., but is greatly diminished in the curves of 100 r., 200 r., and 800 r. Not all the curves of the tested doses are presented here. Curves for 400 r. and 600 r. are almost identical with that of 800 r. and curves for 50 r., 45 r., 40 r., and 10 r. are similar to that of the control. Dotted lines indicate the baseline for each curve. Each division on the ordinate line represents 5 per cent of the cells counted. Cells of more than 80 threshold units are omitted from all the curves because they show little difference from each other.

induced giant cells increase with the increase of radiation dose<sup>7-9</sup>. We expected a parallel change in the cell-size distribution in the electronic counting. To reconcile the discrepancy, we believe that the distribution changes found in counting with a Coulter counter are not due to changes in cell-size, but due to changes in conductivity of the cell caused by cell membrane injury from irradiation, and that changes in conductivity of the cell are triggered off by a critical radiation dose. An increase in cell conductivity is recorded by the counter as a decrease in cell-size since the size of a cell is based on the low-conductivity of the cell and the volume of conductive medium displaced by the cell in the counting with a Coulter counter. This will explain why the 'hump' diminishes at a critical radiation dose-level with an accompanying increase of small cells at the 10–15 unit section of the curve.

Apart from the theoretical considerations, in the practical aspects these findings can be adapted as a test for rapid screening of nuclear irradiated victims from a large population. This test will indicate whether a person received more than a non-lethal dose of radiation (300 r. or below, in the case of *LAF<sub>1</sub>* mice). A person receiving doses below 100–200 r. scarcely requires immediate medical attention.

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## MICROBIOLOGY

Mating in *Pseudomonas aeruginosa*

SEXUAL recombination in *Pseudomonas aeruginosa* was first reported by Holloway<sup>1</sup>. Later, Holloway and Jennings<sup>2</sup> demonstrated that there were similarities between their mating system and that of *Escherichia coli* K12. Fertility in the males was associated with an infectious factor which Holloway and Jennings called FP to distinguish it from the fertility factor, F, in *E. coli*. They were, however, unable to free their cells from the FP factor with acriflavine. Holloway<sup>1</sup> reported 125 recombinants per 10<sup>9</sup> parent cells.

Experiments were designed with the view of using the *Pseudomonas aeruginosa* mating system to assist genetic mapping of isoleucine-valine mutants. FP<sup>+</sup> and FP<sup>-</sup> strains were kindly provided by Dr. Holloway. We used FP<sup>+</sup> and FP<sup>-</sup> strains of strain 1 (1, try<sup>-</sup>, str<sup>r</sup>, chl<sup>r</sup>, FP<sup>+</sup> and 1, leu<sup>-</sup>, str<sup>s</sup>, chl<sup>s</sup>, FP<sup>-</sup>). Overnight cultures of these strains grown in Difco nutrient broth at 37° C were used for mating. The cells were washed once in saline and resuspended to the same concentration (approximately 10<sup>9</sup> cells per ml.). Equal volumes of FP<sup>+</sup> and FP<sup>-</sup> cells were mixed and 0.1 ml. aliquots of the mixture were spread on minimal agar plates of Davis and Mingioli<sup>3</sup> enriched with 0.005 per cent Difco nutrient broth. The plates were incubated at 37° C for 3 days and examined for the presence of prototrophic recombinant colonies.

Unfortunately when mating was carried out as described extreme variability was noticed between replicates from the same mating mixture as well as between mating mixtures prepared on different days. Some days we obtained up to 400 recombinant colonies per plate. On other days we obtained as few as 10 colonies per plate. 400 colonies represent one recombinant per 1.2 × 10<sup>5</sup> FP<sup>+</sup> cells.

During investigations of this variability we noticed one surprising result. In one experiment we mated a 10<sup>-1</sup> dilution of the FP<sup>+</sup> cells with undiluted FP<sup>-</sup> cells and obtained a mean of 386 recombinant colonies per plate with the diluted FP<sup>+</sup> cells but only 64 when the same cells were used undiluted. This observation was extended to include other dilutions of both FP<sup>+</sup> and FP<sup>-</sup> cells and the results of two experiments are set out in the following table. The experiments were carried out on different days and give a clear indication of the variability of the system. In Experiment I the FP<sup>-</sup> cells were diluted 10<sup>-2</sup> and used at that concentration. In Experiment II the FP<sup>+</sup> and FP<sup>-</sup> cells were diluted equally.

Table 1. RECOMBINANTS OBTAINED AFTER DILUTION OF PARENT FP<sup>+</sup> AND FP<sup>-</sup> CELLS OF *Pseudomonas aeruginosa*

	Dilution of FP <sup>+</sup> cells	Dilution of FP <sup>-</sup> cells	No. of recombinants per plate
Exp. I	No dilution	10 <sup>-2</sup>	0
	10 <sup>-1</sup>	10 <sup>-2</sup>	4
	10 <sup>-2</sup>	10 <sup>-2</sup>	79
	10 <sup>-3</sup>	10 <sup>-2</sup>	144
Exp. II	No dilution	No dilution	250
	10 <sup>-1</sup>	10 <sup>-1</sup>	255
	10 <sup>-2</sup>	10 <sup>-2</sup>	313
	10 <sup>-3</sup>	10 <sup>-3</sup>	100
	10 <sup>-4</sup>	10 <sup>-4</sup>	10

These results show that in Experiment I recombination was inhibited when the FP<sup>+</sup> parent was in gross excess and the number of recombinants increased as the number of FP<sup>+</sup> cells decreased. In Experiment II recombination was demonstrated with high concentrations of FP<sup>+</sup> cells provided the FP<sup>-</sup> cells were there in an equivalent concentration. It also shows that the system was much less efficient when there was an excess of both parents. A ten-fold decrease in the concentration of both parents from 10<sup>-2</sup> to 10<sup>-3</sup> resulted in only a three-fold decrease in the number of recombinants. It was only when the cells were diluted from 10<sup>-3</sup> to 10<sup>-4</sup> that the number of recombinants was correlated with the dilution.

Using the figure at the point of maximum efficiency we calculated the number of recombinants per FP<sup>+</sup> parent and found that the figure was greater than that recorded by Holloway<sup>1</sup>. In Experiment II, using the number of recombinants at 10<sup>-3</sup> dilution, we obtained one recombinant for every 500 FP<sup>+</sup> cells. As stated, results do vary from day to day and in one experiment we found one recombinant for 109 FP<sup>+</sup> cells or 0.92 per cent recombination. In the same experiment, 1.7 per cent recombination was obtained when an FP<sup>-</sup> strain was used which had a thiamine marker as well as leucine. 1.7 per cent recombination was obtained with the thiamine marker and 0.92 per cent with the leucine marker.

These results show that the efficiency of recombination in *Pseudomonas aeruginosa* is much greater than previously reported. The system cannot achieve this efficiency when there is an excess of the parent cells. It is not yet clear how the excess cells interfere with the process but the problem is being investigated and will form the subject of a separate communication.

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## Magnesium Sulphate as Stabilizer during Liberation of Yeast and Mould Protoplasts

LIVING protoplasts can be produced from yeast and moulds through treatment with snail or microbial enzymes<sup>1-6</sup>, and numerous investigations have made it evident that the removal of the cell wall does not affect to any large extent most of the biochemical and biological activities of the microbial cell.

Investigations on the permeability of bacterial protoplasts provide evidence that the exchange of matter between the interior of the cell and the external medium is regulated by the cytoplasmic membrane. Similar work on fungi is sparse although investigations on fungal protoplasts have also given evidence of the presence of a semi-permeable but non-rigid membrane with the function of a permeability barrier in these organisms<sup>7</sup>. In intact cells of yeast or moulds the mechanical protection of the cell wall prevents the disintegration of cells suspended in water or in very dilute media. It is well known that if the cell wall is digested the delicate cytoplasmic membrane disintegrates unless an osmotic stabilizer is applied. This stability can be obtained by adding to the medium a substance that does not penetrate, or penetrates very slowly, into the protoplast, although in this connexion it might be worth emphasizing that the stability of free fungal protoplasts is affected by several other factors besides the osmotic pressure of the medium.

Most investigations on protoplast formation and physiology have been carried out in media of comparatively high osmotic pressure since this seems to be required for maintenance of structural integrity. The solute must not be able to penetrate the osmotic barrier of the protoplast at an appreciable rate if the solution is to give protection from lysis. The concentration of the solutes used as stabilizers varies largely; this phenomenon can be correlated to some extent with differences in internal osmotic pressure in different species.

A wide variety of stabilizing media has been used by different workers to obtain protoplasts from fungi. Media containing one of the following solutes have commonly been used: sucrose, maltose, fructose, rhamnose, sorbose, xylose, sorbitol, mannitol, polyethyleneglycol, 'Carbowax-



0.000', KCl, NaCl, and serum albumin, added to a buffer solution at pH between 5.8 and 6.8. The protoplasts lysed rapidly or slowly with other solutes, and they lysed immediately when placed in distilled water. However, mannose<sup>1,3</sup>, mannitol<sup>2</sup>, MgSO<sub>4</sub> (refs. 9 and 10) and KCl (ref. 2) have been preferred by most workers.

We have investigated the properties of some of these solutes using *Candida utilis* and *Fusarium culmorum* protoplasts not only as stabilizers but taking into consideration other important factors, such as the stimulatory effect of the solute on the lytic enzymes and the degree of bacterial contamination of the suspensions, this last factor being a very important one to try to avoid during mass biochemical protoplast preparations. We have concluded that magnesium sulphate at a concentration of 0.8 to 0.10 M is by far the best of all the stabilizers tested, yielding a stable protoplast suspension<sup>9</sup>. Using the MgSO<sub>4</sub> concentrations, it is very unusual to see any microbial contaminants during the experiments and it facilitates the performance of experiments which require long incubation periods. It has also been observed that when microbial enzymes (streptozymes<sup>8,10</sup>) are used the formation of protoplasts takes place more quickly in MgSO<sub>4</sub>.

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### Induced Protection against Wildfire Disease in Tobacco Leaves treated with Heat-killed Bacteria

PLANT tissues infected with viruses and fungi are often protected against re-infection with the same or another pathogen<sup>1-4</sup>. Multiplication in the host tissues of micro-organism(s) of the primary infection appears to be a pre-requisite of these protective actions. Recently Loebenstein succeeded in inducing partial protection of the host against viral infection by treating the host tissues with the protein part of the infective virus particle<sup>5</sup>. This indicates that, at least in some cases, the multiplication of the pathogenic agent is not necessary for the development of the protective effect. A phenomenon somewhat similar to that observed by Loebenstein is described in the present report. It has been shown that the pretreatment of tobacco leaves by heat-killed bacteria induces protection against infection by *Pseudomonas tabaci* (Wolf and Foster) Stevens.

Washed suspensions of *Ps. tabaci* H 17 were used in the experiments. Cell numbers were adjusted densitometrically.

For pretreatment of tobacco tissues half-leaves were injected according to Klement<sup>6</sup> with *Ps. tabaci* cell suspensions kept in a boiling water bath for 5 min and the other halves of the same leaves with water ('con-



Fig. 1. Protection induced by heat-killed bacteria in a tobacco leaf against the wildfire disease. Left half: water control. Right half: tissue pretreated with heat-killed *Ps. tabaci* cells.

trol'). For infection, cell suspensions containing 10<sup>7</sup> bacterial cells/ml. were injected into the leaves.

As the most striking symptom of infection with *Ps. tabaci* was the gradual wilting of the host tissues the degree of protection could be expressed by determining the difference between fresh weight of leaf disks punched from half-leaves pretreated with killed bacteria and from control half-leaves at various intervals after infection.

Intact leaves of *Nicotiana tabacum* var. White Burley plants (4- to 6-leaf stage) were used throughout the experiments. Pretreatment of tobacco tissues was carried out by bacterial suspensions containing 10<sup>9</sup>, 10<sup>7</sup> or 10<sup>5</sup> heat-killed cells/ml. The leaves were infected with *Ps. tabaci* one day after pretreatment. Infected water-pretreated half-leaves wilted and turned yellow within 2 days after infection, and perished within about 6 days. By contrast, half-leaves pretreated with 10<sup>9</sup> heat-killed cells/ml. remained normal for 6 days after infection, that is, a marked delay in symptom development was experienced (Fig. 1). The protection induced by 10<sup>7</sup> or 10<sup>5</sup> heat-killed cells/ml. was less pronounced (Table 1). More efficient protection could be induced in young leaves than in older ones.

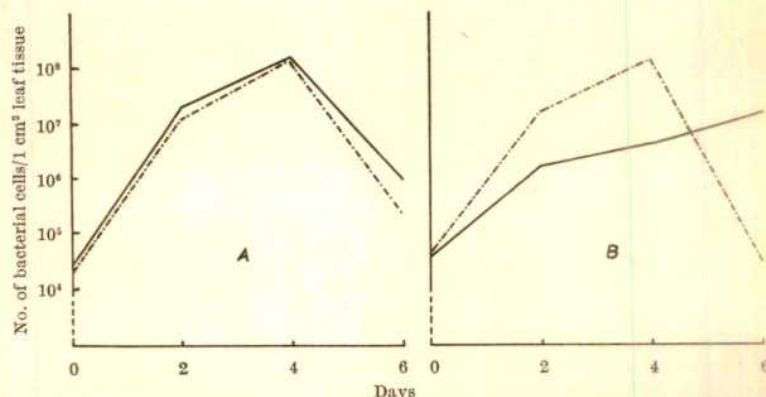


Fig. 2. Multiplication of *Ps. tabaci* in tobacco leaves treated with heat-killed cells of *Ps. tabaci*. A, Leaf injected simultaneously with heat-killed and living bacteria: —, half-leaf injected with living and heat-killed bacteria; — · — · —, half-leaf injected with living bacteria only. B, Leaf injected with living bacteria 1 day after pretreatment: —, half-leaf pretreated with heat-killed bacteria; — · — · —, half-leaf pretreated with water. Suspensions of 10<sup>9</sup> cells/ml. heat-killed cells (100° C for 5 min) and 10<sup>7</sup> cells/ml. suspensions of living bacteria were used for the injections.

Table 1. EFFECT OF NUMBER OF HEAT-KILLED *Pseudomonas tabaci* CELLS INJECTED INTO TOBACCO LEAVES ON THE EXTENT OF PROTECTION INDUCED AGAINST WILDFIRE DISEASE

Pretreatment of half-leaves†	Fresh weight of 4 cm² leaf tissue after infection* (mg)				Ratio of fresh weight of tissues pretreated with H <sub>2</sub> O and with heat-killed bacteria			
	0‡	2	4	6	0	2	4	6
10 <sup>9</sup> cells/ml. H <sub>2</sub> O	57.9 58.6	58.4 39.4	58.1 32.0	59.4 9.1	1.01	0.67	0.55	0.15
10 <sup>7</sup> cells/ml. H <sub>2</sub> O	63.4 64.7	56.5 47.3	57.6 45.6	31.3 7.5	1.02	0.83	0.79	0.23
10 <sup>5</sup> cells/ml. H <sub>2</sub> O	57.4 57.7	45.2 45.0	37.3 38.3	15.9 15.0	1.00	1.00	1.02	0.94

\* Infection of tobacco leaves was carried out 1 day after pretreatment by using *Ps. tabaci* cell suspensions (10<sup>7</sup> cells/ml).

† Pretreatment was carried out by using *Ps. tabaci* cells exposed to 100° C for 5 min.

‡ 0, 2, 4 and 6 days after infection.

All figures are averages of 3 samples.

Table 2. PROTECTION AGAINST WILDFIRE DISEASE IN TOBACCO LEAVES TREATED WITH HEAT-KILLED CELLS OF *Pseudomonas tabaci* AT VARIOUS INTERVALS BEFORE INFECTION WITH *Ps. tabaci*

Treatment of half-leaves	No. of days between pretreatment* and infection†	Fresh weight of 4 cm² leaf tissue (mg)				Ratio of fresh weight of tissues pretreated with H <sub>2</sub> O and with heat-killed bacteria			
		0‡	2	4	6	0	2	4	6
Killed bacteria H <sub>2</sub> O	0	43.9 43.9	42.7 43.3	36.9 36.7	33.2 33.9	1.00	1.01	0.99	1.02
Killed bacteria H <sub>2</sub> O	1	43.9 45.0	44.9 37.2	43.9 15.7	46.9 6.1	1.02	0.82	0.35	0.13
Killed bacteria H <sub>2</sub> O	3	40.1 41.5	42.1 37.2	41.9 33.8	43.1 21.2	1.03	0.88	0.80	0.49
Killed bacteria H <sub>2</sub> O	6	45.2 45.7	44.0 44.4	35.8 32.2	14.7 11.2	1.01	1.00	0.89	0.76

\* Pretreatment of leaves was carried out by using cell suspensions of *Ps. tabaci* (10<sup>9</sup> cells/ml.) exposed to 100° C for 5 min.

† Infection was carried out by cell suspensions of *Ps. tabaci* containing 10<sup>7</sup> cells/ml.

‡ 0, 2, 4 and 6 days after infection.

All figures are averages of 3 samples.

The dependence of the degree of protection on the length of time elapsing between pretreatment and infection was also investigated. As may be seen from Table 2, time is needed for the development of protective action and this indicates the active part played by the host tissue in the process. Full effectiveness of the pretreatment is maintained only for a short period.

Treatment of tobacco leaf tissues with heat-killed bacteria failed to antagonize the effect of the toxin-containing culture filtrates of *Ps. tabaci*. These results suggest that the protective effect induced by heat-killed bacteria in the host is directed towards the living bacteria and not against their products. To test this idea investigations on the effect of pretreatment on the multiplication of bacteria in infected host tissues were undertaken. Determination of cell number was carried out by plate counting as described previously<sup>7</sup>. The results are shown in Fig. 2. It may be seen that bacterial multiplication was not affected by the heat-killed bacteria if the leaves were treated simultaneously with dead and living cells. On the contrary, the number of bacterial cells was less in half-leaves pretreated with heat-killed bacteria than in the controls until the fourth day after infection. Evidently, the pretreatment with heat-killed bacteria evoked unfavourable conditions in the tobacco leaf for the multiplication of *Ps. tabaci*. At the end of the experiment (6th day) cell number was already lower in the 'control' half-leaves than in those pretreated with bacteria. This is explained by the fact that the control half-leaves completely perished by that time and the bacteria were killed, whereas those pretreated with heat-killed bacteria were still free of symptoms and the multiplication of bacteria continued in these tissues at a low but constant rate.

Results of experiments obtained with bacteria exposed to 100° C for 5 or 15 min or for 2 h at 1 atm. pressure indicated that the latter drastic heat treatment results in a reduction of the protective effect which could be induced by heat-killed bacteria. This is probably due to a partial destruction of the cell component(s) responsible for this phenomenon.

Protection of tobacco leaf tissues against *Ps. tabaci* H 17 could be induced by heat-killed suspensions of other strains of *Ps. tabaci*, for example, *Ps. tabaci* H 20, *Ps. tabaci* T (a strongly toxin-producing strain) and by other species, for example, *Ps. syringae* 366 or *Corynebacterium flaccumfaciens* 1449. Evidently, the protective effect of heat-killed bacteria is not species specific.

The question arises as to the nature of physiological processes evoked by heat-killed bacteria in the host tissues. It can be assumed that the formation of phytoalexin-like inhibitory substances is involved<sup>8</sup>. However, further experiments are needed for the elucidation of this problem as it is also possible that the tissues pretreated with heat-killed bacteria are unable to provide substances or circumstances necessary for the optimal multiplication of *Ps. tabaci*.

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### Modification of the Action of Ionizing Irradiation on *Escherichia coli* by 2-Fluoroadenosine

In the course of routine screening for radiation modifiers in bacterial systems, 2-fluoroadenosine<sup>1</sup> has been observed to modify the activity of ionizing irradiation on non-proliferating *Escherichia coli*. The procedures used to determine irradiation-modifying activity have been described<sup>2</sup>. In essence, the procedure used for measuring effects on non-growing cells consists of exposing washed



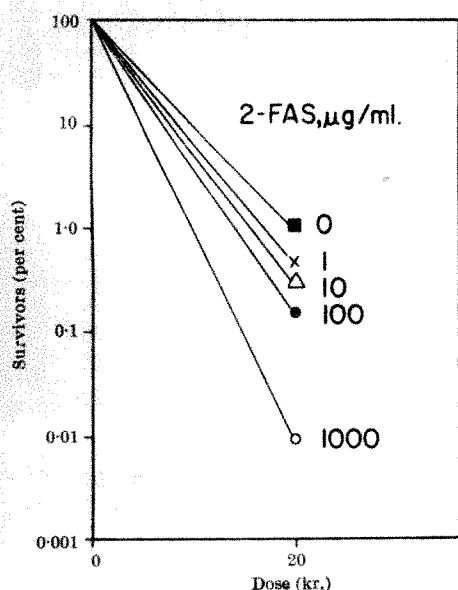


Fig. 1. 2-Fluoroadenosine (FAS) potentiation of the effect of ionizing irradiation ( $^{60}\text{Co}$ ) on non-proliferating cell suspensions of *Escherichia coli* 'ATCC 9637'.

cells (grown on simple glucose-salts medium and resuspended in M/15 phosphate buffer) to irradiation from a cobalt-60 source ( $\sim 1,000$  c., dose rate of 2.0 kr./min) with and without test compound. Experiments with growing cells were performed in which the test compound was added to the glucose-salts medium at the time of inoculation and aliquots of cell suspensions irradiated at varying times during the growth cycle.

Data compiled from several representative experiments which show the potentiating action of 2-fluoroadenosine on  $^{60}\text{Co}$ -irradiation damage of non-proliferating cells of *Escherichia coli* 'ATCC 9637' are presented graphically in Fig. 1. It may be noted that the effect is dose related.

Other non-proliferating wild-type cultures of *E. coli*, namely, *E. coli* 'B' (ORNL) and *E. coli* 'B' (Hill strain), responded differently from *E. coli* 'ATCC 9637' (see Table 1). 2-Fluoroadenosine potentiation of ionizing irradiation was not appreciable in *E. coli* 'B' (Hill), although it was observed in the derived radiation-sensitive strain, *E. coli* 'B<sub>s</sub>' (Hill)<sup>2</sup>. 2-Fluoroadenosine affords a modest degree of protection against the effects of ionizing irradiation on *E. coli* 'B' (ORNL), but the derived irradiation-resistant strain, *E. coli* 'B/r' (ref. 4), was more sensitive to ionizing radiation in the presence of 2-fluoroadenosine than in its absence. *E. coli* 'ATCC 11303' was slightly sensitive to potentiation by 2-fluoroadenosine.

Strains of *E. coli* 'ATCC 9637' resistant to either 2-fluoroadenosine, azaserine, 6-diazo-5-oxo-*l*-norleucine (DON), or nitrogen mustard<sup>5</sup> were found to be sensitive in

varying degrees to the potentiating action of 2-fluoroadenosine on ionizing irradiation when tested as cell suspensions. The strains resistant to 2-fluoroadenosine, azaserine, or nitrogen mustard were less sensitive than the parent culture, *E. coli* 'ATCC 9637', but the DON-resistant strain was approximately 100 times more sensitive than the parent culture. A culture derived from *E. coli* 'B' (Hill), resistant to the antileukaemic alkylating agent, 1,3-bis(2-chloroethyl)-1-nitrosourea<sup>6</sup>, was also more sensitive to the potentiating action of 2-fluoroadenosine on ionizing irradiation than its parent culture.

For potentiation to occur, 2-fluoroadenosine must be present during irradiation. Cell suspensions of *E. coli* 'ATCC 9637' were exposed to 0.1 mg of 2-fluoroadenosine per ml. for 1 h, washed three times by centrifugation, and irradiated. No increase in percentage killed of such cells, as compared to control cells, was observed.

The potentiating activity of 2-fluoroadenosine on ionizing irradiation damage of non-proliferating *E. coli* 'ATCC 9637' is not markedly affected by either cysteine or cysteamine (see Fig. 2). Cysteine (1.2 mg/ml.), for example, under the conditions described here, affords considerable protection to *E. coli* 'ATCC 9637' against an irradiation dose of 20 kr. (5.6 per cent survivors to 20 kr.; 47.6 per cent survivors to 20 kr. + cysteine, 1.2 mg/ml.). 2-Fluoroadenosine, 0.1 mg/ml., in combination with an irradiation dose of 20 kr., results in 0.08 per cent survivors. The combination of 2-fluoroadenosine, 0.1 mg/ml., with an irradiation dose of 20 kr. plus cysteine, 1.2 mg/ml., results in 2.97 per cent survivors. 2-Fluoroadenosine, 0.1 mg/ml., and cysteine, 1.2 mg/ml., either alone or in combination, are non-toxic to *E. coli* 'ATCC 9637'. Similar results were obtained with cysteamine.

The effect of 2-fluoroadenosine on *E. coli* 'ATCC 9637' under conditions which allow cell division, with and without ionizing irradiation, have been investigated (see Fig. 3). Non-toxic concentrations of 2-fluoroadenosine (0.1  $\mu\text{g}/\text{ml}$ .) do not appear to potentiate the damaging action of ionizing irradiation on this bacterium. A concentration of 2-fluoroadenosine which is bacteriostatic does potentiate irradiation effects.

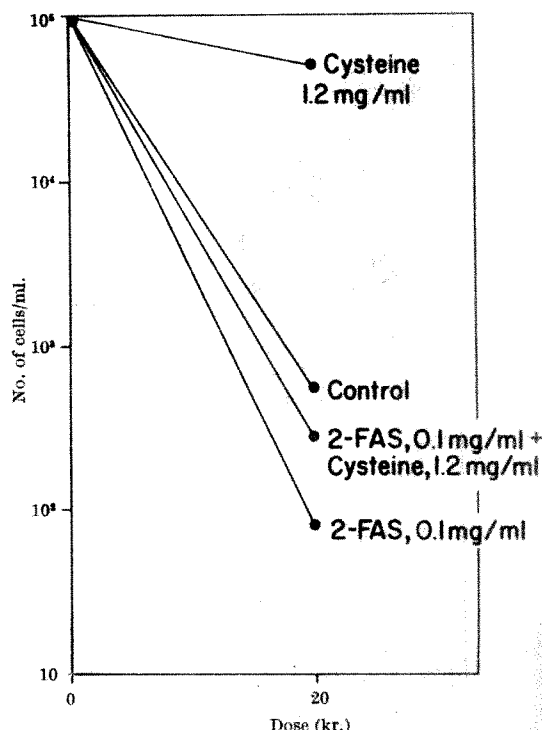


Fig. 2. The effect of cysteine (1.2 mg/ml.) on the potentiation by 2-fluoroadenosine (FAS) of irradiation damage in non-proliferating cell suspensions of *Escherichia coli* 'ATCC 9637'.

Table 1. 2-FLUORADENOSINE (FAS) POTENTIATION OF THE DAMAGING EFFECTS OF IONIZING IRRADIATION ( $^{60}\text{Co}$ ) ON A SPECTRUM OF *Escherichia coli* AND DERIVED RESISTANT STRAINS

Micro-organism	Survival (%)		
	(a)	(b)	Ratio a/b
	20 kr.	20 kr. + FAS, 100 $\mu\text{g}/\text{ml}$ .	
<i>Escherichia coli</i> 'ATCC 9637'	2.10	0.05	42.00
<i>Escherichia coli</i> 'ATCC 9637'/FAS*	0.54	0.22	2.45
<i>Escherichia coli</i> 'ATCC 9637'/AZA	16.40	5.55	2.97
<i>Escherichia coli</i> 'ATCC 9637'/DON	0.42	<0.009	>4,666.0
<i>Escherichia coli</i> 'ATCC 9637'/HN <sub>2</sub>	12.22	1.61	7.58
<i>Escherichia coli</i> 'B' (ORNL)	0.12	0.20	0.62
<i>Escherichia coli</i> 'B/r'	80.50	7.30	11.02
<i>Escherichia coli</i> 'B' (Hill)	0.06	0.06	1.0
<i>Escherichia coli</i> 'B' (Hill)/BCNU	8.12	1.27	6.3
<i>Escherichia coli</i> 'B <sub>s</sub> ' (Hill)	0.13	0.06	2.1
<i>Escherichia coli</i> 'ATCC 11303'	0.08	0.06	1.37

\* Resistance to a growth inhibitor indicated thus: name of parent culture/ name of inhibitor to which strain is resistant.

FAS = 2-Fluoroadenosine

HN<sub>2</sub> = Nitrogen mustard

AZA = Azaserine

r = Radiation

DON = 6-Diazo-5-oxo-*l*-norleucine

BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea



## VIROLOGY

## Antigenic Variability of the Strains of Rous Sarcoma Virus

At present the variability of biological properties of the strains of the Rous sarcoma virus (RSV) is well known. This variability concerns first of all their oncogenic activity<sup>1,2</sup>.

Simons and Dougherty<sup>3,4</sup> were the first to note the antigenic differences between the three strains of RSV in the neutralization reaction. Moreover, antigenic differences were accompanied with considerable differences in biological properties<sup>1</sup>.

It has been shown that the Carr strain of RSV is able to cause haemorrhagic disease or tumours in rats<sup>5,6</sup>, rabbits<sup>7</sup> and other animals, including monkeys<sup>8</sup>. The Bryan strain exerted no oncogenic activity for mammals<sup>9</sup>. It was of interest to compare the antigenic properties of the two mentioned strains. The results of examining Carr and Bryan strains in the neutralization reaction with sera prepared against Carr strain follow.

The Carr strain was kindly supplied by Dr. G. Y. Svet-Moldavsky, and the Bryan strain by Prof. A. A. Smorodintsev. Both strains have been investigated for many years in the U.S.S.R. and are passed on chickens. The titres of virus in tumours were  $10^5$ – $10^6$  oncogenic doses ( $OD_{50}$ )/g tumour.

Thirty per cent tumour suspensions in Hanks's solution with 10 per cent calf serum were used as virus-containing material. The tumour suspensions were centrifuged twice at 3,000 r.p.m. for 20 min and the supernatant was kept at  $-50^\circ\text{C}$ .

The sera were taken from chickens the tumours of which, caused by RSV, had resolved. Before bleeding the chickens were given several additional injections of homologous RSV strain with an interval of 1–2 weeks.

The method of the neutralization reaction was as follows: 10-fold dilutions of virus suspension in saline were mixed with constant (1:5) dilution of test or control (normal calf) serum. The mixtures were incubated for 2 h at room temperature and 0.2 ml. was injected into the wing web of 2-week-old White Leghorn chicken (4–5 wing webs/mixture). The chickens were under observation up to 3 weeks. The neutralization index was calculated by the Reed-Muench method.

The results of a typical experiment where the serum against Carr strain distinctly neutralizes the homologous virus and is completely inactive against Bryan strain are given in Table 1.

The indexes of neutralization of Carr and Bryan strain by chicken sera against Carr strain are given in Table 2.

Table 1. RESULTS OF NEUTRALIZATION TEST OF RSV, CARR AND BRYAN STRAINS WITH CHICKEN SERUM NO. 9/2 AGAINST CARR STRAIN

Serum	Strain	Log of virus dilution				Neutralization index (NI)	NI (Carr) / NI (Bryan)
		2.5	3.5	4.5	5.5		
Immune	Carr	0/5*	0/5	0/5	0/5	$\geq 100$	
Control	Carr	5/5	5/5	0/5	0/5		
Immune	Bryan	4/4	4/4	2/4	1/4	0.3	$\geq 333$
Control	Bryan	5/5	5/5	1/4	0/4		

\* Numerator, No. of wing webs with tumours out of the No. of injected wing webs (denominator).

Table 2. INDEXES OF NEUTRALIZATION OF RSV, CARR AND BRYAN STRAINS BY IMMUNE CHICKEN SERA AGAINST CARR STRAIN

No. of the serum against Carr strain	No. of the test	Neutralization index (NI) of strain		NI (Carr) / NI (Bryan)
		Carr	Bryan	
9/2	1	$> 25$	$< 1.8$	$> 14$
9/2	2	$> 100$	0.3	$> 333$
9/5	1	10,000	3.15	3,200
7/2	1	$> 10$	0.25	$> 40$
7/2	2	$> 100$	$< 3$	$> 33$
7/2	3	1,000	0.5	2,000
7/5	1	1,000	0.3	3,333
4/5	1	1,000	1.8	556
10/5	1	1,000	1.0	1,000
4/2	1	10	0.25	40
4/2	2	12	8	1.5

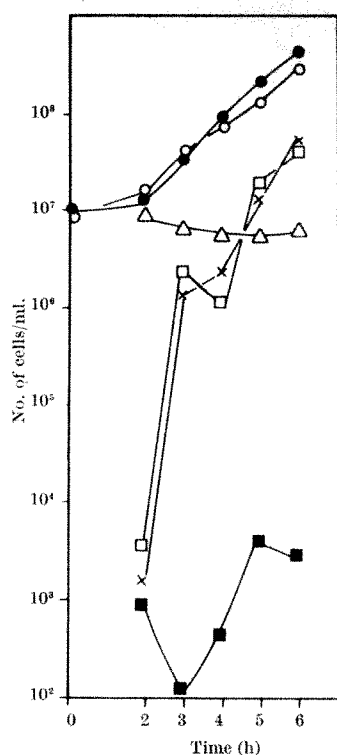


Fig. 3. The effect of 2-fluoroadenosine (FAS) on the damaging effects of ionizing irradiation ( $^{60}\text{Co}$ ) on growing cells of *Escherichia coli* ATCC 9537. ●, Growth control; ○, 2-FAS, 0.1  $\mu\text{g/ml}$ ; △, 2-FAS, 1.0  $\mu\text{g/ml}$ ; ×, survivors to 30 kr.; □, survivors to 30 kr. + 2-FAS, 0.1  $\mu\text{g/ml}$ ; ■, survivors to 30 kr. + 2-FAS, 1.0  $\mu\text{g/ml}$ .

It has been reported<sup>7</sup> that cells deficient in purines have an increased sensitivity to the damaging effects of ionizing irradiation. The possibility that 2-fluoroadenosine exerts its irradiation-potentiating effect by inhibiting purine biosynthesis *de novo*—known to occur in non-proliferating *E. coli* ATCC 9637 (ref. 8)—was investigated. Cell suspensions of *E. coli* ATCC 9637 prepared from inocula grown in a glucose-salts medium supplemented with non-toxic (0.1–0.5  $\mu\text{g/ml}$ ) or slightly toxic (1.0  $\mu\text{g/ml}$ ) concentrations of 2-fluoroadenosine were no more sensitive to ionizing irradiation than were cell suspensions prepared from inocula grown in the same medium without 2-fluoroadenosine.

The mechanism by which 2-fluoroadenosine exerts its potentiating effect is unresolved. It is interesting to note, however, that under our conditions of test none of the following purine analogues affected the activity of ionizing irradiation on *E. coli* ATCC 9637: 6-mercaptopurine, 8-azaguanine, 8-azaxanthine, or 6-thioguanine. Surprisingly, 2-fluoroadenosine was also without effect.

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From this table one can see that sera Nos. 9/2, 9/5, 7/2, 7/5, 4/5, 10/5 distinctly neutralize the homologous strain and are completely inactive against Bryan strain; serum No. 4/2 neutralizes weakly Carr strain and has no influence on Bryan strain. (The numerator of the number of the serum refers to the number of the chicken and the denominator shows the number of additional injections received by the chicken after the resolution of the tumour.)

The results obtained suggest that between Carr and Bryan strains there are distinct antigenic differences which are easily determined in the neutralization reaction. Thus, the principal differences between these two strains concerning their oncogenicity for mammals correspond to clear differences in the antigenic structure of virus protein.

We obtained preliminary results showing the wide antigenic variability of RSV strains: Bryan strain used in the work recorded here proved to be not identical with the standard Bryan strain obtained in 1964 through the courtesy of Dr. W. R. Bryan. The Schmidt-Ruppin strain also had its antigenic peculiarities.

Hanafusa *et al.*<sup>10</sup> showed that antigenic properties of RSV are due to the properties of the helper viruses pertaining to the avian leucosis complex. There are reasons for supposing that RSV strains which we have examined have not identical helper viruses.

As during the passage of RSV on chickens the strains can be contaminated by different avian leucosis viruses which are RSV helpers and which determine its properties, one of the mechanisms of RSV variability becomes clear.

Further investigations of antigenic variability of RSV are of great interest. They should lead to the serological classification of RSV and helper virus strains.

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## Antibacterial Activity of a Variety of Viruses

It has been previously noted that growth of a number of bacterial species is inhibited *in vitro* by herpes simplex virus propagated in a variety of antibiotic-free cell lines<sup>1</sup>. 0.1 ml. of such tissue culture fluid, applied to 1 cm<sup>2</sup> of sterile thick filter paper, placed on a Petri plate surface-seeded with 0.2 ml. of a standardized inoculum of a 6-h bacterial culture on a medium containing equal parts of mixed 1 per cent trypticase in 2 per cent agar and monkey kidney B medium Melnick, was found to produce a distinct inhibitory zone around the virus-impregnated filter square with bacterial strains belonging to a number of different species after 16–18 h incubation at 37° C, providing sufficient concentration of virus is present. Allantoic fluid derived from 9-day-old chick embryos the chorioallantoic membranes of which had been infected with 2 herpes simplex virus strains failed to suppress the bacterial growth. Control tissue culture fluid, not inoculated with virus, was similarly ineffective in this regard.

A number of other viral species, randomly selected from among available stock without regard to titre, have been tested for antibacterial capability by the same procedure as an extension of the foregoing observation. These were propagated in antibiotic-free WI-38 human embryonic lung cell line and in some instances in the allantoic cavity of the chick embryo in order to ascertain whether or not viruses derived from a source other than tissue culture might also exert the same effect.

As may be noted in Table 1, some of the viral species did prove to be capable of inhibiting growth in a number of strains belonging to a variety of bacterial species, as evidenced by the production of a clear zone of varying diameter around the virus-impregnated filter paper square. Included among those which were active were some viruses which had been propagated in the allantoic cavity of the chick embryo. The possibility that some of the ineffective viruses might have proved to be active had they been of higher titre must be given consideration. Control tissue culture fluid and allantoic fluid were inactive. Thus, bacterial inhibition is a capability possessed by a number of different viruses including some cultivated in the allantoic cavity of the chick embryo as well as in tissue culture and is not limited to herpes simplex virus grown in tissue culture.

With regard to relative effectiveness of the different viruses, the broad activity of influenza strain PR8 and of the influenza A2 strain in eliciting bacterial inhibition is particularly noteworthy. As for the bacterial species involved, a high degree of refractoriness to viral inhibition may be particularly observed with all 5 *Pseudomonas aeruginosa* strains as well as with the 5 *Streptococcus faecalis* strains. The former was resistant to the entire gamut of test viruses as was the latter with the exception of some

Table 1. EFFECT OF VARIOUS VIRUSES ON GROWTH OF DIFFERENT BACTERIAL SPECIES

Virus	Test source*	<i>Staphylococcus aureus</i>	<i>Streptococcus faecalis</i>	<i>Escherichia coli</i>	<i>Aerobacter aerogenes</i>	<i>B. proteus</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella</i>
Herpes simplex—S	TC	3/4†	0/5	3/5	2/5	2/4	0/5	0/5
Adenovirus 7	TC	3/4	0/5	4/5	2/5	0/5	0/5	0/4
Coxsackie A—Type 9 (PB-BOZEK)	TC	4/5	0/5	3/5	0/5	0/5	0/5	0/5
Coxsackie B—Type 3	TC	2/5	0/5	2/4	2/5	0/5	0/5	0/5
ECHO—Type 7	TC	1/5	0/5	—	2/5	1/4	0/5	0/5
ECHO—Type 9	TC	3/4	0/5	4/5	2/5	1/5	0/5	0/5
Encephalomyocarditis—strain Col. SK1	TC	3/5	—	2/5	2/5	0/5	0/5	0/5
Influenza A—strain Jap. 305	AF	0/4	—	—	—	—	—	—
Influenza A—strain PR-8	AF	—	5/5	—	—	5/5	—	5/5
Influenza A2	AF	2/5	3/4	5/5	2/5	2/4	0/5	3/5
Influenza B—strain GL	AF	4/5	—	0/5	0/5	0/5	—	0/5
Influenza—swine	AF	0/5	—	0/5	0/5	—	—	—
Mumps—enders	TC	2/5	0/5	1/3	—	0/5	0/5	0/5
Mumps—flower	AF	0/5	—	0/2	—	—	—	—
Newcastle disease virus strain Michigan	AF	0/5	—	2/5	—	—	—	—
Strain NS ROAKIN	TC	4/4	0/5	—	—	0/5	—	—
Poliomyelitis 2—strain MEF 1	TC	1/4	0/5	1/4	0/5	0/5	0/5	0/5

\* TC, tissue culture; AF, allantoic fluid.

† No. of strains inhibited/No. of strains tested.

of the influenza viruses. One *Staphylococcus aureus* and one *Aerobacter aerogenes* were also unaffected by any of the viruses. The reason for the refractoriness of some bacterial species and strains as well as the underlying mechanism responsible for the ability of viruses to effectuate bacterial inhibition remains to be elucidated.

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### Further Simplification of the Purification Procedure of Influenza Virus on Barium Sulphate

A METHOD for the purification of influenza virus by adsorption to and elution from BaSO<sub>4</sub> with subsequent ultracentrifugation of the eluate has been published by Mizutani<sup>1</sup>. We have found that this method can be further simplified by separation of the citrate (necessary for elution but interfering with adsorption) in the form of its calcium salt after elution from BaSO<sub>4</sub> has been achieved. By this step the ultracentrifugation of the eluate is eliminated and repeated purification cycles on the BaSO<sub>4</sub> can be performed.

Allantoic fluid of eggs was infected with PR8, Singapore, and Lee strains of influenza virus maintained in this Institute. CCA was established according to Salk<sup>2</sup>, and infectivity by titrating serial dilutions of the preparations in embryonated eggs; N was determined by the Kjeldahl method as modified by Schulek<sup>3</sup>. In the adsorption stage the prescriptions of Mizutani were strictly adhered to but the time needed for adsorption was reduced to 1 h after it had been confirmed that during this period the virus content in the supernatant did not decrease, that is, the adsorption to BaSO<sub>4</sub> was complete. CaCl<sub>2</sub> solution, in quantities equivalent to the citrate content, was added to the eluate, the pH of which was adjusted to 7.2. After standing for 1 h in a refrigerator at 4° C Ca-citrate crystals separated. According to confirmatory tests these did not adsorb the virus. After sedimentation of the crystalline material by centrifugation a second cycle of adsorption to and elution from BaSO<sub>4</sub> could easily be carried out in the supernatant. The results are shown in Table 1.

It can be seen that in the case of the PR8 strain the results are similar to those of Mizutani. With the other two strains, however, the rate of recovery and the effi-

ency of purification were considerably lower. Mizutani's findings also indicate that the CCA/N value of strain 'Adachi' is five times greater than that of strain 'Setagaya'. Obviously differences concerning adsorption to and elution from BaSO<sub>4</sub> do exist between different strains. The data presented in Table 1, however, reveal that these differences are likely to originate from the different adsorptive capacity of the various strains to BaSO<sub>4</sub>. Thus adsorption to and elution from BaSO<sub>4</sub> show a similar pattern to the adsorption to and elution from Al(PO<sub>4</sub>) used by Miller and Schlesinger<sup>4</sup>. Some damaging effects on the adsorption of the yet unknown ballast substances in the allantoic fluid were also noted. There is no doubt that the peculiarities of the behaviour toward an adsorbent are determined by the surface of the viral particles, and might be changed by its variations.

The N/CCA values of the PR8 strain shown in Table 1, representing the efficiency of the purification process, compare favourably with the corresponding values found by Miller with his purest influenza virus preparations without substantial loss of infectivity. In view of the simplicity, effectiveness and technological advantages of autoclaving the adsorbent and eluent, further investigation of some other factors, such as pH, ionic strength, etc., influencing the adsorption to and elution from BaSO<sub>4</sub> of the various influenza virus strains seems to be justified.

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## CYTOLOGY

### Chloroplast Replication: Evidence for 5-Bromouracil Incorporation and Plastid Mutation in *Euglena gracilis*

THERE is now growing experimental support for the hypothesis that nucleoprotein genetic determinant located outside the nucleus confer photosynthetic activity on plastids. The first clear indication of nucleoprotein involvement in plastid mutation was derived from the action spectrum for ultra-violet inactivation of *Euglena* plastids which showed peaks at 260 and 280 mμ. The ultra-violet sensitive plastid determinant is photoreactive<sup>1</sup>, and evidence for its cytoplasmic nature has been reported<sup>2-3</sup>. Comparison of wild-type and ultra-violet induced mutants of *Euglena* strongly suggests that DNA of different base composition from the nucleus is uniquely associated with the ability to form plastids. Similar findings have been reported for plastids of other algae<sup>4-8</sup> and higher plants<sup>9,10</sup>. The recent observation that cells which incorporate azathymine in place of thymine exhibit increased resistance to ultra-violet inactivation of plastids is consistent with the report that plastids have associated with them a specific DNA and serves as a guide to the rational search for chemical agents that might be expected to induce plastid mutations. By analogy with other nucleoprotein genetic systems, we have postulated that incorporation of base analogues into the genetic material that determines plastids should lead to mutational changes, and that such changes would be reflected in altered plastid structure and function. This communication presents evidence for incorporation of 5-bromouracil into cells of *Euglena gracilis*, and describes conditions for obtaining stable colourless plastid mutants during replication of plastids.

Table 1

Strain		Haemagglutinating units (HU)	Recovery virus (%)	N (mg)	HU/mg N	mg N/HU	Concentration
PR8	1	102-400	—	30.3	3,413	2.9 × 10 <sup>-4</sup>	—
	2	800	0.8	29.5	27.1	3.7 × 10 <sup>-2</sup>	—
	3	61-440	59.5	3.67	16-742	6.0 × 10 <sup>-3</sup>	—
	4	0	—	1.26	0	0	—
	5	92-160	90.0	0.70	131-660	7.6 × 10 <sup>-3</sup>	38.5
Singapore	1	51-200	—	31.8	1,610	6.2 × 10 <sup>-4</sup>	—
	2	3,000	5.9	24.8	129	7.2 × 10 <sup>-2</sup>	—
	3	15-360	29.9	3.79	4,053	2.5 × 10 <sup>-4</sup>	—
	4	600	1.17	1.69	355	2.8 × 10 <sup>-3</sup>	—
	5	11-520	22.6	1.49	7,732	1.3 × 10 <sup>-4</sup>	4.8
Lee	1	12-800	—	36.9	346	2.9 × 10 <sup>-3</sup>	—
	2	1-600	12.5	25.8	62	1.6 × 10 <sup>-2</sup>	—
	3	3-840	30.0	4.5	853	1.2 × 10 <sup>-3</sup>	—
	4	600	4.63	2.44	245.9	4.1 × 10 <sup>-3</sup>	—
	5	5,760	45.1	1.86	3,096	3.2 × 10 <sup>-4</sup>	9.0

1, Crude infected allantoic fluid.

2, Supernatant of No. 1 after adsorption to BaSO<sub>4</sub>.

3, Eluate from BaSO<sub>4</sub>.

4, Supernatant from II adsorption to BaSO<sub>4</sub>.

5, Eluate from II adsorption to BaSO<sub>4</sub>.



eterminants in the presence of 5-bromouracil and sulphanilamide.

The mutagenic effect of base analogues is strongly dependent on the concentration of the natural base, and mutagenesis can be observed only when the synthesis of the natural base is suppressed. For maximum incorporation of 5-bromouracil, cells of *Euglena gracilis* Z were grown in the presence of sulphanilamide in a sugar-containing medium<sup>11</sup> supplemented with a combination of metabolites (Table 1) to satisfy deficiencies induced by sulphanilamide. Preliminary experiments were performed to determine the optimum concentrations of sulphanilamide and bromouracil (Tables 2 and 3). The results indicate that growth is completely suppressed at concentrations more than 30 mg per cent sulphanilamide, and that at lower levels of sulphanilamide, growth could be restored by addition of *p*-aminobenzoic acid. When the concentration of sulphanilamide was lowered to 15 mg per cent, addition of 5-bromouracil partially reversed the growth inhibition, but not as effectively as *p*-aminobenzoic acid. Fig. 1 shows the growth response of *Euglena* to sulphanilamide alone and in combination with 5-bromouracil. It is apparent that the presence of the analogue permits considerably more growth than with sulphanilamide.

Colourless mutants of *Euglena* were first observed when cells grown in darkness with sulphanilamide and 5-bromouracil were plated on a basal medium (not containing either bromouracil or sulphanilamide). Under these conditions the frequency of mutant colonies which appear

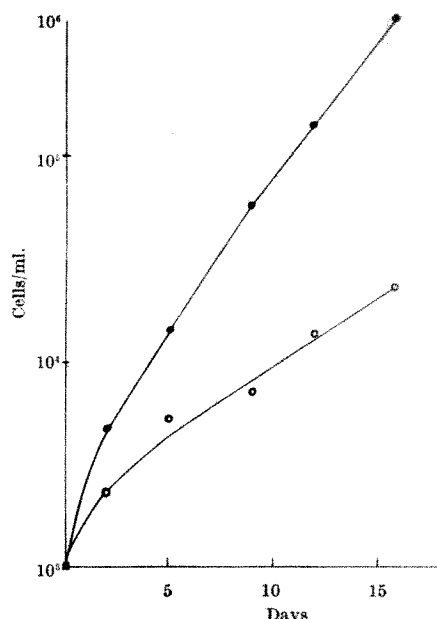


Fig. 1. Growth of *E. gracilis* at 25°-28° C in darkness on complete medium in the presence of 15 mg per cent sulphanilamide (O) and 15 mg per cent sulphanilamide + 200 mg per cent 5-bromouracil (●). Cell counts were determined by means of a haemocytometer.

Table 1. SUPPLEMENT TO BASAL MEDIUM<sup>11</sup>

Compound	Final conc. (%)
DL-Methionine	0.02
L-Histidine	0.02
DL-Serine	0.02
L-Leucine	0.01
DL-Valine	0.01
Adenosine	0.01
Guanosine	0.01
Inosine	0.02
Uracil	0.02
Calcium pantothenate	0.1 mg
Pyridoxine	0.1 mg

The basal medium supplemented as above constitutes the complete medium.

Table 2. GROWTH INHIBITION OF *Euglena gracilis* BY SULPHANILAMIDE

Additions to basal medium	No addition	Optical density <i>p</i> -Aminobenzoic acid 10 µg %
1. No addition	1.4	2.0
2. Sulphanilamide 3 mg %	0.2	1.0
3. Sulphanilamide 10 mg %	0.1	0.2
4. Sulphanilamide 30 mg %	0.0	0.0

Cell growth measured as optical density was determined with a Bausch and Lomb Spectronic 20 colorimeter at 550 mµ after 7 days in continuous light at 25°-28° C.

Table 3. EFFECT OF 5-BROMOURACIL AND SULPHANILAMIDE ON THE GROWTH OF *Euglena gracilis*

Additions to complete medium	No addition	Sulphanilamide 15 mg %
1. No addition	2.5	0.1
2. 5-Bromouracil 50 mg %	4.9	0.1
3. 5-Bromouracil 200 mg %	2.4	0.6
4. <i>p</i> -Aminobenzoic acid 10 µg %	3.0	2.1

Cell growth measured as optical density was determined with a Beckman spectrophotometer model B at 550 mµ after 14 days in continuous light at 25°-28° C. The inoculum consisted of 3-day-old light-grown cells (100 cells/ml) grown in basal medium.

Table 4. EFFECT OF 5-BROMOURACIL ON THE FREQUENCY OF STABLE COLOURLESS MUTANTS OF *Euglena gracilis*

Additions to complete medium	Average No. of mutant colonies (%)	Range (%)	Total No. of colonies scored
No addition	0.7	0-3.4	1,175
5-Bromouracil 200 mg %	0.7	0-2.7	772
Sulphanilamide 15 mg %	1.4	0-5.7	755
5-Bromouracil 200 mg % + sulphanilamide 15 mg %	8.3	3.2-15.0	568

Experimental conditions: the inoculum consisted of 3-day-old light-grown cells (1,000 cells/ml.). After 14 days incubation at 25°-28° C in darkness approximately 100 cells in 0.1 ml. were spread on basal medium + 2.0 per cent agar plates using the overlay method described by Lyman *et al.*<sup>1</sup>.

as white or yellow-white, when plated, markedly increases above the spontaneous level. Table 4 summarizes the results of several experiments designed to determine the contribution of 5-bromouracil and sulphanilamide to plastid mutagenesis. The frequency of plastid mutants observed with either 5-bromouracil or sulphanilamide falls within the range of spontaneous mutation; however, in the presence of both the analogue and sulphanilamide, the mutant yield was considerably above the spontaneous level. A large number of these plastid mutants have been isolated, replated, and have thus far maintained their mutant identity through numerous sub-cultures. Analysis of mutant production in actively growing cells as compared with cells in resting medium provides evidence that plastid mutagenesis is strictly dependent on replication in the presence of 5-bromouracil and sulphanilamide; the mutant frequency of resting cells exposed to otherwise mutagenic conditions appears to fall within the spontaneous range.

The reversal of sulphanilamide inhibition by 5-bromouracil and the increased frequency of plastid mutants produced from cells grown in the presence of 5-bromouracil and sulphanilamide can be taken as evidence of incorporation of the analogue. Appearance of colourless plastid mutants (presumably lacking photosynthetic pigments such as chlorophylls) that do not revert to the normal green condition when grown in the absence of sulphanilamide or 5-bromouracil provides experimental support for their genetic stability. The observation of plastid mutation associated with 5-bromouracil incorporation is consistent with the hypothesis that plastid replication is under the control of a DNA that is susceptible to genetic alteration by base analogues.

The mutagenic effect of base analogues such as 5-bromouracil has been attributed to base pair transitions<sup>12</sup>. According to this view, incorporation of the analogue *per se* is insufficient to cause mutagenesis, and requires replication of the DNA strand containing the 5-bromouracil<sup>13</sup>. That induction of plastid mutagenesis is dependent on growth in the presence of 5-bromouracil and sulphanilamide argues in favour of a plastid determinant that must be replicated for the mutagenic event to occur, and suggests that the mechanism of base analogue mutagenesis of the plastid genome may parallel that proposed for other DNA-containing genetic systems.

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## FORESTRY

### Pathways of Water Movement in Young Conifers

EXPERIMENTS on the uptake of dyes by conifers have suggested that water is transported vertically in the earlywood of the annual ring<sup>1</sup>. Few systematic investigations have been made, however, to determine how this pattern of water movement is achieved, whether there is any lateral transport between the earlywood regions of different annual rings or, indeed, whether this pattern is constant under different environmental conditions.

A series of experiments has been made in the greenhouse on young plants of Corsican pine (*Pinus nigra* var. *calabrica* Loud.), Japanese larch (*Larix leptolepis* Murr.), Norway spruce (*Picea abies* L. Karst.) and Scots pine (*Pinus sylvestris* L.), up to 4 years of age, to determine the pattern of water transport in roots, stems and twigs under different environmental conditions. The effective water-conducting tissues were identified by immersing whole root systems or individual roots of intact plants in aqueous solutions of various dyes, including acid fuchsin, reduced basic fuchsin and safranin. Experiments were made on actively transpiring plants and on plants in which transpiration was reduced by enclosing the shoots in perforated polythene bags.

After immersion in the dye for periods of 1-5 days, the plants were dissected and cross- and longitudinal-sections made at intervals along the roots, main stem and major branches: these sections were rapidly washed in 95 per cent ethyl alcohol, washed twice in absolute alcohol and finally mounted in Canada balsam.

For detailed investigations acid fuchsin proved unsuitable since it did not adequately stain the xylem walls and often diffused out after the sections were cut. In contrast, 0.1 per cent basic fuchsin that had been almost decolorized with 10 per cent sodium metabisulphite<sup>2</sup> stained the xylem walls intensely and did not usually diffuse out. When freshly cut sections of stems that had not previously been treated with the dye were immersed in reduced basic fuchsin, both the xylem and phloem showed a marked affinity for the dye. The observation that the intensity of staining of such sections in weak solutions of the dye (0.1-0.001 per cent) appeared to be a function of the length of exposure to the dye offered, in addition, a semi-quantitative measure of dye uptake. For example, examination of sections of branches at various heights on the main stem showed a higher intensity of staining in the xylem of lower whorls than of upper ones, indicating, therefore, a greater velocity of flow through the lower

branches. This may have been due to the very much larger number of needles on the lower whorls.

In young plants with up to four annual rings, all or most of these rings were concerned with water transport, and generally this occurred mainly in the earlywood of each ring. In short-term experiments the latewood was commonly not stained at all. This pattern of upward movement of water along a series of concentric earlywood circles confirms the findings of Harris<sup>1</sup> based on the uptake of safranin by seedlings of *Pseudotsuga taxifolia*, *Pinus nigra* and *Pinus radiata*. Our investigations suggest, however, that there is no sharp differentiation in the pathway of water transport within an annual ring. Observations of a grading off in dye intensity from the larger diameter, earlywood cells to the smaller diameter, latewood cells indicate a gradual decrease in the rate of translocation as the component tracheids decrease in size within the annual ring. Under conditions of low transpiration, less of the annual ring was involved in water transport than when transpiration was high. That smaller elements may be brought into use for the conduction of water under conditions of high transpiration is supported by Scholander's<sup>3</sup> observations that upward water movement in trees follows a path of least resistance; when larger elements were blocked by air, water moved through smaller ones.

In most of our experiments, the medullary rays were very heavily stained with dye, suggesting a highly efficient radial conduction of water; in some cases the dye was seen throughout most of the ray extending across the latewood of the outermost annual ring into the phloem ray cells. In contrast, tangential movement of water from the rays into the latewood of the annual ring appeared to be limited by restricted transport within the latter. Staining usually was observed only in the single row of latewood tracheids adjacent to the ray.

Examination of cross and longitudinal sections of treated plants below the root collar revealed that the separation of the vertical water path into concentric circles, such as was observed in the stem, had already occurred below ground; in other words water was conducted from the root in more than one annual ring.

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## OCEANOGRAPHY

### Slicks associated with *Trichodesmium* Blooms in the Sargasso Sea

NATURAL slicks are apparently formed by the rippling damping action of monomolecular layers of organic matter which have an effective strength of only a few dynes/cm or less and are visible against the adjacent rippled water only in relatively calm weather<sup>1-4</sup>. One of the few correlations of slick with source was made by Grantved<sup>5</sup> when an oily slick of several square miles in the North Sea was found to contain a high oily, senescent and dense patch of the diatom *Coscinodiscus concinnus*. The existence of blue-green algae of the genus *Trichodesmium* in the open ocean<sup>6</sup>, and its wide distribution<sup>7</sup> and ability to discolor

water as well as some of its ecology<sup>9,10</sup> has long been recognized. The purpose of this communication is to describe blooms in which well-defined and surfactant slicks were associated with dense patches of *Trichodesmium* species.

During R.V. *Trident* cruise 10 (September 7-20, 1963) in the Sargasso Sea to continue an investigation<sup>11</sup> of the pelagic Gulf weeds, two marked areas of surface slicks were observed. The water, both rippled and slick, lacked the usual clarity of tropical oceanic water. The presence of brownish-red specks appeared more prevalent in the slick areas. An examination of bucket samples revealed the presence of two species of *Trichodesmium* which were more abundant in the slick areas than in the adjacent rippled waters. The dominant organism was *Trichodesmium erythraeum* Ehrenberg ex Gomont (*Skujaella erythraea* (Ehrenberg) De Toni); *Trichodesmium thiebautii* Gomont (*Skujaella thiebautii* (Gomont) De Toni) was also present.

The slicks ranged in size from about 75 m ovoid patches (area 1: 30°19' N, 60°08' W) to long windrows approximately 0.25 km by 3 km (area 2: 33°19' N, 63°05' W). These slicks were observed over a distance of some 200 km in both areas. Since a series of spreading oils of the Adam's variety<sup>12</sup> prepared and calibrated by the Surface Chemistry Branch of the U.S. Naval Research Laboratory, Washington, D.C., were aboard, an attempt was made to determine if the slicks were associated with surfactant activity. Some vials dipped in the calibrated oils were thrown ahead of the bow and the equilibrium spreading pressures of the mid-portion of rippled and slick areas were noted. Bucket samples were taken from the other side of the ship and 375-ml. aliquots were used to determine the algal and bacterial content. Three samples of each water type in area 1 and six samples of each type in area 2 were examined. Due to the variable number of filaments in each *Trichodesmium* bundle (usually 12-23 with an extreme of 80) the bundles were teased and the number of filaments were counted. Bacterial counts were obtained from surface inoculated sea water agar plates incubated at 27° C for three days. The results are given in Table 1. There was a small but measurable increase in the equilibrium spreading pressure in the slick areas. The apparent concentration of *Trichodesmium* in the slick areas was confirmed.

Table 1. COMPARISON OF EQUILIBRIUM SPREADING PRESSURE (E.S.P.), *Trichodesmium*, AND BACTERIAL POPULATIONS IN RIPPLED AND SLICK SURFACE WATERS OF BLOOM AREAS OF THE SARGASSO SEA\*

Area	Water surface	E.S.P. dynes/cm	<i>Trichodesmium</i> filaments No./375 ml.	Bacteria No./ml.
1	Rippled	<1	11	5
		<1	15	
		<1	19	
	Slick	1-2	55	59
		1-2	66	
		1-2	76	
2	Rippled	<1, 1-2	0, 0	20
		<1, 1-2	0, 0	
		<1, 1-2	0, 18	
	Slick	<1, 2-3-3	7, 18	20
		1-2, 2-3-3	24, 68	
		1-2, 3-3-4-5	69, 172	

\* Mean of three observations per water type in area 1 and of six in area 2.

Although the bacterial counts were higher in the slicks of area 1 there was neither marked enhancement nor inhibition of the natural population. Since blue-greens have been noted for their inhibitory activities<sup>13,14</sup> a plankton net concentrate from area 1 (with gross zooplankton removed, filtered on a clean towel to give approximately 0.5 g algal cells, and ground with 1 ml. of sterile sea water) was filter pad assayed against a strain each of *Achromobacter*, *Pseudomonas*, *Vibrio* and *Sarcina*. There was only minimal activity against the *Vibrio*, which is the dominant genus found associated with algal material. Agar plate enzyme assays on the same material for lipase, gelatinase and amylase showed marked activity for the latter. The results are given in Table 2. This activity against a polysaccharide is of interest due to the known

Table 2. THE ENZYMATIC AND ANTIBACTERIAL ACTIVITIES OF A *Trichodesmium* CELL CONCENTRATE (1:3 DILUTION W/V)

Enzyme activity	Antibacterial activity
Zone mm	Zone mm
Lipase ('Tween-80')	<i>Sarcina</i>
Gelatinase	<i>Pseudomonas</i>
Amylase	<i>Achromobacter</i>
	<i>Vibrio</i>
	1-0

ability of blue-greens to excrete appreciable quantities of these substances<sup>15,16</sup>. It seems possible that the slicks associated with *Trichodesmium* may be due at least in part to the surfactant property of excreted carbohydrates.

The occurrence of large blooms of *Trichodesmium* in tropical and subtropical oceanic waters and the ease of its detection and handling may make it an ideal test organism for examining the algal excretion of organic compounds and their role in surface slicks.

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## STATISTICS

### An Epidemic Process in an Open Population

In general an epidemic process can be characterized as a time-dependent process of transition by the members of a population, where the state transitions are caused by exposure to some influence called infectious material. The members of the population can belong to one of three basic states at a given point in time: (a) *Infective*, those members of the population who are host to the infectious material; (b) *Susceptible*, those members of the population who can become infectives given effective contact with infectious material; (c) *Removal*, those members of the population who have been removed from circulation for one of a variety of reasons such as death, immunity, hospitalization, etc.

These latter members may have been either susceptibles or infectives at the time of their removal.

As the process develops, an individual is exposed to infectious material either by direct contact with an infective or through some intermediary host or vector. The exposed person may either be resistant to the incoming organism, in which case the organism is rejected, or may be infected by it, in which case the invading organism proceeds on a course of development. The time-interval in which the development takes place is called the latency period, that is, the interval of time necessary for a susceptible to be transformed into an infective. In other words the latency period is the time-interval between the receipt of infectious material by a susceptible and the time at which he is in a position to transmit infectious



material to another susceptible, thus repeating the process.

There has been extensive development of mathematical representations of epidemic processes<sup>1,2</sup>. Such a process may be treated deterministically, in which case it can be represented by a system of differential equations; or it may be treated stochastically, in which case the process is represented by a finite state Markov process of either discrete or continuous parameter depending on the physical situation. (The discrete parameter is applicable where the latency period is constant, in which case infectives occur in generations.)

Mathematical treatments of epidemic processes, both deterministic and stochastic, which have appeared in the literature have usually dealt with processes involving closed populations; that is, a population  $N$  which remains constant throughout the development of the process.

The purpose of this note is to point out a property of an epidemic process in which the population is open; that is, in which  $N$  is not constant. In such a process new supplies of susceptibles and infectives are introduced into the population as the process proceeds on its course of development.

Consider a population  $N$ , consisting of  $S$  susceptibles,  $I$  infectives, and  $R$  removals, and in which the infectious material is transferred by direct contact.  $N$ ,  $S$ ,  $I$ , and  $R$  are all functions of time  $t$ . Let  $\beta$  be the rate of infection in  $N$  and  $\gamma$  the rate of removal of both susceptibles and infectives. (Although this assumption may not be very realistic in describing the spread of infectious disease, it may be realistic in the treatment of other physical situations as epidemic processes, particularly the information spread<sup>3</sup>.) Let  $\mu$  be the rate at which new susceptibles are introduced into  $N$  and let  $\nu$  be the rate at which new infectives are introduced.

If homogeneous mixing among members of the population and a zero latency period are assumed, the process can be described deterministically by the following system of differential equations:

$$\begin{aligned} \frac{dS}{dt} &= -\beta SI - \gamma S + \mu \\ (E) \quad \frac{dI}{dt} &= \beta SI - \gamma I + \nu \\ \frac{dR}{dt} &= \gamma(S + I) \end{aligned}$$

A threshold density of susceptibles can be obtained if initial conditions of a single infective introduced into the population at time  $t_0$  is assumed. Hence:

$$N_0 = S_0 + 1$$

That is, the initial population  $N_0$  consists of  $S_0$  susceptibles, one infective, and zero removals.

For an epidemic to develop from time  $t_0$  the rate of change of the number of infectives must be positive. Hence:

$$\begin{aligned} \beta S_0 I &> \gamma I - \nu \\ \text{or} \\ S_0 &> \frac{\gamma - \nu}{\beta} = \rho \end{aligned}$$

Thus an epidemic can develop from time  $t_0$  only if the number of initial susceptibles  $S_0$  exceeds the threshold  $\rho$ .

By the existence and uniqueness theorems for systems of ordinary differential equations<sup>4</sup>, a solution to the system (E) exists and is unique.

$R$  can be obtained by direct integration as follows:

$$\begin{aligned} \text{Since } N &= S + I + R \\ \frac{dR}{dt} &= \gamma(N - R) \end{aligned}$$

$$\text{and } \frac{dR}{dN} = \lambda(N - R) \quad \text{where } \lambda = \frac{\gamma}{\mu + \nu}$$

$$\text{Then } R = \frac{(\lambda N - e^{\lambda N}) + e^{\lambda N_0}(1 - \lambda N_0)}{\lambda e^{\lambda N}}$$

$$\text{or } R = \frac{(\gamma t + \lambda N_0 e^{\gamma t}) + (1 - \lambda N_0)}{\lambda e^{\gamma t}}$$

$$\text{and } S + I = \frac{e^{\gamma t} + \lambda N_0 - 1}{\lambda e^{\gamma t}}$$

This process will reach its peak at the point in time for which the rate of change of susceptibles and infectives is maximal.

$$\text{Hence: } \frac{d^2(S + I)}{dt^2} = 0$$

in order for the process to peak.

Now

$$\frac{d^2(S + I)}{dt^2} = \frac{[\gamma N_0 - (\mu + \nu)]\gamma}{e^{\gamma t}}$$

Hence:

$$\frac{d^2}{dt^2}(S + I) = 0$$

either for  $t = \infty$  or when  $N_0 = \frac{\mu + \nu}{\gamma}$

but the  $\lim_{t \rightarrow \infty} (S + I) = \frac{\mu + \nu}{\gamma}$

Thus if the process attains a peak and stabilizes itself the number of susceptibles and infectives at its initial point will equal the number of susceptibles and infective at its terminal point.

In fact from the system of equations (E) it can easily be seen that in this event:

$$S + I = \frac{\mu + \nu}{\gamma}$$

for all values of  $t$ .

Hence the process (E) will reach a maximum in finite time if, and only if, the sum of susceptibles and infective is constant.

In the general case, that is, where  $\delta \neq \gamma$  is the rate of removal of susceptibles, it follows directly from the system of differential equations that if

$$\frac{d^2(S + I)}{dt^2} = 0$$

then  $\frac{dR}{dt} = \delta S + \gamma I$  equals a constant. But if  $\delta S + \gamma$

equals a constant then  $\frac{d(S + I)}{dt}$  equals a constant

Hence  $\frac{d^2(S + I)}{dt^2} = 0$  and

**Theorem 1.** The general process (E) will reach a maximum in finite time if, and only if, the rate of change of removals is constant.

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<sup>3</sup> Goffman, W., and Newill, V. A., *Nature*, **204**, 225 (1964).

<sup>4</sup> Hurewicz, W., *Lectures on Ordinary Differential Equations* (Mass. Inst. Tech. Press, 1958).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, February 22

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. Emrys Jones: "The London Atlas".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. A. Saxton, Mr. J. A. Lane, Mr. R. W. Meadows and Mr. P. A. Matthews: "Layer Structure of the Troposphere".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. S. Kaplan (Stanford): "Unfolding Concepts of Mechanisms in Leukemogenesis and Carcinogenesis".\*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Transport in the Year A.D. 2000".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUBGROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. R. Calvert: "Curing Systems".

## Tuesday, February 23

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. L. L. Jones and Mr. G. D. Base: "Test of a One-Twelfth scale Model of the Dome Shell Roof for Smithfield Poultry Market"; and Mr. P. Ahm and Mr. E. J. Perry: "The Design of the Dome Shell Roof for Smithfield Poultry Market".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "What is systems Engineering?" opened by Mr. K. L. Smith.

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. J. Avery: "Automatic Instrument manufacture".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. I. M. Glynn: "The Transport of Sodium and Potassium Across Cell Membranes". (Twelfth of sixteen lectures on "The Scientific Basis of medicine" organized by the British Postgraduate Medical Federation.)\*

INSTITUTION OF MECHANICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6.30 p.m.—Dr. C. Wright: "Design and Development of Modern Guns".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. R. A. Shaw: "Some aspects of Non-Metallic Inorganic Polymers".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Norwood Technical College, Knight's Hill, Norwood, London, S.E.27), at 7 p.m.—Lt.-Col. B. D. Haw: "Explosives".

## Wednesday, February 24

ROYAL SOCIETY OF MEDICINE (joint meeting with the Society for Endocrinology, at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 10 a.m.—Meeting on "Salt and Water".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 2 p.m.—Dr. H. E. M. Kay: "Possibilities of Transplantation of Bone Marrow".\*

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY AND FOOD GROUPS (at the School of Pharmacy, University of London, Brunswick Square, London, W.C.1), at 2.30 p.m.—Meeting on "Modern Trends in Food Preservation".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. M. Kaufman: "New Plastics and Their Application".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. M. Cowan and Mr. L. L. Preston: "Parallel Operation of Transformers with On-Load Tap Changes and Negative-Reactance-Compounding Control".

UNIVERSITY OF LONDON (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 5.30 p.m.—Dr. S. R. Pele: "Electron Microscope auto-Radiography". (Second of three lectures on "The Scientific Basis of entistry" organized by the British Postgraduate Medical Federation.)\*

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 6 p.m.—Prof. W. D. Robertson (Yale University): "Design Criteria for the Development of New Alloys".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Meeting on "A Low Cost Video Tape Recorder for Professional Applications".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. H. F. Padbury: "Oxygen Steelmaking processes: Consideration of Some Operational and Engineering Aspects" (first John Player Lecture).

INSTITUTION OF ENGINEERING INSPECTION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.15 p.m.—Mr. E. Newton: "Investigation of Aircraft Accidents—The Engineering and Inspection aspects".

OFFICE OF HEALTH ECONOMICS (at the Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1), at 8.30 p.m.—Dr. J. Yule Bogue: "The International Pattern of Pharmaceutical Research".\*

## Thursday, February 25

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 a.m.—Mr. D. Brennan, Mr. M. J. Graham, Mr. F. H. Hayes and Mr. D. O. Hayward: "The Energy and Configuration of the Adsorbed Layer with special reference to the Adsorption of Krypton, Xenon, Carbon Monoxide and Carbon Dioxide on Metals".

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on

"The Hydrological Aspects of the Work of the Water Resources Board" introduced by Mr. N. A. F. Rowntree.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. I. S. Longmuir: "Tissue Oxygen Requirements". (Thirteenth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

CHEMICAL SOCIETY (in the Edward Lewis Lecture Theatre, Middlesex Hospital Medical School, London, W.1), at 6 p.m.—Prof. F. Lynen: "Biosynthesis and Function of Biotin-Enzymes" (Centenary Lecture).

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Borough Polytechnic Chemical Society), at the Borough Polytechnic, Borough Road, London, S.E.1, at 7 p.m.—Prof. C. H. Bamford, F.R.S.: "Some New Developments in Polymerization".

## Thursday, February 25—Friday, February 26

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Symposium on "Gyros".

## Friday, February 26

BIOCHEMICAL SOCIETY (joint meeting with the Medical Research Society, at the London Hospital Medical College, London, E.1), at 9.40 a.m.—Scientific Papers followed by a Colloquium on "Structure and Clinical Significance of Immune Globulins".

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2 p.m.—Geophysical Discussion on "The Magnetosphere".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 4 p.m.—Dr. A. Goldberg: "Reassessment of the Diseases of Porphyrin Metabolism".\*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Logarithmic h.f. Aerials" opened by Mr. P. A. C. Morris, Mr. M. F. Radford, Mr. M. Telford and Mr. D. E. Watt-Carter.

ROYAL INSTITUTE (at 21 Albermarle Street, London, W.1), at 9 p.m.—Prof. R. L. F. Boyd: "The Lure of the Moon".

## Saturday, February 27

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. John F. Potter: "300 Million Years of London's Past".\*

## Monday, March 1

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (in the Main Lecture Theatre, Department of Electrical Engineering, Exhibition Road, London, S.W.7), at 5.30 p.m.—Mr. L. Hix: "The Application of Automation to Railways".\*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Control of Room Storage Heaters" opened by Mr. B. B. Rowson.

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. Stewartson: "The Boundary Layer".\*

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Professional Engineer and Man-Management" opened by Sir Wm. Carron.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Microbiology Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. K. Mills: "Research and Brewing" (Jubilee Memorial Lecture).

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Audrey Butt: "Peoples of the Forest and the Rivers".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (preferably with experience in the field of mammalian biochemistry) in BIOCHEMISTRY—The Registrar, Room (X), The University, Hull (February 24).

LECTURER or ASSISTANT LECTURER Grade B (graduate of a British university or with equivalent qualifications, and preferably some teaching experience) in MATHEMATICS—The Principal, Norwood Technical College, Knight's Hill, London, S.E.27 (February 27).

LECTURER or ASSISTANT LECTURER (with particular interests in palaeontology and stratigraphy, and research or other professional postgraduate experience) in GEOLOGY—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting ref. 395/2 (February 27).

RESEARCH ASSISTANT (graduate in medicine or biology and preferably experience in tissue culture) in the DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/442/N (February 27).

ASSISTANT LECTURER and a COLLEGE LECTURER (with the degree of M.B. and Ph.D. or equivalent) in the DEPARTMENT OF PHYSIOLOGY—The Secretary and Bursar, University College, Dublin, Republic of Ireland (February 28).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS in BIOCHEMISTRY, CHEMISTRY, ENGINEERING, OCEANOGRAPHY or PHYSICS (experimental or theoretical)—The Deputy Secretary, The University, Southampton (February 28).

LECTURER or ASSISTANT LECTURER in MATHEMATICS—The Secretary, The University, Edinburgh (March 1).

SENIOR LECTURER in the DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leicester (March 6).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (March 8).

LECTURER with a good honours degree and/or a higher degree in sociology or a cognate discipline, and preferably a knowledge of survey techniques and statistics) in SOCIOLOGY in the DEPARTMENT OF EDUCATION—The Registrar, University College of North Wales, Bangor, North Wales (March 8).

LECTURER in ORGANIC CHEMISTRY—The Registrar, The University, Leicester (March 10).

**CHAIR OF MATHEMATICS (The M'Laurin Chair)**—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (March 15).

**LECTURER or ASSISTANT LECTURER in EXPERIMENTAL PSYCHOLOGY**—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (March 19).

**SENIOR LECTURER or LECTURER in PHYSICAL and INORGANIC or ANALYTICAL CHEMISTRY** at the University of Natal, Durban—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, March 22).

**ASSISTANT LECTURER or DEMONSTRATOR in BOTANY**—The Secretary, Wye College (University of London), near Ashford, Kent (March 31).

**PROFESSOR of ZOOLOGY** at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, March 31).

**RESEARCH ASSISTANT** (graduate in botany or agricultural botany) in the DEPARTMENT of AGRICULTURE, to work on the micro-ecology of fungal infections of leaves—The Registrar, The University, Leeds, 2 (March 31).

**IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS** (preferably with the degree of Ph.D. or equivalent research experience and below the age of 30) in BIOCHEMISTRY, CHEMISTRY, CHEMOTHERAPY, ENGINEERING, METALLURGY, PHARMACOLOGY or PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 1).

**LECTURER or ASSISTANT LECTURER in the DEPARTMENT of PHYSICS**—The Registrar, The University, Leeds, 2 (April 1).

**ASSISTANT LECTURER or a LECTURER in ORGANIC CHEMISTRY**—The Registrar, University College of Swansea, Singleton Park, Swansea (April 5).

**LECTURER or ASSISTANT LECTURER in the DEPARTMENT of METALLURGY**—The Registrar, The University, Leeds, 2 (April 20).

**LECTURER** (preferably with biochemical interests) in INSECT PHYSIOLOGY in the DEPARTMENT of ZOOLOGY and APPLIED ENTOMOLOGY—The Professor's Secretary, Department of Zoology, Imperial College of Science and Technology, London, S.W.7 (April 30).

**ASSISTANT LECTURERS** (preferably with some experience in the field of natural products) in ORGANIC CHEMISTRY—The Clerk to the Council, School of Pharmacy, University of London, 29-39 Brunswick Square, London, W.C.1.

**ASSISTANT PROFESSOR in the DEPARTMENT of BACTERIOLOGY**, to teach both undergraduate and postgraduate students and to initiate and develop an active research programme in either a medical or a non-medical field—The Chairman, Department of Bacteriology, University of Alberta, Edmonton, Alberta, Canada.

**ASSISTANT PROFESSORS** (with a Ph.D. or equivalent, and preferably with research interests in biophysics, magnetic resonance, cosmic radiation, upper atmosphere physics, or theoretical physics) in the DEPARTMENT of PHYSICS—Prof. C. E. Chalice, Department of Physics, University of Alberta, Calgary, Alberta, Canada.

**GRADUATE RESEARCH ASSISTANT** at the Regional Rheumatism Research Centre to work with medical staff in a well-equipped laboratory—The Administrative Officer, Stoke Mandeville Hospital, Aylesbury, Bucks.

**MATHEMATICIAN/SCIENCE GRADUATE** (preferably with experience of programming and an interest in biology) for a research project to identify bacteria using computer techniques—The Director, Central Public Health Laboratory, Colindale Avenue, London, N.W.9.

**RESEARCH DEMONSTRATORS** (with a good degree in botany or horticulture) in the DEPARTMENT of HORTICULTURE, to assist with practical classes in horticulture or plant physiology and carry out research in one of those subjects—Prof. O. V. S. Heath, F.R.S., University of Reading Horticulture Research Laboratories, Shinfield Grange, Reading, Berkshire.

**RESEARCH FELLOW** (physicist or inorganic chemist with the degree of Ph.D. or several years relevant industrial experience) in the DEPARTMENT of PHYSICS, to join a group working on the electrical properties of aluminium nitride and related compounds—The Registrar, University College of North Wales, Bangor, North Wales.

**SENIOR BIOCHEMIST** (preferably with honours B.Sc. and several years experience in hospital laboratories) in the METABOLIC UNIT—The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

**TEMPORARY LECTURER or TEMPORARY ASSISTANT LECTURER** (pure or applied mathematician) in the DEPARTMENT of MATHEMATICS (may be a permanent appointment later)—The Secretary, University College, Gower Street, London, W.C.1.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

**University of Cambridge.** Titles of Dissertations Approved for the Ph.D., M.Sc., and M.Litt. Degrees in the University of Cambridge during the Academic Year 1963-1964. Pp. 27. (Cambridge: Board of Research Studies, The University, 1964.) [3011]

**University of Oxford.** Report of the Committee appointed by the Hebdomadal Council to make Detailed Proposals for Carrying Out the Policy Contained in the Further Report on the Closer Integration of University Teaching and Research with the College System. Pp. 10. (Supplement No. 1 to the *University Gazette*, November 1964.) (Oxford: The University, 1964.) [3011]

**Planning.** Vol. 30, No. 483 (November, 1964): Firms and Their Exports. Pp. 279-318. (London: Political and Economic Planning, 1964.) 6s. [3011]

**Experiment in Higher Education.** By Sir John Fulton. (The Tavistock Lecture 1963, delivered at University College, London, 4th December 1963.) Pp. 16. (London: Tavistock Publications, 1964.) 3s. 6d. net. [3011]

**London County Council.** Public Health Department: Scientific Branch. Annual Report of the Scientific Adviser, 1963. Pp. 84+6 photographs. (London: London County Council, 1964.) [3011]

**Philosophical Transactions of the Royal Society of London.** Series A: Mathematical and Physical Sciences. No. 1076, Vol. 257 (19 November, 1964): The Thermodynamic Properties of Solid and Fluid Helium-3 and Helium-4 Above 3 K at High Densities. By J. S. Dugdale and J. P. Franck. Pp. 1-29. 10s. 6d.; 1.55 dollars. Series B: Biological Sciences. No. 743, Vol. 248 (26 November, 1964): The Foetal Membranes and Placenta of the African Elephant (*Loxodonta africana*). By Prof. E. C. Amoroso and Dr. J. S. Perry. The Structure and Development of the Reproductive Organs of the Female African Elephant. By Dr. J. S. Perry. Pp. 1-51+plates 1-19. 45s.; 6.75 dollars. No. 745, Vol. 248 (26 November, 1964): The Late-Glacial and Post-Glacial Escarpment near Brook, Kent. By M. P. Kerney, E. H. Brown and T. J. Chandler. With Appendixes by J. N. Carroek, Caniffa A.

Lambert, J. F. Levy and A. P. Millman. Pp. 135-304+plates 19-22. 32s. 4.80 dollars. (London: The Royal Society, 1964.) [301]  
Science and Technology Bill. Pp. ii+12. 1s. 6d. net. Machinery (Government Bill. Pp. ii+7. 1s. 3d. net. (London: H.M. Stationer Office, 1964.) [301]

### Other Countries

**Travaux et Mémoires du Bureau International des Poids et Mesures.** Tome 22, Fascicule 2: La Mesure des Fils Géodésiques au Bureau International des Poids et Mesures. Par A. Bonhoure. Pp. 61. (Paris: Gauthier Villars et Cie., 1964.) [301]

**Effective Use of the Science Citation Index:** a Programmed Text. Pp. 52 (Philadelphia: Institute for Scientific Information, 1964.) [301]

**The University of the State of New York—The State Education Department.** Educational Leaflet No. 16: Bird Art in Science—The Growth of Tradition. By R. L. Scheffel. Pp. vi+30. (Albany, N.Y.: New York State Museum and Science Service, Education Building, 1964.) 50 cents. [301]

**Institut for Atomenergi:** Kjeller Research Establishment. Kjeller Report No. 74: New Type of Safety Amplifiers for Reactors. By Eina Jakobsen. Pp. iii+7+5 figures (Kjeller, Norway: Institutt for Atomenergi Kjeller Research Establishment, 1964.) [301]

**International Pacific Salmon Fisheries Commission.** Bulletin No. 15: The Migration and Exploitation of Pink Salmon Runs in and adjacent to the Fraser River Convention Area in 1959. By E. H. Vernon, A. S. Hourston and G. A. Holland. Pp. 296. (New Westminster, B.C.: International Pacific Salmon Fisheries Commission, 1964.) [3011]

**Australia: Commonwealth Scientific and Industrial Research Organization CSIRO Sixteenth Annual Report, 1963-64.** Pp. 187. Annual Report of the Division of Dairy Research, 1963-64. Pp. 26. Annual Report of the Division of Forest Products, 1963-64. Pp. 65. Annual Report of the Chemical Research Laboratories, 1963-64. Pp. 123. Annual Report of the Division of Tribophysics, 1963-64. Pp. 17. Annual Report of the Fodder Conservation Section, 1963-64. Pp. 9. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1964.) [3011]

**World Meteorological Organization, and Anti-Locust Research Centre.** Technical Note No. 54: Meteorology and the Migration of Desert Locusts. Applications of Synoptic Meteorology in Locust Control. By R. C. Rainey. Pp. 115. (WMO-No. 138. TP. 64.) (Geneva: World Meteorological Organization; London: Anti-Locust Research Centre, 1964.) 25 Sw. francs; 42s. [3011]

**British Guiana: Geological Survey Department.** Report on the Geological Survey Department for the year 1961. Pp. 46. 1 dollar (W.I.). Bulletin No. 32: Bibliography of the Geology and Mining of British Guiana. By C. G. Dixon and H. K. George. Pp. iv+87. 2.40 dollars (W.I.). Bulletin No. 34: Bauxites and Laterites of British Guiana. By Dr. D. Blackley. Pp. 156 (21 plates). 6 dollars (W.I.). Bulletin No. 35: The Geology of the Bartica Assemblage. By Dr. R. T. Cannon. Pp. 83 (12 plates). 4.80 dollars (W.I.). (Georgetown, British Guiana: Geological Survey Department, 1964.) [3011]

**Commonwealth of Australia.** Department of National Development: Bureau of Mineral Resources, Geology and Geophysics. Geological Map of South Alligator River Area, Northern Territory. Geological Maps—1 Mile Geological Series. Sheet 68, Zone 4: Mount Hayward, Northern Territory. Sheet 69: Zone 4: Tipperary, Northern Territory. 1:250,000 Geological Series—Explanatory Notes. Sheet G/50-A, Australian National Grid: Glenburgh, W.A. Compiled by M. A. Condon. Pp. 22+map. Sheet SE/53-2, Australian National Grid: Tanumbirini, N.T. Compiled by A. G. L. Paine. Pp. 15+map. Sheet SE/53-8, Australian National Grid: Calvert Hills, N.T. Compiled by H. G. Roberts, J. M. Rhodes and K. R. Yates. Sheet E/53-12, Australian National Grid: Mount Drummond, N.T. Compiled by J. W. Smith and H. G. Roberts. Pp. 17+map. (Canberra: Bureau of Mineral Resources, Geology and Geophysics, 1963 and 1964.) [3011]

**International Council for Building Research, Studies and Documentation.** C.I.B. Report No. 3: A Master List of Properties for Building Materials and Products. (Prepared by C.I.B. Working Commission W.31.) Pp. 20. (Rotterdam: General Secretariat, C.I.B., P.O. Box 299, 1964.) [3011]

**South Australia.** Institute of Medical and Veterinary Science—25th Annual Report of the Council, July 1962—June 1963. Pp. 74. (Adelaide: Institute of Medical and Veterinary Science, 1964.) [3011]

**Bibliographien des Deutschen Wetterdienstes.** Nr. 15: Neuere Arbeiten zur örtlichen Wettervorhersage (1960-1963). Bearbeitet von Rudolf Anfol. Pp. 11. Mitteilungen des Deutschen Wetterdienstes. Nr. 51 (Band 5): Die Buchenmast in Schleswig-Holstein und ihre Abhängigkeit von der Witterung. Von Walter Hase. Pp. 52. 4.80 D.M. Nr. 52 (Band 5): Synoptisch-Statistische Untersuchungen. Pp. 44. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1964.) [3011]

**University of California Publications on Zoology.** Vol. 75, No. 1: An Analysis of Color Changes and Social Behavior of *Tilapia Mossambica*. By E. H. Neil. Pp. 1-58 (5 plates). (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1964.) 1.25 dollars. [3011]

**Fiji: Department of Agriculture.** Bulletin No. 43: Annual Reports of Specialist Officers, 1961. Pp. iii+116. (Suva, Fiji: Government Press, 1962.) 4s. [3011]

**National Science Foundation, Washington.** From Egg to Man: Biological Studies of Cell Differentiation. Pp. 10. (NSF Newsfeature.) (Washington, D.C.: National Science Foundation, 1964.) [3011]

**New Zealand: Department of Scientific and Industrial Research.** Information Series, No. 42: DSIR Handbook, 1964. Compiled by the Information Service. Pp. 104. (Wellington: Government Printer, 1964.) [3011]

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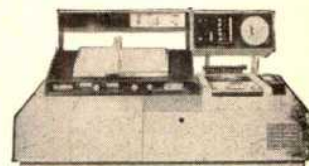
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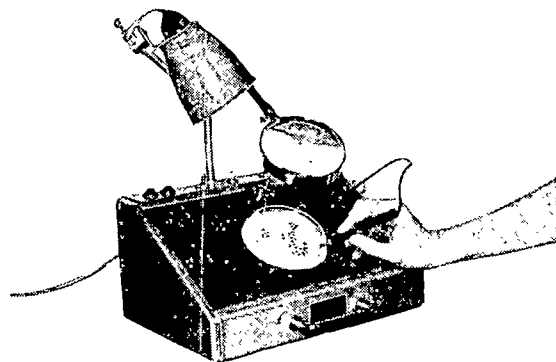
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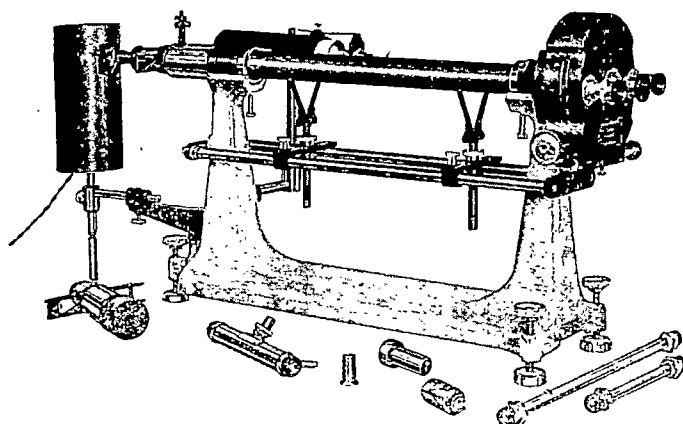
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## NEW THINKING IN UNDERGRADUATE TEACHING

THE final report\* of the Committee appointed by the University Grants Committee in March 1961, under the chairmanship of Sir Edward Hale, "to make a comparative study of undergraduate teaching methods and practices current in the universities and colleges of Great Britain in the fields of arts and pure and applied science", starts from the assumption that the main object of an undergraduate course should be the development of a student's capacity to think for himself and to work on his own. Although it reaches comparatively few specific conclusions, and definite recommendations are equally sparse, its thorough and sensitive discussion of problems that are of increasing importance is an invaluable contribution to the task of ensuring that university expansion is realistic and efficient. It demonstrates the complexity of the whole series of problems, the danger of facile generalization, and the folly of allowing organizational or administrative convenience to override the personal and human issues.

An interim report on the use of vacations by students was presented in June 1963, in which the Committee found that for a large proportion of students the long vacation is, academically speaking, time largely wasted. That was duly noted by the Robbins Committee when it reported later that year. However, the Robbins Committee, while expressing the opinion that students should be expected to spend a substantial part of their vacations on work related to their fields of study and that grants should be assessed on the assumption that students will be occupied with their studies during ten months of the year—though not necessarily exclusively with academic work—was reluctant to recommend changes in the academic year. In particular, it was opposed to a general lengthening of first-degree courses to four years, and it should be noted that its emphasis on responsibility, on the part of the teacher and of the student, was placed in the context of its remarks on vacations.

The Hale Committee in its final report, however, is led by this loss of time in vacations to suggest that sessional and final examinations should take place in November and that the academic year should begin in January, largely because of the salutary effect which the change would have on the use of the long vacation and the effective lengthening and better utilization of the three-year courses taken by most undergraduates. The Committee presents strong arguments for such a change, but does not consider the possibilities which have been strongly urged by Prof. B. R. Williams and Mr. C. F. Carter of rearranging undergraduate teaching to permit six eight-week terms for two intakes a year. This change is advocated on the ground that it would allow the number of undergraduates to be increased by 30 per cent with only a 10 per cent increase in building: the capital cost of the expansion up to 1980–81 recommended in the Robbins Report would be reduced from about £1,420 million to about £600 million. While this is urged as essential on economic grounds, it is also pressed from the point of view

of the misuse of vacations noted by the Hale Committee, and the consequent slight reduction in the length of term in most universities is not dismissed without consideration.

The Hale Committee in its final report departs in other respects from some of the conclusions of the Robbins Report, but before considering these the general framework of the Report should be noted. The comments on the use of vacations and the changes recommended in the university year are to be found in a chapter on the structure of degree courses and the university year. This is followed by two chapters on problems of organization and the introduction of students to the university. Information collected on the use students make of their time, and on student opinion, is summarized in the next two chapters, and then the question of lectures and discussion periods is considered at length, followed by a chapter on practical classes and another on examinations. A chapter on equipment of teaching gives rather more weight than did the Robbins Committee to the importance of adequate university libraries and of effective instruction in the use of libraries, while a final chapter deals with university teaching as a matter for training and study.

All this is preceded by an introductory chapter in which some general considerations are set forth which reveal the whole spirit of the Report. The Hale Committee has been impressed by the interest in, and concern for, undergraduate teaching shown by those university teachers who gave evidence. The testimony of this Report to the extent to which university teachers are already accepting the formidable challenge of the present situation should be set against many facile reproaches. It is manifest that many university teachers do seek to teach their subjects in such a way that they not only serve as a basis of professional skill but also stimulate and broaden the mind. It is also salutary to be reminded that the challenge cannot be met successfully without reference to the content of courses as well as to the methods by which students are taught.

Here the Committee refers especially to the way in which first-degree courses tend to become overloaded with fact, as a consequence of the growth in the volume and complexity of knowledge. In consequence, the student is in danger of spending too much of his limited time memorizing facts, and has insufficient time at his disposal to master the principles underlying his subject and to develop his powers of thought. Since the Committee believes, as already indicated, that the most important purpose of a university education is to teach the student to think for himself, this is a serious danger, for which it suggests that the first remedy is to keep the nature and content of courses under constant review in order to eliminate all unessential or obsolete material. This is not simply a matter of detailed pruning; it may on occasion demand a re-examination of the whole approach to a subject in undergraduate courses.

Here is a point on which the Hale Committee parts company with the Robbins Committee, which was inclined to favour a further increase in postgraduate work, partly to reduce the content of specialized knowledge required in first-degree courses. On the contrary, the

\* University Grants Committee. Report of the Committee on University Teaching Methods. Pp. v+173. (London: H.M.S.O., 1964.) 10s. net.

Hale Committee deplores any tendency to design and teach undergraduate courses merely as a preliminary to work for a higher degree. It is convinced that the essential purpose of the first-degree course is to give the student a preparation for his professional life which does not depend for its value on the addition of a postgraduate course. An undergraduate course should not aim solely or even primarily to equip the student with knowledge; it should also, and even more important, teach him to think for himself and work on his own.

That conviction inspires the Hale Committee's assessments of the methods and practice of university teaching, and in the context of university expansion to-day they are unquestionably right. Any other course is only too likely to lead to a mass of dissatisfied, unemployed, even unemployable, graduates. Moreover, concentration on postgraduate teaching has dangers for the university teacher, who may well become subject-centred and not student-centred. In the increasing size of universities and with growing numbers of students drawn from homes and backgrounds with no tradition of learning, only the utmost determination and enlightened administration are likely to keep undergraduate teaching centred, as it should be, on the student.

This administrative problem is explored in the chapter on problems of organization, and here the Committee differs from Lord Bowden in rejecting the view that all the advantages are with large departments. Fully recognizing that a department which is weak in research is thereby weakened for teaching, it nevertheless sees some truth in the dictum that the efficiency of an organization tends to vary inversely with its size, particularly on the pastoral and administrative side rather than in teaching in the strict sense. The existence of these problems is recognized by the universities, and the difficulties may become more acute as university departments become larger, but, while the Report outlines some of the problems to be considered, it makes no concrete proposals itself other than commending the practice of most universities of assigning each student to one member of staff for advice on his studies as a whole. The Committee is particularly concerned about the incompatibility between full departmental autonomy and the satisfactory conduct of multi-subject courses, and suggests that this requires further consideration. A student who belongs fully to no department is liable to be under a serious disadvantage, and the member of staff to whom he is assigned needs special opportunities of meeting the student for serious discussion.

The student's introduction to university life is related to such problems, and here one is impressed by the sensitive insight of the Committee. It recognizes that first-year students do get rather more personal supervision than other students, but all their teaching, whether by lectures, classes or discussion groups, tends to be in larger groups than that of other students. Moreover, it is in the student's first year that the risk of failure is greatest, and although the causes of first-year failure are not always academic, the Committee considers that sometimes failure could be prevented by timely intervention by a member of the academic staff who knows that the student possesses his confidence.

The Committee stresses the importance of every first-year student having opportunities, which he does not have to make for himself, to discuss his problems with a member of staff who is not a stranger to him. Preferably the person to whom he is assigned for advice should also

be the same one he meets regularly in a discussion period to be effective, staff-student contacts must be about some shared activity, the teacher-learner relation being the best. Further, the Committee points out that it is in the first year that the student should break through from the mentality of the schoolboy to that of the university student, and this cannot be done by lectures alone, where reliance on authority may tend to perpetuate the immaturity which the student should be discarding.

This aspect is considered more fully in the chapter in which the Committee discusses specifically the functions of lectures and discussion periods and their use in the universities. The Committee does not accept without qualification the view of the Robbins Committee that formal lectures delivered to small audiences have little value. It argues that some lectures could, with advantage, be eliminated, and that a large audience may have a stimulating effect, but it does not think that the size of the university affects the need for highly specialized lectures. These, the Committee suggests, are a necessary concomitant of highly specialized courses and may be a valuable means of communicating a scholar's enthusiasm to his students, though the case for highly specialized lectures cannot be considered simply from the point of view of undergraduate needs. It directs attention to other points which call for consideration when the justification for lectures to small audiences is being assessed, and it emphasizes that if a higher proportion of undergraduates are to receive a broader education, a higher proportion of first graduates will probably also tend to demand specialized postgraduate courses, some of which must necessarily be given by lecturers to relatively small audiences.

The pros and cons of the lecture as a means of university teaching are very fairly set out, and before considering the use of discussion periods the Committee makes two important comments. First, now that most students are receiving grants from public funds, and have been selected to the exclusion of other candidates, neither university authorities nor grant-making authorities can be indifferent to whether a student is availing himself of the teaching given. If it is clearly reasonable that authority should know whether a student is attending lectures, it is also reasonable that students should be informed if a series of lectures is one at which their attendance is considered necessary. This is the Committee's second recommendation, and while there may be practical difficulties in keeping a reliable attendance register for a large audience, that is no reason for leaving students in doubt as to the importance attached to a particular series of lectures.

On the functions and use of discussion periods the Hale Report is again at issue with the Robbins Report, which regarded the tutorial system used at Oxford and Cambridge as too exacting for most students, too costly and too demanding on teaching resources: its introduction into other British universities, it is believed, would be both impracticable and undesirable. These conclusions are challenged by the Hale Committee, which once again maintains the importance of the individual student whose needs are met so successfully in the individual tutorial. It questions whether this is necessarily so costly as the Robbins Committee alleged, if it is used to reduce the time given to other forms of teaching and to make the student do more for himself. Even if the figures given for Oxford and Cambridge are accepted—on which the



Hale Committee has reservations—the balance might not be expensive and, apart from the fact that some universities are believed to have used tutorials similar to those used at Oxford without costs becoming out of line with the national average, it refers to the possibility of the use of graduate students in tutorials to reduce the heavy demand on College Fellows.

As to whether the tutorial is too exacting, the Hale Committee insists that the purpose of a tutorial is to be as exacting as possible, and every student needs a system which evokes from him the greatest intellectual effort of which he is capable and which stretches his ability to the utmost. It is the merit of the one-to-one tutorial that the teaching can be suited to the pupil's ability and that he can be taken at his own best pace. Nevertheless, the Committee thinks that, whereas the amount of teaching by discussion which can be given to a student is limited, the work required for each tutorial or seminar should be substantial. Rightly used, teaching by discussion is a way of making the student do for himself what is too often done for him, and if it is used in this way the Hale Committee thinks that it would not be unduly expensive or burdensome on staff.

The Report suggests that at a time of expansion a critical study of the role of practical classes is required, subject by subject. With regard to examinations, the Hale Report recognizes the experimental innovations which are being introduced or proposed in some university examinations, and it emphasizes that continued study and experiment are important for two reasons. Examinations should measure, so far as possible, not only the candidate's knowledge but also the qualities and habits of mind for which his degree should vouch. Their effect on the student's education should be beneficial and encourage good teaching.

The subject of teaching equipment is one which at the present time will arouse the keenest expectations and interest. The importance of the university library has already been mentioned; the Committee refers also to the importance of students buying books for themselves, but questions whether any practical increase in the allowance for book purchase in students' awards would suffice by itself to bring about the desired increase in books so as to reduce the undue dependence of students on oral teaching. On accommodation, many complaints were found concerning that available for teaching by tutorial or seminar, and while most university teachers consulted were satisfied with lecture rooms, there was considerable complaint about laboratories, particularly for biology and geography. Many were dissatisfied with the available secretarial and technical assistance.

Since the Brynmor-Jones Committee is now independently examining the potentialities of closed-circuit television and other audio-visual aids in teaching science, it is natural that the Hale Committee's observations on the use of such aids should be limited, and it merely summarizes the replies to the questions about the supply and use of such aids, including sound-recorded material, other than sound films. On the other hand, the possible use of programmed learning in universities is discussed in some detail. This form of teaching it regards as sound in principle and *prima facie* applicable to some forms of university work. The most promising field at present appears to be in application to introductory courses in subject-matter of a factual kind, but its value requires testing by trial and error. Moreover, the preparation of a programme is most exacting and time-consuming, and,

to be economic, the programme must be widely used. For this, inter-university co-operation with *ad hoc* finance would be necessary.

This comment is closely linked with what is said in the final chapter on university teaching as a matter for training and study. The Hale Committee believes that if the expansion recommended by the Robbins Committee is to be achieved, and the standard of university teaching maintained without excessive claims on national resources and on the limited pool of ability, ways must be found of making better use both of university buildings and equipment and of the time of university teachers. For this it suggests that operational research is necessary, and particularly a policy of well-directed experiment in university teaching organized on an inter-university basis. This would involve, for example, a standing committee, with a full-time secretary, to promote and steer a concerted programme of experiment; adequate finance for the additional costs, to be administered by this committee; and publication of the results in such a way as to reach all those for whose teaching the results might be significant. The Hale Committee also envisages this body becoming a centre of information in this field, as well as exercising advisory functions.

The Hale Committee has furnished a Report of great value, not least in its correction of some hasty and ill-informed criticism of the universities. It should not only give pause to some of those obsessed with the advantages of size but also stimulate an insight into the needs of students and of teachers. Certainly it should encourage the understanding between teacher and taught and the sense of responsibility which the Robbins Report desiderated and which is all too often overlooked in discussion. There may well be impatience in some quarters with the caution of the Hale Committee, but its wisdom and imagination deserve attention. If the standards of university education are to be maintained, and if Britain's need for trained man-power and woman-power is to be met in quality as well as in quantity—without gross waste of her limited resources either of finance, of materials or of man-power—the expansion of higher education must be geared closely to the further enquiries which the Hale Committee suggests and take due account of all the factors which are discussed with such vision as well as understanding.

## CARDIO-VASCULAR HISTORY

### Circulation of the Blood

Men and Ideas. Edited by Dr. Alfred P. Fishman and Prof. Dickinson W. Richards. Pp. xiv+859. (New York and London: Oxford University Press, 1964.) 144s. net.

THE two editors of this weighty volume, Alfred P. Fishman, director of the Cardiorespiratory Laboratory, Columbia University Division, Bellevue Hospital, New York, and Dickinson W. Richards, Lambert professor of medicine emeritus, Columbia University College of Physicians and Surgeons, New York, have produced an authoritative history of cardio-vascular physiology. The former writes on the cerebral circulation, the latter (with William F. Hamilton) on the output of the heart. Contributions on different aspects of the subject are given by other cardio-vascular experts, nine American, two English, one Belgian and one Swedish.

Prehistoric man realized that the heart was a vital organ, for it is indicated in many cave drawings as the aim of the hunter. The ancient Egyptians also knew this and the organ's relation to the whole body through the blood vessels. The Chinese studied the heart and the

pulse and its variations. The Greeks counted the pulse by a water-clock. Hippocrates regarded the heart as a source of bodily heat. Herophilus (c. 300 B.C.) showed that the brain) and not the heart, was the seat of intelligence and sensation; he distinguished arteries from veins but held that the former contained air. This mistake persisted until Galen (c. A.D. 131–200) showed that arteries contained blood. He taught that the heart had three chambers with a porous septum. Galen advanced knowledge in many respects, but his errors persisted for centuries through dogmatic teaching. Then, with the Renaissance, Leonardo da Vinci depicted the four-chambered heart, and Vesalius in his *De fabrica* described its anatomy accurately.

As early as the thirteenth century something was known of the lesser circulation through the lungs. Ibn Nafis, an Arabian physician (1210–88) of Damascus and Cairo, questioned Galen's views on the ebb and flow of the blood in the body to some extent. In a manuscript he stated that the interventricular septum is solid and that part of the blood is aerated in the lungs. In the sixteenth century that martyr of science, Michael Servetus (1511–53), described the pulmonary circulation, as did Realduo Columbus in 1558. Fabricius, Harvey's teacher, in 1603 described the valves in the veins. Then in 1628 William Harvey published *De motu cordis* showing that the whole of the blood circulates through the human body. From this can be dated the sciences of experimental physiology and experimental medicine generally and the basis of a scientific approach to cardio-vascular problems.

Many investigators entered through the door thus opened by Harvey. Aselli (1581–1626) discovered the lymphatic circulation, Pecquet the thoracic duct in 1647. Malpighi (1628–94) confirmed Harvey's belief that blood passed from the arteries to the veins by discovering the capillaries. Thomas Willis (1621–75) described the arterial circle at the base of the brain known by his name. Here it may be observed that it was not until 1908 that C. E. Beevor (1854–1908) gave a complete description of the arterial supply to all parts of the brain (*Phil. Trans. Roy. Soc.*). The Rev. Stephen Hales (1677–1761) recorded and measured the blood pressure and investigated the dynamics of the circulation. Morgagni (1682–1771) first described heart-block, elaborated at a later date as Stokes-Adams disease by Adams and Stokes. Effective pulse-watches, percussion, and auscultation by the stethoscope increased knowledge of the heart and circulation. The microscope and chemical advances led to the study of the constituents of the blood, histology of heart muscle and structure of arteries and veins. The blood-pressure was more accurately estimated by special instruments, as was the velocity of blood-flow by the Weber brothers in the nineteenth century. They also confirmed Volkmann's discovery that the vagus nerve had an inhibiting action on the heart, and its centre was localized in the medulla a few years later.

From the earlier history the book proceeds to more modern discoveries. Of these only some selected instances can be given here. In 1907 Keith and Flack demonstrated the sino-atrial node in the right auricle which initiates the heart impulse. Mackenzie's work with the polygraph correlated arterial and venous pulsation with the heart-beat and differentiated between trivial and serious irregularities of the heart rhythm. The researches of Einthoven led to the use by Thomas Lewis of the electrocardiograph for the clinical investigation of heart disease and its aid in diagnosis and prognosis. Before this, perfusion of the isolated heart had led to further knowledge. Starling and Lovatt Evans had investigated the metabolism of cardiac muscle, and the last-named also the hydrogen-ion concentration of the blood. One of the ablest chapters (Chapter 8) in the book is written by Sir George Pickering, Regius professor of medicine, University of Oxford, on systemic arterial hypertension, a subject to which he has devoted many years of research.

The bibliographies appended to each chapter are good and the book is well indexed. The numerous illustrations including portraits of the earlier and more recent investigators who have made cardio-vascular history, constitute another admirable feature of the volume.

## NEW LIGHT ON WILLIAM HARVEY

### The Anatomical Lectures of William Harvey

*Prelectiones Anatomie Universalis De Musculis*. Edited with an Introduction, Translation and Notes, by Dr Gweneth Whitteridge. Pp. lxiv + 504 + 7 plates. (Edinburgh and London: E. and S. Livingstone, Ltd., 1964. 147s.

THIS book is a very satisfying, interesting and educative presentation of William Harvey's anatomical lectures and of the problems they present.

It should be known that they were delivered at the Royal College of Physicians during 1616–28. In 1582 Lord Lumley had established in perpetuity a lectureship in surgery which was to present "in good order all the whole course of the Art and Science of Surgery". In addition, the Lumleian Lecturer was enjoined to hold a public anatomy in the winter of each year. Harvey was appointed in 1615. His notes that survive are contained in two manuscripts in the Sloane Collection in the British Museum. One manuscript has been published before the other, *De musculis*, is printed for the first time.

The original notes are in Latin with many abbreviations. Harvey added later notes and comments and phrases in English. The work of transcription, of translation and of presentation is obviously difficult and complex. Dr Whitteridge prints her transcription on the left-hand page and her translation on the right-hand one. The translation is rendered as running prose.

It is impossible to review this book without comparing it with the only other attempt at translation, published in 1961 by Drs. O'Malley, Poynter and Russell. Their book gives their translation but not their transcription, and the translation is in a disjointed form and so does not make pleasant or easy reading. Dr. Whitteridge's presentation is much more readable and the printing and arrangement are more attractive.

An assessment of the comparative merits of the scholarship of the two books is difficult and could only be achieved with certainty by reference back to the original text. It can be said that the impression gained is that Dr. Whitteridge's transcription is the more reliable. Certainly it makes easier sense. Thus O'Malley's translation states: "In quantity the heart of man is large, wherefore timid, wherefore courage from a common intelligence". Whitteridge renders the same passage: "The heart is large in men, and for this reason they are timid by nature and their courage comes from their intelligence".

In the description of the testes Whitteridge renders "The testes are so-called because they attest virility Worthy to be made a Pope". The Latin text she gives is "*Dignus Papari*", and her notes state that in order to avoid any repetition of the scandal of election of "Pope Joan" the cardinals ordained that the sex of the candidates should be determined by examination of the genitalia. It is somewhat startling to find in O'Malley's version "The testes which attest virility, worthy to be eaten"!

In addition to the translation of the lectures there is an excellent introduction of some sixty pages that is interesting and valuable in presenting many Harveian facts, notably a section on the state of Harvey's ideas on the circulation of the blood as expressed in these lectures. The argument is convincing that at the time they were delivered Harvey's ideas were far from fully formed and had not been crystallized by his later experiments. The notes that have been taken to indicate he understood the

true nature of the circulation are shown probably to have been added later, about 1627 or 1628. This section will repay careful study. Dr. Whitteridge points out that it is not the similarities with *De motu cordis* which are of importance in determining the state of Harvey's knowledge in 1616 but the dissimilarities.

Another interesting section deals with Harvey's interest in surgery and pathology. It presents clearly the relationship between physician and surgeon at that time and how the instruction of surgeons rested with the physicians. She completely rejects the statement by O'Malley, Poynter and Russell that Harvey practised surgery himself and had actually performed lithotomy.

The final paragraph of the introduction states "these lecture notes show Harvey primarily as an observer rather than as an experimentalist. . . . The rigorous discipline of argument and experiment of the *De motu cordis* was yet to come. That work was to be seen later as one of the first examples of modern scientific method. By comparison the lectures are backward looking. From Antiquity, through the Middle Ages and the Renaissance, medical education is unbroken in its tradition and in this tradition the lectures are enshrined. They do not reach beyond it to the dawning of the scientific attitude of the second half of the seventeenth century".

The book is handsomely and finely produced in a manner fitting for such a scholarly, impressive and instructive presentation of Harvey's lectures. It deserves great praise and is very strongly recommended.

RUSSELL BROCK

## PATHO-PHYSIOLOGICAL PHARMACOLOGY

Kurzes Lehrbuch der Pharmakologie

Von Prof. G. Kuschinsky und Prof. H. Lüllmann. Pp. viii + 332. (Stuttgart: Georg Thieme Verlag, 1964.) 33 D.M.

THIS text-book attempts to present pharmacology and those parts of toxicology which are relevant to clinical medicine on the basis of patho-physiological considerations, stressing structure-activity relationships. By describing the actions of drugs as they affect physiochemical processes, in addition to giving an account of their effects on the organs of the body, pharmacological observations are evaluated in their relationship to biochemical and biophysical data. Considered from this angle, pharmacology is seen to deepen the understanding of processes as they occur in health and disease, in contrast to the notion that it consists merely in a description of a vast variety of drug effects on various parts of the body.

The references are mainly confined to review articles which would guide the reader to the original papers. Unfortunately, quite a few of the reviews quoted are ten or more years old. On the other hand, the authors have in many cases succeeded in integrating recent findings and modern theories into the presentation of their subject. For example, on the basis of the hypothesis of the storage mechanism of biogenic amines in the tissues, a brief and concise explanation is offered of the apparently contradictory influence of cocaine on the action of catecholamines, which is intensified, and on the effect of epinephrine and tyramine, which is diminished (p. 24). Tracings are well chosen and structural formulae of many compounds and their generic and trade names are included. Practical implications of the pharmacological actions, clinical usage and doses are indicated. Thus the chapter dealing with the pharmacology of smooth muscle ends with an appendix which outlines the treatment of bronchial asthma, hypertension and migraine. Although these surveys are necessarily kept very short, phaeochromocytoma and unilateral

kidney disease should have been included as curable types of hypertension.

Sections which have received a thorough and up-to-date treatment include those dealing with the pharmacology of the autonomic nervous system and the kidney. Other parts are less comprehensive or frankly deficient. Some of them may be mentioned here: The clinician or student will scarcely be able to grasp the meaning of the brief paragraph which states that for a given stage of anaesthesia thermodynamic activity is approximately the same for the various anaesthetics, while solubility coefficients, oil-water distribution coefficients and partial pressures of the gases in the respiratory air show marked differences. The attitude to the use of amidopyrine is that common in parts of the European Continent and contrasts sharply with the fear of agranulocytosis that limits its use in other countries. The description of insulin does not include the principles involved in the preparation of the different kinds, nor does it mention that the lente type should not be mixed with other preparations and the reason for it. Treatment of diabetic coma by 50-100 units insulin intravenously and by subcutaneous injection, in addition to glucose, water and electrolytes, is surely inadequate. Though the significance of glucose-6-phosphate-dehydrogenase deficiency for idiosyncrasy to certain drugs and for the development of some haemolytic anaemias is stressed, the genetically determined differences in sensitivity to suxamethonium are not discussed. The actions of ACTH are only considered very briefly and its effect on cholesterol and ascorbic acid levels is not explained; its therapeutic uses are not sufficiently described, while half a page is allotted to thyrotropic hormone. In the section dealing with chemotherapeutic agents and antibiotics nitrofurantoin should have been included, and paramomycin and perhaps the ampicillin treatment of typhoid mentioned.

The index is well compiled and includes the names of many preparations. In spite of the uneven treatment of the different chapters, the book is an up-to-date source of pharmacological knowledge and deserves a place on the shelf of medical practitioners and students.

L. WISLICKI

## INFORMAL AND INFORMATIVE ASTRONOMY

The Universe

Karl Taylor Compton Lectures. By Otto Struve. Pp. ix + 159 (figures). (Cambridge, Mass: The M.I.T. Press (Massachusetts Institute of Technology), 1962.) 44s.; 4.95 dollars.

THROUGH his numerous articles in *Sky and Telescope* Prof. Otto Struve has created a distinctly personal style of semi-popular writing in astronomy. These articles were intended to give to the informed public whatever happened to be the latest among professional astronomers. Physical ideas were set forth with lucidity, accompanied by ample arithmetical illustrations, but the mathematics was never allowed to go beyond an odd square root here and there. The style, though informal, was by no means facile, because of an almost visible desire to be informative on the part of the author. Ideas succeeded one another with little regard for linguistic fluency, but this very shortcoming, like the occasional stammer of a great speech maker, enhanced rather than diminished the power of persuasion. This informal and informative style found its full expression in the 1959 Karl Taylor Compton Lectures, now available in book form.

Of the six lectures, that on binary stars is outstanding. Such an illuminating account could only have come from someone who had a deep sense of personal involvement



in the subject. Prof. Struve's own contribution in this field was not mentioned; the limelight was on the discovery of the binary nature of Nova Herculis, and what it implied in our search for the missing link in stellar evolution. The other lectures were on stellar evolution, origin of the Solar System, galaxies and radio astronomy. In all these Prof. Struve can be depended on for being up to date (in 1959)—and this means the lecture on radio astronomy is already somewhat out of date. The last lecture is an odd-man-out. It has for its title "Man and the Universe", but turns out to be a plea for carrying out more astronomy; the reasons, apparently, are (1) the Russians had fired the first satellite; (2) astronomy had been useful to physics; (3) there may be other intelligent beings outside our planet.

There is a large number of diagrams and photographs in the book—too many photographs, I feel. A large assortment of lantern slides, each shown for a brief moment, may be just the thing in public lectures, but to reproduce them all in print, which seems to be the case, is sheer extravagance. The diagrams are all right because they serve a particular purpose, but the photographs, most of them familiar anyway, simply take up a lot of space and put up the cost of the book out of proportion to their usefulness.

T. KIANG

## SPACE SCIENCE

### Interplanetary Dynamical Processes

(Interscience Monographs and Texts in Physics and Astronomy, Vol. 8.) By E. N. Parker. Pp. viii+272. (New York and London: Interscience Publishers, a division of John Wiley and Sons, 1963.) 95s.

### Astrophysique

(Cours à l'usage de la Licence.) Par E. Schatzman. Pp. 150. (Paris: Masson et Cie, 1963.) 29 F.

### Radio Astronomy

By J. Steinberg and J. Lequeux. Translated by R. N. Bracewell. Pp. xii+260. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1963.) 77s.

*INTERPLANETARY Dynamical Processes* is devoted to a discussion of the theory of coronal expansion or the theory of the 'solar wind'. This is a question which has acquired great importance since the first introduction of this subject more than ten years ago. The problem is introduced and the observational evidence presented and discussed in the first three chapters. The next six chapters are concerned with establishing the mathematical techniques used, that is, the necessary hydrodynamical equations and their solutions under various conditions. The part played by magnetic fields, irregularities and the propagation of high-energy particles in the expanding corona are discussed. The book is concluded by a discussion of the question of coronal expansion for stars other than the Sun.

This is a particularly interesting account of the theory of coronal expansion. It summarizes in one volume the hydrodynamical theory of such expansion and presents the arguments and evidence in support of this method of approach. The accounts of the work were previously scattered in the literature. While expensive, the price is not unduly high for a specialized monograph which must have appeal limited to the fields of solar and space dynamics.

*Astrophysique (Cours à l'usage de la Licence)* begins by discussing the means of obtaining astrophysical information before discussing the Sun, double stars, variable stars, stellar distributions and motions, interstellar material and extragalactic nebulae. The subject is covered in a very concise way. The compactness is of such a

degree that it would be difficult to use this text independently either of the course of lectures on which it is based or a set of reference texts.

However, there is a need for this type of text where there is more demand for astrophysics courses. The book allows rapid refreshment of the memory and allows the student to obtain a grasp of the subject as a whole. In this respect, however, the price of the book is somewhat high. It would be desirable to see more of this type of document associated with courses given in Britain. While the expense of the book precludes its use by students, it should find a place in astronomical libraries, where it can be used to good effect in the planning and presentation of new courses.

The translation of J. Steinberg and J. Lequeux's book brings a fine text to the English-speaking readership. The standard at which *Radio Astronomy* is written will make it an excellent text to supplement a course of lectures on radio astronomy at degree level. The topics covered are: the role of the atmosphere; thermal radiation; simple radio telescopes; interferometers and aperture synthesis; spectral observations; mechanism of the emission of radio waves; radio emission from the Sun; radio emission from the Solar System; galactic radio emission; galactic radio sources; extragalactic radio sources. The treatment is kept simple, but no endeavour is made to avoid mathematical treatment at the expense of clarity. The section on the mechanism of radio emission is of particular interest.

While useful as a text-book, its value should not end there. It is perfectly comprehensible to advanced general readers who wish to gain some insight into the techniques and the results of radio astronomy. There are few books of this level on radio astronomy and it is indeed welcome that this excellent French text should have been translated by such a well-known radio astronomer as Dr. R. N. Bracewell.

D. McNALLY

## PRESENT STATE OF HISTONE STUDIES

### The Nucleohistones

Edited by Prof. James Bonner and Dr. Paul Ts'o. Pp. xvii+398. (San Francisco and London: Holden-Day, Inc., 1964.) 12.75 dollars.

THIS book is the outcome of the first World Conference on Histone Biology and Chemistry in April 1963. It is worth stating at the outset that this is a very useful work as it contains most of the present data on histones and, unlike many *Proceedings* of international meetings, is likely to be consulted for some time to come.

It is inevitable in a work of this nature that there will be considerable overlapping of the individual contributions, and the student wishing to ascertain the present-day views on histone function will need to work carefully through with a notebook and then cross-reference his own notes. This is not a criticism of the work of the editors who have tidied the contributions into sections which are, to some extent, self-contained. They have also avoided verbatim reports of discussions, which can be merely distracting to the reader who does not know the participants. The difficulty in assessing the present standing of histones reflects the diversity of opinions expressed in the book, all founded on well-documented experiments; it is probably a salutary introduction for any student entering the field of molecular biology.

The book opens with a very readable chapter on the history of histone chemistry, starting with the work of Hoppe-Seyler and ending with the Kossel school. This is followed by ten chapters by various authors, dealing with details of histone chemistry. The heterogeneity of

histones from a single source, the characteristics of histones from different sources, techniques and results of analyses, are fully discussed, making this a useful reference section. The rapid development of this topic is, incidentally, made obvious by the confusion of nomenclature and the plea for a unified system.

The structure of nucleohistones opens with a chapter by Zubay in which he discusses in detail the essential feature of the problem, the function of histones. After a review of the previously published evidence and an exposition of the Jacob-Monod theory of the regulation of protein synthesis, he concludes that most histones are involved solely as structural proteins concerned with the supercoiling of DNA and its availability as a source of information during interphase. It is clear from subsequent papers that, although much has been discovered about the properties of isolated histones, for example the  $\alpha$ -helix content, the nature of their interactions with DNA is relatively unknown.

The remainder of the book is largely concerned with dynamic aspects. In the chapters concerned with histone metabolism results of turnover investigations lead several authors, for example Dounce *et al.* and Busch *et al.*, to the conclusion that specificity requirements for gene inhibition cannot be met by the histones. In the following section on enzymology the control function of the histones is further discussed. Huang and Bonner, and Allfrey and Mirsky, report experiments showing that histones inhibit DNA-primed RNA synthesis, thus providing experimental evidence for the Stedmans' inhibition hypothesis, while Billen and Hnilica have shown some specificity of inhibition of DNA synthesis *in vitro* by different histone fractions.

The role of histones in development covers examples of function in one cell cycle and aspects of differentiation. The problem of specificity is raised again by Bloch, as a consequence of his conclusion that probably any histone could combine with any DNA, and by Zalokar, who considers that removal of histone from incompetent genes does not make the genes active but makes them susceptible to the action of inducers.

The last section of the book is entitled "Thoughts for the Future", and in five chapters the various authors speculate on the developments as they see them. This will be to many readers the most thought-provoking section. In a final chapter the editors set out in a logical sequence the various possibilities for histone behaviour within the cell and discuss specific experiments designed to test them. This forms an elegant essay demonstrating the way in which to decide on the information required from an experiment and how to approach the interpretation of results.

The reader will have realized that this review does not do justice to all the authors. By mentioning the points raised by a few of them an attempt has been made to illustrate the coverage of the work. It will be apparent that although a great deal is known this has mainly resulted in a realization that the structure and functions of histones is a more complicated problem than was thought a decade ago.

A. R. CRATHORN

## KARL POPPER'S CRITICAL RATIONALISM

The Critical Approach to Science and Philosophy  
Edited by Prof. Mario Bunge. (Essays in Honor of Karl R. Popper.) Pp. xv + 480. (New York: The Free Press of Glencoe, a Division of the Macmillan Company; London: Collier-Macmillan, Ltd., 1964.) n.p.

KARL POPPER is a philosopher who, during a period of philosophical disillusionment—a "time of drought", as Karl Löwith has called it—has had the

courage to put forward a comprehensive view that has relevance for most of the branches of philosophy as well as for mathematics and the natural sciences and for history and the social sciences. His view was first presented in *Die Logik der Forschung* in 1934, where he argued that scientific theories are not established inductively but must be regarded as hypotheses—he later used the term "conjectures"—which hold the field so long as they have not been shown to be false when put to the test.

Theories are not established or verified, but withstand or fail to withstand attempts to refute them, so that the path to truth is through the rejection of error. In subsequent writings, notably in the *Poverty of Historicism*, which appeared in *Economica* in 1944, and in *The Open Society and its Enemies* published in 1945, Popper developed his non-inductivist, non-verificationist view into a general defence of rational criticism in opposition to the methods of dogmatism and authority. He argued that in morals the prevention or lessening of suffering takes precedence over the production of happiness, and gave reasons for preferring piecemeal reform to total planning. Like Comte, Popper holds that science and morality are closely interlocked though not identical, but, unlike Comte, he holds that large-scale prediction of the future of society is in principle impossible.

This collection of essays in his honour is divided into four parts. Part 1 contains eight essays under the general heading of "The Critical Approach to Philosophy". Of these we may specially notice the first, in which W. W. Bartley III argues that Popper's critical method implies the inadmissibility of any attempt to 'justify' a theory, or, I suppose, a belief, practice or institution. For justification, according to Bartley, requires an appeal to some ultimate authority, whereas criticism, with its reliance on falsification, needs no such extraneous support. Paul Bernays, in a paper entitled "Reflections on Karl Popper's Epistemology", while in agreement with Popper's main tendency, suggests that testing is not such a pessimistic thing as Popper assumes when he takes it as the attempt to overthrow the conjecture being tested. Bernays's critical examination of testing and of criticism itself is brief but fruitful. Part 2 contains four essays under the general heading of "The Critical Approach to Logic and Mathematics". We may here note W. C. Kneale's defence, against Popper, of the view that the necessity of laws of Nature is similar in kind to the necessity of laws of logic. There are other papers on logic by H. B. Curry, N. Rescher and G. Stahl. Part 3, entitled "The Critical Approach to Science", contains twelve papers. Feigl criticizes instrumentalism and incidentally discusses the quantum theory, as does O. R. Frisch in a paper on that subject. A. Grünbaum, in a paper on irreversibility, criticizes Popper's view, stated in *Nature* in 1956, 1957 and 1958, that the 'arrow of time' is not closely connected with and not dependent on the law of entropy. Popper's and Kneale's views on natural necessity are briefly discussed at the end of Yourgrau's paper on the reality of elementary particles. The fourth part of the book is called "The Critical Approach to Society and History", and of the five essays that make it up we may mention Pieter Geyl's appreciative review of Popper's *Open Society*. The book concludes with a bibliography of Popper's writings.

There is, of course, plenty of support for Popper in this volume, but it is far from being a collection of laudatory testimonials. In some of the papers he is not discussed at all, and in others, as we have seen, he is honoured by being criticized. He is also honoured by the galaxy of talent who have contributed to the book, for it is a tribute to the interest that his views have aroused, as well as to the skill of the editor, that space only allows me to mention contributions by Gombrich and by Hayek and forces me to leave many others unmentioned altogether.

H. B. ACTON

### The Oxford Book of Birds

By B. Campbell and D. Watson. Pp. xvi+207 (illustrations). (London: Oxford University Press, 1964.) 35s.

AMONG the many current books on British birds, this may be acclaimed the best at its particular level, between the pocket guide to identification and the advanced text-book. It is indeed excellent for identification purposes, but it also gives a satisfying amount of solid information on distribution, habitat and behaviour. Each of the 96 colour plates depicts several species, and two or more examples where there are important differences in plumage according to sex, age or season. All species that have occurred in the British Isles are figured except a few rare vagrants, and even some of these and of introduced species that have become locally naturalized. Mr. Watson is skilful with his brush and obviously knows most of his subjects in life. These are shown in a 'natural' setting; although this raises occasional difficulties of scale and involves some incongruities of juxtaposition, it has the advantage of immediately relating the bird to its habitat and at the same time produces a pleasing picture. The standard of colour reproduction is high, although in certain instances some predominant tint appears to be too strong.

The text is thoroughly reliable and up to date. It is conveniently arranged with from one to three paragraphs about each species on the page facing its illustration. Dr. Campbell has succeeded in compressing a great deal of information into limited space, by careful selection and by using an economical style; the result is pleasantly readable. He has also avoided overloading the text with information that is irrelevant to the British status of the species, such as the breeding habits of those that are not natives. For the commoner birds the treatment ends with an ingenious single-line calendar—a series of numbers, with typographic variations, that shows at a glance in which months the species is (a) present, (b) nesting, and (c) in song.

At the beginning and end of the book there are useful brief chapters on general subjects such as classification, anatomy, flight, behaviour and breeding, migration and numbers. Black-and-white drawings illustrate these and also show the appearance of various waders and ducks on the wing.

LANDSBOROUGH THOMSON

### Intersexuality in Vertebrates Including Man

Edited by C. N. Armstrong and A. J. Marshall. Pp. xi+479. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 90s. net.

THE scope of this excellent text-book is logical but unusual, in combining a series of review articles directed chiefly to zoologists, with two outstanding clinical reviews. In the context of biology it is eminently reasonable to discuss intersexuality in vertebrates by phyla, ending with a special discussion of it in man; it is none the less a relatively new gesture by publishers in the direction of an interdisciplinary human biology; this approach is so useful that one must hope that the clinical sections are not overlooked by medical readers, coming as they do after a sizable body of zoology.

Intersexual phenomena, normal and pathological, are a good deal commoner in vertebrates than had been realized until recently—the number of known examples of hermaphroditism in fish, for example, has been growing steadily since the beginning of the present century. The bulk of this book is taken up with a series of good specialist reviews—of fishes (J. W. Atz), amphibia (C. L. Foote), reptiles (T. R. Forbes), birds (E. Taber) and non-human mammals (J. Bruner-Lorand), prefaced by a full discussion of vertebrate chromosome deviations by R. A. Beatty, excluding those in man. There is also an informative and entertaining editorial foreword by A. J. Marshall. Human intersexuality is then dealt with in full clinical detail,

together with the present state of knowledge about human chromosome anomalies, by C. N. Armstrong. This article is well illustrated with case photographs and histories, and preceded by a series of paragraphs on the legal and logical definitions of maleness or femaleness. Both as a summary of sex-determination in man, and as a short primer of an obscure but important clinical problem in differential diagnosis, this would be hard to better. Especially welcome, however, is the good judgment of the editors in following this essay with another, by M. Roth and J. B. Ball, on the behavioural types of intersexuality in man (homosexuality, trans-sexualism, transvestism) depending on the mis-learning of a gender role, which should serve once and for all to end the confusion between these states and that of physical intersexuality. An incidental but important use of the reviews of phyla is that they provide most valuable data for the selection of experimental animals. Bibliographies are adequate without being inflated, illustrations are good, and the style of the whole is readable. The book is a most useful addition to the library of any department or worker concerned with cytology, zoology, veterinary surgery, or clinical medicine—or with the teaching of these at pre- and post-graduate levels. ALEX COMFORT

### The Natural Radiation Environment

Edited by Prof. John A. S. Adams and Wayne M. Lowder. Pp. xiv+1069. (Chicago and London: University of Chicago Press, 1964. Published for William Marsh Rice University.) 105s.

THE proceedings of scientific conferences or symposia are not usually the best material for a text-book, since there is no reasoned account of the fundamental parts of the subject and the papers tend to be heterogeneous in outlook. International symposia tend to suffer from a further disadvantage: presentation of a paper is frequently the passport to the meeting, with the result that many relatively poor papers are written. It is only the major workers in the field, and as a rule the contributors from abroad, who can be relied on for really good papers, the latter primarily because the expense of sending them must be justified by a significant contribution to the conference. This is evident in *The Natural Radiation Environment*, where the average standard of the American papers is well below that of the European contributions. The main criticism that can be levelled at some of the authors is that their topics are parochial in the extreme and make little contribution to the overall knowledge of the subject. The book as a whole—and presumably, therefore, the symposium—suffers from too wide a diversity of interest and it is occasionally difficult to see how a paper fits into the general scheme.

The subject-matter is divided into two main sections: the first, entitled "Environmental Radiation Sources", includes radioactivity in the earth, in water and in the atmosphere,  $\alpha$ -radioactivity (on which one of the best papers has been written) and cosmic radiation. The second part, "Environmental Radiation Measurements", includes instrumentation and techniques, aerial surveys and ground measurements and surveys. Apart from one or two notable exceptions, the second part probably contributes more to our knowledge of the subject than does the first.

A short section is devoted to an intercalibration experiment carried out on the day before the symposium. In this, six different instruments, five from the United States and one from Great Britain, were used in three environments of widely differing natural radiation-levels. The results are interesting, but more could be made of them.

This is certainly not a general text-book on environmental radiation. It has perhaps much for the specialist, but its diversity and its price will fail to attract a wide audience.

H. D. EVANS



## NEW HIGH ANNUAL AWARDS IN AUSTRALIA

By OSCAR A. GUTH

School of Physics, University of Sydney

FIVE new Australian annual national awards—in science, medicine, literature, education and art—were presented in Canberra for the first time on January 21. The awards are among the richest in the world, each consisting of a gold medal and citation and £A5,000 (tax free). Named the Britannica Australia Awards after their sponsors, Encyclopaedia Britannica Inc., Chicago, the awards are administered and adjudicated by an entirely independent body in Australia, comprising some thirty-six of Australia's leading experts in the five award fields.

The Britannica Australia Awards are intended as a stimulus to science and culture in Australia and to reduce the country's 'brain drain'. They are made for work of outstanding merit in the respective award fields involving an original contribution or development and having a direct connexion with, or benefit for, Australian life. The awards are open to Australian nationals whether living at home or abroad, or non-Australian nationals who were living in Australia at the material time.

The first five awards—for 1964—were presented during an impressive ceremony in the Council Room of the Australian National University by the University's vice-chancellor, Sir Leonard Huxley, who is chairman of the Britannica Australia Awards General Council. The choice of the first six winners—the medical award was made jointly to two recipients—provides a good illustration of the character of the new awards.

The 1964 award for natural and applied sciences went to Dr. J. Griffiths Davies, who is chief of the Division of Tropical Pastures, Commonwealth Scientific and Industrial Research Organization (Australia). Dr. Davies established the Division of Tropical Pastures in Brisbane, Queensland, in 1952 to meet the challenge of a programme of pasture research leading to the establishment of satisfactory pastures in tropical areas. Suitable pasture plants, particularly legumes, were sought and found, and his Division has acquired a high reputation at home and abroad for its theory and practice of pasture science in the tropics.

The medicine award for 1964 was shared by Sir Norman Gregg, of Sydney, and by Dr. Kate Isobel Campbell, of Melbourne. Sir Norman, in 1940, in the course of his work as an ophthalmologist, recognized that congenital cataract was seen in a quite unusually large number of infants. He discovered that this serious condition was occurring only in children of mothers who had suffered an attack of German measles (rubella) during the early months of pregnancy. His completely unexpected finding in 1940 has led to the opening up of an important new field in medical science—the pathology of the unborn child. More directly it was followed in Australia and elsewhere by detailed investigations of the effect of rubella in pregnancy, which showed that, in addition to cataract and blindness, the virus could give rise to deafness, malformation of the heart and other less clearly definable conditions.

Dr. Kate Campbell, an acknowledged authority on disease in the first months of life, was the first in the world, in 1951, to show that an important cause of blindness in premature babies, retrolental fibroplasia, was due to over-treatment with oxygen. During her research she found that nurseries which used more elaborate apparatus to maintain a constant high level of oxygen had a much higher incidence of eye trouble than occurred in institutions where oxygen was given with simple or makeshift

apparatus, and at lower concentration. Dr. Campbell realized then that benefit to the blood must be balanced against possible harm to the eyes, and her analysis of the situation has since saved many children from blindness.

The literature award for 1964 went to Miss Judith Wright, who over the past twenty years has made a wide-ranging and permanent contribution to Australian literature. Best known as a lyrical and meditative poet of great technical mastery, she has also published outstanding short stories, a chronicle of family life based on her own pioneering ancestors, children's books and a considerable amount of literary criticism. The citation of her award particularly refers to her book *Five Senses* published during the past year.

The education award for 1964 went to Dr. Harold S. Wyndham, director-general of the State Department of Education in New South Wales since 1952. Dr. Wyndham received the award mainly for his leadership of a committee the investigations and final report of which led to major changes and reforms in the New South Wales secondary school curriculum. One of the main features in the new "Wyndham Plan" education—as it has become known in Australia since its implementation in 1962—is that it requires all high-school students to study an integrated course of science during the first four years of their six-year secondary education. This four year science course breaks new ground in its insistence on the teaching of physics, chemistry, biology and geology as one integrated subject and on the inculcation of the student to learn to think and understand science and scientific method as a whole rather than being presented with science in 'bits'. Dr. Wyndham's citation said that his new pattern of education had not been limited in its effect to his home State, but that it was also being closely studied throughout Australia, as well as abroad, and would serve as a test for other systems. Dr. Wyndham therefore stood out as a leader in the field of modern Australian secondary education.

The 1964 art award went to the already famous Australian painter and portraitist, William Dobell. Mr. Dobell, now sixty-five years old, returned to Australia from London in 1939, and by his steadfast example and his quiet insistence on the professional status of the painter, has significantly helped to establish the Australian artist as an integral part of the country's community. His citation said that his paintings and his name were now indelibly woven into the fabric of Australian life.

The first presentation ceremony of the Britannica Australia Awards at the Australian National University was marked by messages of goodwill from both the Governor-General of Australia, Lord De L'Isle, and the Prime Minister, Sir Robert Menzies. The chairman, Sir Leonard Huxley, said in his address that the awards would give great encouragement to creative work in the arts and sciences in Australia. He recalled that this new award scheme, second in richness and prestige only to the Nobel Prize, had been announced only a short six months previously, and he commented on the remarkable speed with which the scheme had been launched and put into operation, "reflecting great credit on the members of the award committees who, recognizing the great national importance of this scheme and serving in an entirely honorary capacity, have given freely of their time and experience to ensure its success". Sir Leonard also directed his thanks to the sponsoring Company whose

vice-president, Mr. L. J. Maher, had flown to Canberra from Chicago for the inaugural awards presentation.

The new awards themselves are based on a carefully drawn-up constitution. They are made by a General Council on the recommendation of each of five adjudicating award committees established under the scheme, one for each field. Each award committee comprises 5-9 members distinguished in its field and elects its own chairman. The General Council itself has a membership of seven, comprising the five chairmen of the award committees, the chairman of the organizing committee and, *ex officio*, the general manager of the Australian branch of Encyclopaedia Britannica Inc. The administration of the awards is thus left in the entirely independent hands of experts and the sponsoring Company is only nominally represented by one vote on the General Council. Nominations of candidates of the awards can be made by anyone to the respective award committees and meritorious nominations are then discussed by the committees at as many meetings as may be required for each committee to decide on a single candidate or very short list of candidates for final decision and endorsement by the General Council.

The composition of the Britannica Australia Award Committees is as follows:

#### GENERAL COUNCIL

Sir Leonard Huxley, vice-chancellor, Australian National University, Canberra (*chairman*); Sir Macfarlane Burnet, director, Walter and Eliza Hall Institute, Melbourne, professor of experimental medicine, University of Melbourne; Mr. H. Missingham, director of the Art Gallery of New South Wales, Sydney; Dr. W. C. Radford, director, Australian Council of Educational Research; Mr. C. W. Semmler, assistant general manager (programmes), Australian Broadcasting Commission; Mr. O. A. Guth, executive assistant in the School of Physics, University of Sydney; Mr. J. J. Salmon, general manager, Encyclopaedia Britannica Inc. (Australia) (*ex officio*); Mr. G. I. Carr (non-voting co-ordinator), Gabriel I. Carr and Associates.

#### SCIENCE AWARD COMMITTEE

Sir Leonard Huxley (*chairman*); Prof. M. Chaikin, dean of the Faculty of Applied Science, and head of the school of Textile Technology, University of New South Wales, Sydney; Prof. T. M. Cherry, president, Australian Academy of Science, professor of mathematics, University of Melbourne; Dr. D. Martyn, chief officer, Upper Atmosphere Section, Commonwealth Scientific and Industrial Research Organization, Camden, New South Wales; Prof. D. Mellor, head of the School of Chemistry, University of New South Wales, Sydney; Prof. H. Messel, head of the School of Physics, University of Sydney, and director, Nuclear Research Foundation within the University of Sydney; Sir Mark Oliphant, director of the School of Research in Physical Sciences, Australian National University, Canberra; Sir Harold Raggatt, secretary, Commonwealth Department of National

Development; Prof. R. Robertson, professor of botany, University of Adelaide.

#### MEDICINE AWARD COMMITTEE

Sir Macfarlane Burnet (*chairman*); Sir John Eccles, professor of physiology, John Curtin School of Medical Research, Canberra; Sir Clive Fitts, consulting physician, Royal Melbourne Hospital and Royal Women's Hospital; Sir Ian Douglas Miller, dean of the Clinical School, St. Vincent's Hospital, Sydney; Major-General W. D. Refshauge, Commonwealth director-general of health, Canberra; Prof. V. M. Trikojus, professor of biochemistry, University of Melbourne.

#### LITERATURE AWARD COMMITTEE

Mr. C. W. Semmler, assistant general manager (programmes), Australian Broadcasting Commission (*chairman*); Mr. V. T. Buckley, senior lecturer, Department of English Language and Literature, University of Melbourne; Mr. Geoffrey Dutton, Adelaide, South Australia; Prof. S. L. Goldberg, professor of English literature, University of Sydney; Mr. W. S. Hamilton, controller of news, Australian Broadcasting Commission; Prof. C. Quentin, associate professor of drama, University of New South Wales, and director, National Institute of Dramatic Art.

#### EDUCATION AWARD COMMITTEE

Dr. W. C. Radford, director, Australian Council for Educational Research, Melbourne (*chairman*); Prof. G. W. Bassett, professor of education, University of Queensland, Brisbane; Prof. C. A. Gibb, professor of psychology, Australian National University, Canberra; Prof. C. Sanders, professor of education, University of Western Australia, Nedlands; Dr. L. N. Short, Educational Research Unit, University of New South Wales, Sydney; Mr. W. Wood, director of special education services, Department of Education, Brisbane, Queensland; Prof. W. M. O'Neil, professor of psychology, and chairman of the professorial board, University of Sydney.

#### ART AWARD COMMITTEE

Mr. H. Missingham, director, Art Gallery of New South Wales, Sydney (*chairman*); Dr. H. C. Coombs, governor of the Reserve Bank of Australia, chairman, Australian Elizabethan Theatre Trust, chairman, Reserve Bank Board, and pro-chancellor, Australian National University; Sir Daryl Lindsay, member of the Commonwealth Art Advisory Board; Dr. Bernard Smith, Department of Fine Arts, University of Melbourne; Mr. E. Westbrook, director of the National Gallery of Victoria.

#### ORGANIZING COMMITTEE

Mr. O. A. Guth, executive assistant in the School of Physics, University of Sydney (*chairman*); Miss D. Sherwin, graduate assistant to the vice-chancellor, Australian National University; Mr. G. I. Carr, Gabriel I. Carr and Associates.

## SCALE STANDARDS IN GEOGRAPHICAL RESEARCH: A NEW MEASURE OF AREAL MAGNITUDE

By P. HAGGETT, R. J. CHORLEY and D. R. STODDART

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### Dynamic Character of Scale

WHEN one compares the nature of work by geographers which has been conducted within areal frameworks of different size, it becomes readily apparent that areal magnitude is not a passive quality merely distinguishing

large-scale from small-scale investigations which in other respects are similar, but that size itself imposes other indissolubly yoked characteristics which do much to colour the whole work. Indeed, the whole question of the analysis of regional and local components<sup>1,2</sup> implies that, at different scales, different parameters become

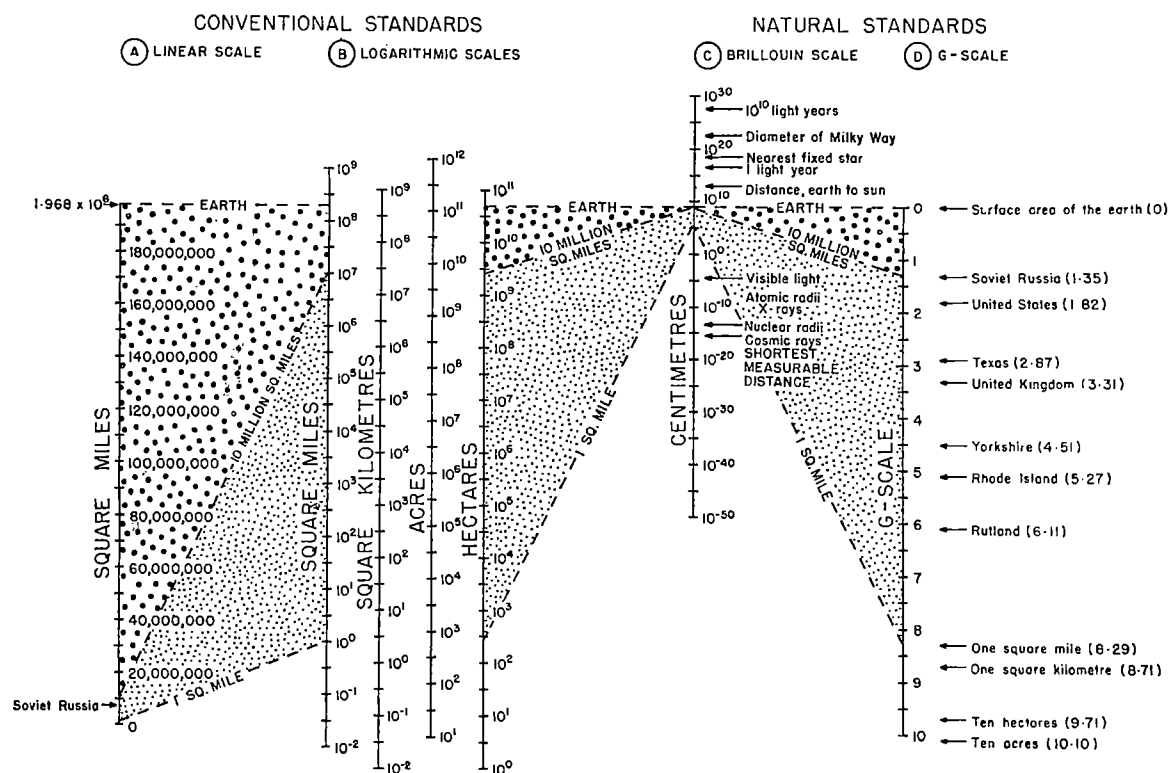


Fig. 1. Relation of the G scale to conventional measures. Scale G, the Brillouin scale, is linear and is therefore not directly comparable with scales A, B, and D. Figures in brackets after the regions indicated in scale D give the appropriate G values

dominant, different levels of generalization may be attempted, and even different problems identified.

The dynamic character of linear scale ( $A \propto L^2$ ) can be illustrated in an exaggerated but productive manner by considering the distortions involved in the reproducing of real events in model form. The inability of the model-builder to match the linear and kinematic ratios in his model is well known, and it is clear that a change of linear scale has involved drastic changes in the relationships between many of the component factors such that in many respects the model is quite unlike the real world situation it is intended to simulate. Although linear scale differences with which geographical investigations are concerned are less radical, it can be shown that here too scale is a dynamic property. A recent hydrological work by Kennedy and Brooks<sup>3</sup> has shown convincingly that whether variables in a fluvial system are identified as independent or dependent is largely a matter of the temporal or areal scales of magnitude concerned. Exploiting this work, Schumm and Lichty<sup>4</sup> have pointed out that cause-effect relationships may be obscured or even reversed depending on the span of time or areal extent under consideration in geomorphology and that, in a similar way, the system itself may be differently described. Schumm and Lichty suggest, for example, that, while a whole drainage basin may not be profitably viewed in steady-state terms, yet certain basin components may be.

It is thus possible to propose a view of areal magnitude which enables one to recognize that the scale of investigation adopted, whether consciously or unconsciously, influences the whole systematic framework of the investigation itself<sup>5</sup>. In this view, with changing areal magnitude, component factors vary in relative importance, the basic dependence or independence of the factors may change, the way in which problems are formulated may vary, the significance and opportunities of a given problem may appear in very different lights, and even different dominant problems emerge. Increasing areal coverage necessarily implies greater discarding of information and more generalization, but in even more subtle ways areal

magnitude seems to pervade and influence every feature of geographical work in such a natural manner that its influence is usually unrecognized.

### Problems of Range and Standard

In addition to the general philosophical problem of magnitude, there is the more immediate difficulty of finding a standard scale of measurement. Geographers are concerned with subjects which range in scale from continents (1,000,000–10,000,000 sq. miles) through parishes (1–10 sq. miles) to even smaller units, and although many of the more antique areal units have been dropped, the variations between the four conventional units (square miles, square kilometres, acres and hectares) are sufficiently great to make easy comparison impossible. Although in practice we may use conventional natural standards (for example, 1,000,000 acres is about the size of the County of Somerset, England; 100,000 sq. miles about the size of the State of Colorado, U.S.A., and so on) or rapidly convert the various units through tables<sup>6</sup>, the method remains cumbersome and difficult. Physical geographers, moreover, may be concerned with the measurement of objects ranging in scale down to individual sediment particles in the silt-clay range. Faced with handling sediment calibre varying over a million-fold range of sizes, sedimentologists have used various logarithmic transformations, such as Krumbein's  $\phi$  scale ( $\phi = -\log_2 D$  mm), in which large boulders ( $-10\phi$ ) and clays ( $+10\phi$ ) may both be easily accommodated<sup>7</sup>. Recent investigations of surface texture of particles using the electron microscope<sup>8</sup> have increased the linear range of geographical interest. The success of the  $\phi$  scale in sedimentology suggests that logarithmic scales provide a convenient way of collapsing differences in magnitude on to a convenient yardstick. Such scales have also been used, for example, in astronomy for measuring stellar magnitude, and in pedology for measuring pH values.

The range of interest of geographical enquiry in absolute terms may be seen with reference to a linear diagram



published by Brillouin<sup>9</sup> (Fig. 1C). This consists of a logarithmic scale ranging from  $10^{30}$  to  $10^{-50}$  cm, and includes the largest and the smallest distances so far measured (approximately  $10^{27}$  and  $10^{-13}$  cm, respectively). Within this spectrum, the area with which geographers are concerned occupies a median position, ranging from  $4.01 \times 10^9$  cm (the circumference of the Earth) down, in the case of sediments, to  $10^{-3}$ – $10^{-4}$  cm. The question arises whether any absolute units may be used to define the range of geographic interest, and to replace the arbitrary conventional units now in use.

Brillouin<sup>10</sup> has suggested we might use as a base an absolute minimum linear distance, operationally defined as that distance below which no wave or other measuring device exists to be used as a standard. The wave-length of cosmic rays is of the order of  $10^{-15}$  cm; below this length the energy quanta involved become so great as to be impossible of realization. Thus Brillouin calculates that a wave-length of  $10^{-50}$  cm would involve a mass equivalent of  $2 \times 10^7$  metric tons ( $10^{13}$  g). "Every interaction between such a wave and any physical system would involve at least one such quantum, either absorbed or emitted, and provoke an immediate catastrophe. Needless to say, this short calculation is sufficient to prove the absolute impossibility of measuring  $10^{-50}$  cm; and if he cannot measure it, a physicist will never dare speak of it"<sup>10</sup>.

However appropriate this natural standard may be for the physical sciences, there is a good case for regarding an equally fundamental unit—the surface area of the Earth—as the appropriate natural standard for geographic measurement. As Hettner argues, "geography interprets the earth as the largest region"<sup>11</sup>, and Hartshorne defined its primary concern as "... to provide accurate, orderly and rational description and interpretation of the variable character of the earth surface"<sup>12</sup>. The total surface of the globe is approximately estimated as  $1.96836 \times 10^8$  square miles (Fig. 1A), and geodetic revision seems unlikely to alter the first few integers of this estimate. The Earth's surface is thus both a natural standard and one of sufficient stability (at least in relation to the span of human history) to provide a logical unit for geographical standardization of area.

### Proposed Measure of Areal Magnitude

We propose therefore that the standard of geographical measurement shall be the Earth's surface area ( $G_a$ ), and that a scale of measurement (the  $G$  scale) shall be derived by successive subdivisions of this standard area by the power of ten. Table 1 shows the general progression of  $G$  values, while Fig. 1D shows the relation of the  $G$  scale to conventional standards of areal measurement.

$G$ scale value	Subdivision of $G_a$	Area (sq. miles)
0	$G_a$	$1.968 \times 10^8$
1	$G_a (10)^{-1}$	$1.968 \times 10^7$
2	$G_a (10)^{-2}$	$1.968 \times 10^6$
3	$G_a (10)^{-3}$	$1.968 \times 10^5$
4	$G_a (10)^{-4}$	$1.968 \times 10^4$
5	$G_a (10)^{-5}$	$1.968 \times 10^3$
$n$	$G_a (10)^{-n}$	$1.968 \times 10^{(8-n)}$

In practice, we suggest the general technique for comparing the standard (the Earth's surface) with the area under investigation is to use tables of logarithms to base 10. In view of the limited accuracy of the natural standard, values of  $G$  beyond two decimal places are unlikely to be needed in practice for which four- or five-figure tables of logarithms are likely to be sufficiently accurate.

The value of  $G$  may be given by the general formula:

$$G = \log (G_a/R_a)$$

where both  $G_a$  (the Earth's surface) and  $R_a$  (the area under investigation) are measured in the same areal unit

(for example, acres). In practice, it is more convenient to compute the value of  $G$  by subtracting the logarithm of  $R_a$  from a constant equal to the logarithm of the Earth's surface. Table 2 gives constants for four common areal standards.

Table 2

Conventional areal standard	Appropriate formula for calculation of $G$
Square miles	$8.2941 - \log R_a \text{ (miles}^2\text{)}$
Square kilometres	$8.7074 - \log R_a \text{ (km}^2\text{)}$
Acres	$11.1003 - \log R_a \text{ (acres)}$
Hectares	$10.7074 - \log R_a \text{ (hectares)}$

The advantage of the  $G$  scale as a geographical reference system stems from four characteristics. First, it uses a natural standard, the surface of the Earth, rather than existing arbitrary standards. Secondly, through its logarithmic nature it reduces a very wide range of values to a simple scale, for example running from zero (the Earth) through 10 (for an area roughly the size of Trafalgar Square) to smaller units. Thirdly, it allows ready comparison of the relative size of areas, in that regions which are different in area by a factor of 10 have values 1 unit apart on the  $G$  scale, regions which are different in area by a factor of 100 are 2 units apart, and so on. Fourthly, it simplifies the present confusion of conventional standards (Fig. 1A and B) and substitutes a simpler scale of natural values (Fig. 1D).

Against these advantages we must set the disadvantage of all new measures, unfamiliarity, and the fact that small  $G$  values indicate large areas, and vice versa, whereas we intuitively expect the reverse to be true. Other measures,

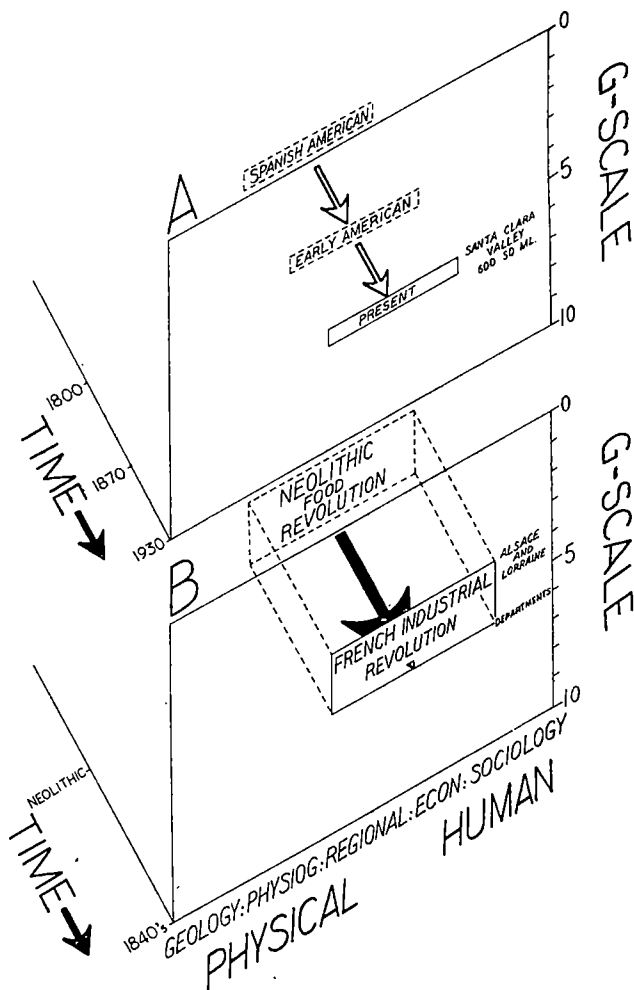


Fig. 2. Comparison of regional studies by Broek<sup>14</sup> (A) and Vidal de la Blache<sup>15</sup> (B) presented with reference to the  $G$  scale, the range of subject treatment, and the time-span under consideration

for example, British Standard sieve sizes in sediment analysis, have survived a similar problem. We do not think one solution proposed for the  $G$  scale, its reciprocal, is viable, since it makes the present scale unnecessarily complex.

### Implications for Geographical Research

Application of the  $G$  scale to existing geographical areas shows some interesting results (Fig. 1D). Values for continents range from  $G=1.06$  to  $G=1.83$  and help to correct some of the distorted size concepts which arise both from the Mercator projection and from relative accessibility (for example, Hägerstrand's<sup>13</sup> hypothesis that 'distant' areas are underestimated in size, whereas we exaggerate the relative size of nearby areas). There are, however, more fundamental implications for the whole character of geographical research.

One of the many illustrative, and even analytical, potentialities presented by the  $G$  scale is shown by a comparison of two important regional works given in Fig. 2. The investigation by Broek<sup>14</sup> of the Santa Clara Valley, California, appears here (Fig. 2A) as three regional time-slices, linked by short textural segments, in which physical and economic aspects of the area are knit into a regional treatment. Although some ranch-scale information is given, the areal scale of the investigation, at all historical periods, is fundamentally that of the 600 square mile Santa Clara Valley ( $G=5.52$ ). Contrast is provided in Fig. 2B by a similar representation of Vidal de la Blache's<sup>15</sup> classic treatment of eastern France. Here,

bounded by the two hiatuses of the Neolithic Food Revolution and the French Industrial Revolution, the region of Alsace-Lorraine is treated in the context of a time-continuum. Carrying his balanced treatment from this scale ( $G=4.12$ ) down to *Département* levels ( $G=c.6$ ), Vidal de la Blache integrates the physical and human aspects of eastern France, carrying his emphasis more deeply into the field of sociology than did Broek.

It is not difficult to imagine how the economical, standardized  $G$  scale, when used in such a comparative manner, can assist not only the convenient illustration of the scope of geographical works but also provides a tool which will aid in the analysis of their methodological bases.

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<sup>8</sup> Krinsley, D., and Takahashi, T., *Science*, **135**, 923 (1962).

<sup>9</sup> Brillouin, L., *Scientific Uncertainty, and Information*, New York, 85 (1964).

<sup>10</sup> Brillouin, L., *Scientific Uncertainty, and Information*, New York, 32 (1964).

<sup>11</sup> Schmittner, H., *Geogr. Z.*, **47**, 52 (1941).

<sup>12</sup> Hartshorne, R., *Perspectives on the Nature of Geography*, New York, 21 (1959).

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## ANTIBODY FORMATION AND THE CODING PROBLEM

By PROF. FELIX HAUROWITZ

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SINCE antibodies have been recognized as typical globulins, antibody formation is essentially formation of protein. Modern views on protein biosynthesis are stated in the simplest form in the central dogma<sup>1</sup> of molecular biology which postulates that the primary structure, that is, the amino-acid sequence of the proteins, is determined by the nucleotide sequence of the nucleic acids, and that information of this type is transmitted in only one direction, from nucleic acids to proteins, and not in the reverse direction. Frequently it is also assumed that the conformation of the native protein is uniquely determined by its amino-acid sequence and that no further genetic information is required for the folding of the peptide chain<sup>2</sup>.

Theories of antibody formation were formulated before the advent of modern concepts of the biosynthesis of proteins. They have usually been classified as: (a) selective theories; (b) template or instructive theories. According to the former, proposed first by Ehrlich (1900), the antigen selects from a number of pre-formed 'receptors' those which can specifically combine with the antigen; combination of these receptors with the homologous antigen stimulates their regeneration and the secretion of free receptors into the circulation where they appear as antibodies. In the template theories<sup>3-7</sup> it is claimed that the antigen molecules interfere with the normal process of globulin formation in such a manner that the newly formed globulin molecule (antibody) fits complementarily to the specific determinant group of the antigen molecule.

Numerous theories of antibody formation have been advanced, each of them modifying one or more of the statements of the original selective or template theory. Most of the immunological phenomena can be explained satisfactorily by any of the many variants proposed. However, in spite of intensive experimental work in this

field of research, it has not yet been possible to make a final decision between the selective role of the antigen and its role as a template. These two functions of the antigen are fundamentally different when applied to the biosynthetic process. If the antigen has merely to select between different types of preformed 'receptors', then it is not necessary to postulate interference of the antigen in the process of globulin biosynthesis, or any modification of this process. On the other hand, if the antigen interferes in this process in such a manner that antibodies are formed instead of normal globulins, the question arises in which phase of protein synthesis this interference occurs and how the complementary conformation of the combining group of the antibody is accomplished. The purpose of this present article is to answer these questions and to show that the template theory can be expressed in terms of modern molecular biology.

### Template Role of the Antigen and the Coding Problem

Breml and Haurowitz in 1930<sup>8</sup> attributed the formation of antibodies to interference of the antigen with the biosynthesis of serum globulins from their amino-acid precursors. Similar views were advanced independently by Alexander<sup>4</sup> and Mudd<sup>5</sup>. The high specificity of the antigen-antibody interaction was attributed by me<sup>6</sup> to the presence in antibodies, but not in normal serum globulins, of groups which are "spatially adjusted to the determinant groups of the antigen and therefore can approach it very closely". Pauling<sup>7</sup> in 1940 suggested that the antigenic determinant interferes only with the folding of the peptide chains which form the antibody molecules, but that it does not alter their amino-acid composition. The work of Porter<sup>9</sup> and Edelman<sup>10</sup> has

revealed that the typical 7S antibody molecule consists of two heavy *A* (or *H*) and two light *B* (or *L*) chains; their molecular weights are approximately 50,000 and 25,000 respectively. They are held together by disulphide bonds. Karush<sup>11</sup> assumes that the conformation of the antibody molecule is determined by the formation of these disulphide bridges and that their position is different in antibodies of different specificity.

*A priori* a template molecule might interfere with any of the principal steps in protein biosynthesis. These are: (a) the replication of DNA molecules; (b) the transcription of the DNA code into the nucleotide sequence in RNA molecules; (c) the translation of the latter into amino-acid sequences; (d) the folding of the peptide chains which results in the formation of the specific combining site of the antibody molecule; (e) the combination of the two *A* (or *H*) chains and the two *B* (or *L*) chains to form the antibody molecule of the composition  $A_2B_2$  (or  $H_2L_2$ ).

The last of these events (phase *e*) can be excluded from our discussion since it has been demonstrated that the four-subunits recombine *in vitro* spontaneously to yield biologically active antibody molecules<sup>12-14</sup>, and that this process does not require the presence of antigen.

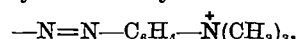
We can also exclude interference of the antigen in phase *a* (replication of DNA) since this would involve mutagenic action of the antigen. The principal objection to such a process is the failure of antibody-forming cells to produce antibody-forming daughter cells in the absence of antigen. When antibody-forming cells of a sensitized animal are transferred to a new-born or irradiated animal which has not the ability to form antibodies, they continue to multiply in the recipient and to form antibody, but lose this ability after a number of transfers from one to another recipient<sup>15,16</sup>; antibody formation is resumed after the administration of a small amount of the antigen. Similar observations have been made in antibody-forming tissue cultures<sup>17</sup>. Evidently, the presence of antigen is necessary for the production of antibody in the daughter cells of antibody-forming mother cells. We have therefore no reason to assume a mutagenic action of the antigen.

Modification of phase *b* (transcription from DNA to RNA) or *c* (translation from RNA to amino-acids) should result in changes of the composition and sequence of the amino-acids in the antibody molecule. Occurrence of such changes was indicated first by small but significant differences in the peptide maps of antibodies against human and horse serum albumin<sup>18</sup>, and against different types of pneumococcal polysaccharides<sup>19</sup>. Since these antigens contain a number of different determinant groups, the antibodies formed by their injection are heterogeneous mixtures of antibodies directed against the different determinants. It is therefore difficult to decide whether the differences in the peptide maps reflect chemical differences in the antigenic determinants or merely reflect different distribution of the injected antigens in the organism and antibody formation in different organs or cells. Some of these difficulties are avoided by the isolation of hapten-specific antibodies which combine with small well-defined determinant groups, and by a comparison of their amino-acid composition and sequence. Antibodies of this type have been produced in my laboratory by injecting rabbits with bovine serum proteins coupled to the anionic diazobenzenearsonate (Ars) or the cationic diazobenzene-*N*-trimethylammonium ( $R_4N$ ) groups and separation of the hapten-specific from the protein-specific antibodies. Koshland and Englberger<sup>20</sup> found small but significant differences in the amino-acid composition of anti-Ars and anti- $R_4N$ . Similarly we found differences in the peptide maps of the two hapten-specific antibodies<sup>21-23</sup>. These differences were extremely small; most of the peptide spots of the tryptic digests of anti-Ars and anti- $R_4N$  were identical. The observed differences cannot be caused by genetic differences, because they were also found when the two azoproteins were injected into

the same animal<sup>20-24</sup>, or when a doubly-labelled azoprotein containing both Ars and  $R_4N$  groups was injected<sup>24</sup>. The latter experiment excluded differences in the organ distribution pattern of the two antigens. It has not yet been possible to decide whether the small differences in the amino-acid composition and similar small differences in the peptide maps of the two hapten-specific antibodies reflect differences in their specific combining groups. The near-identity of the peptide maps indicates clearly that the anti-Ars and the anti- $R_4N$  antibody molecules have numerous identical amino-acid sequences in their molecules.

Although the differences between the two hapten-specific antibodies are small, they seemed to indicate interference of the antigen in the phases *b* (transcription from DNA to RNA) or *c* (translation of the code). Since we do not know of any long-range forces in biological systems, we would have to postulate the presence of antigenic determinants in the vicinity of the sites of amino-acid incorporation. This postulate is in agreement with the finding of isotopically labelled antigenic determinants in the microsomal and mitochondrial fractions of spleen and liver cells<sup>25-27</sup>, and with combination of antigen fragments with sRNA or other RNA fractions<sup>28,29</sup>. However, even if we assume a modification of the amino-acid sequence by interference of the antigenic template with the phases *b* and *c*, it is still difficult to understand why such a change in amino-acid sequence should result in the production of a peptide chain which spontaneously would fold in such a manner that the complementary combining group would be formed.

In considering this problem it must be kept in mind that the conformation of the combining site of the antibody is determined principally by the three-dimensional shape of the antigenic determinant, much less by its charge, polarity and other properties. This conclusion is based on the ability of antibodies to differentiate between *o*-, *m*- and *p*-isomers, between *L*- and *D*-isomers and also between *cis*- and *trans*-derivatives of otherwise identical antigenic determinants<sup>30</sup>. The great importance of the shape of the antigenic determinant is clearly demonstrated by the observation that antibodies produced by the injection of an azoprotein containing the positively charged azophenyl-*N*-trimethylammonium group,



combine also specifically with the uncharged azophenyl-trimethylmethane group,  $-N=N-C_6H_4-C(CH_3)_3$  (ref. 31), since the shape of the  $-C(CH_3)_3$  group is identical with that of the  $-\overset{+}{N}(CH_3)_3$  group. The question to be answered by a satisfactory theory of antibody formation is, therefore: In which phase of the biosynthetic process is information concerning the shape of the antigenic determinant transmitted to the sites of protein biosynthesis, and how does this transmittance occur?

In view of the absence of long-range forces, it must be postulated that the folding of the peptide chains (phase *d* of the biosynthetic process) occurs in the immediate vicinity of the antigenic determinant. This is merely a restatement of Pauling's<sup>7</sup> original postulate that the antigenic determinant acts as a template for the folding of the peptide chains. At first sight this postulate seems to be incompatible with the observed differences in amino-acid sequence and also with the claim that the conformation of the peptide chain is determined by its amino-acid sequence<sup>2</sup>, which in turn depends on the nucleotide sequence of the messenger-RNA molecules. Most of these difficulties disappear when the statement concerning dependence of the conformation on the amino-acid sequence is replaced by the statement that conformation and amino-acid sequence are interdependent, and that changes in conformation caused by a template might secondarily lead to changes in the amino-acid sequence.



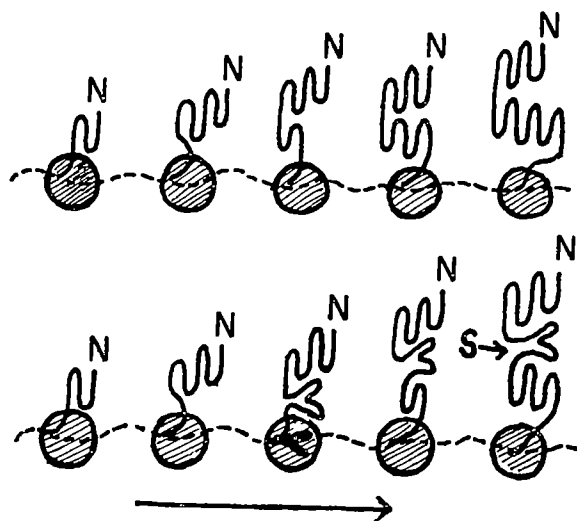


Fig. 1. The diagram shows five ribosomes linked to each other by a long thread of messenger-RNA (broken line). The upper part of the diagram represents the biosynthesis of a peptide chain of normal  $\gamma$ -globulin, the lower part the analogous process in the presence of an antigenic determinant (dark area on the third ribosome). The end of the growing peptide chain which carries the terminal  $\alpha$ -amino (or acetylamino) group is marked by the letter N, the specific combining site of the growing chain of the peptide chain; it may just as well be near the N- or C-terminus. In the diagram the transfer of information concerning only the shape of the antigenic determinant is delineated. If the determinant contains an anionic or cationic group, electrostatic interaction may result in the incorporation of an amino-acid which contains a group the  $pK$  value of which is high or low, respectively. A mechanism of this type has been suggested earlier<sup>42</sup> and is supported by experimental work of Pressman *et al.*<sup>48</sup>

We assume, in accordance with these views, that antigenic determinants are bound to some of the ribosomes or to the messenger RNA molecules as shown in Fig. 1, and that the determinant group, which is usually rigid and polar, affects the biosynthetic mechanism in two ways: (1) dipole induction and other intermolecular forces originating from the template induce a small portion of the newly formed peptide chain to fold over the surface of the antigenic determinant and thus to produce a complementary pattern; (2) while most of the aminoacyl residues are incorporated into the peptide chains of the antibody molecule in the sequence dictated by the nucleotide sequence of the messenger-RNA, deviations from the code occur during the formation of the combining site; only those aminoacyl residues are incorporated which allow the peptide chains to fold complementarily over the antigenic determinant, whereas other aminoacyl-sRNA complexes are rejected. Since the process of amino-acid incorporation is exergonic and practically irreversible, the rejection of any unsuitable aminoacyl residue must occur before the next amino-acid is incorporated. The incorporation process has been described appropriately as displacement of the terminal tRNA residue of an sRNA-peptide ester by the incoming aminoacyl-sRNA complex<sup>32,33</sup>. The rejection mentioned obviously may be considered as the inability of an unsuitable aminoacyl-sRNA complex to displace the terminal RNA residue.

Antibody formation, according to this view, involves primarily direct interference of the antigenic template in the case of folding of peptide chains of the biosynthetic process and, by rejection of unsuitable aminoacyl residues, indirectly with the translation process (case c). Most probably, the process occurs in the same cells in which the normal  $\gamma$ -globulins are formed, and is controlled by the same genetic apparatus, the same types of DNA and RNA, as shown by the occurrence of the same genetic markers in antibodies and in normal globulins<sup>44</sup>.

Our postulate that the antigenic template, by dictating definite conformation, may alter the normal amino-acid

sequence is contradictory to the simple form of the original central dogma and may appear heretical. However, modifications of the coding process by external factors have been demonstrated by several authors<sup>35-37</sup> and may be quite frequent. Processes of this type have been discussed extensively by Dean and Hinshelwood<sup>38</sup> and by Commoner<sup>39</sup>. The possibility of deviations from the central dogma in the biosynthesis of antibodies has been pointed out earlier by Spiegelman<sup>40</sup> and Me<sup>41</sup>.

The theory presented here does not involve the claim that each antigenic determinant induces in all organisms formation of the same complementary group of identical amino-acid composition. Peptide maps of hapten-specific antibodies against the azophenylarsenate residue are different in chicken and in rabbit antibodies<sup>23</sup>. Differences may also exist between antibodies formed in different organs or cells of an animal. Evidently, the complementary pattern of the combining group can be produced by different amino-acid sequences. It would be strange if the process of template action were limited to antibody formation. 'Physiological' templates may be responsible for complementary patterns in enzymes, hormones and other biologically active protein molecules.

### Objections Raised against the Template Role of the Antigen

The principal objections raised against the template theory of antibody formation are that it cannot explain: (a) antibody formation after elimination of all the antigen; (b) the characteristic differences between the primary and the secondary response; (c) the ability of the organism to differentiate between self and non-self<sup>42</sup>. The following paragraphs (a)-(c) show that these objections are based on different interpretations of experimental results rather than on divergent results.

(a) The claim that antibody formation occurs after elimination of all the antigen was originally based on the assumption that all the antigen is rapidly eliminated. While it is true that cellular or bacterial antigens do not survive as such, experiments in my laboratory<sup>25-27</sup> and in other laboratories<sup>28,29</sup> have shown that isotopically labelled determinant groups of the antigen persist in the organs of the reticuloendothelial system for many months. In experiments with <sup>35</sup>S- or <sup>14</sup>C-labelled azoprotein antigens the half-time of antigen elimination from the spleen increased from a few days shortly after a single injection to 5-7 weeks a few months after the injection<sup>26</sup>. Since the antibody titre in these experiments decreased at a similar rate<sup>43</sup>, we have every reason to assume that retention of antigen and extent of antibody formation are correlated with each other.

(b) It is a well-established experimental fact that the primary antibody response depends on the form in which the antigen is administered, and that the intensity of response to soluble antigens is increased when these antigens are made insoluble by adsorption to adjuvants. The higher antigenicity of the insoluble antigen particles is evidently due to the fact that only these stimulate phagocytosis and are thus rapidly incorporated into macrophages and other phagocytic cells, whereas the soluble antigens circulate for a long time in the body fluids. The intensity of the anamnestic response is frequently attributed to the immunological memory of the cells stimulated in the primary response. This view is based on the fact that the precipitin test becomes negative a few weeks after a primary reaction. However, continuous formation of small amounts of antibody in the circulation for more than a year after a single injection of bovine serum albumin or ovalbumin can be detected by means of the highly sensitive passive haemagglutination reaction, that is, the agglutination of antigen-coated erythrocytes<sup>50</sup>. Re-injection of the antigen a few weeks or months after the first injection results in the immediate

ormation of antigen-antibody complexes which, in contrast to the soluble antigen molecules, are rapidly eliminated from the circulation, trapped in phagocytic cells, and therefore elicit a much stronger antibody response than the primary injection of soluble antigen<sup>43</sup>. The 'memory cells', according to this view, are merely cells which, owing to their content of antigen fragments, continue to elicit the formation of small amounts of antibody.

(c) 'Self-recognition' is a more recent term for the phenomenon designated by Ehrlich as "horror autotoxicus", that is, the inability of the organism to form antibodies against its own proteins. This idea has lost its general validity by the discovery of auto-antibodies against the eye lens, the brain, testis, thyroid gland, kidney and other organs the proteins of which, owing to injury, penetrate into the circulation. In some instances these tissue proteins may undergo denaturation and become 'foreign'<sup>44</sup>. Frequently, auto-antibody formation is a physiological process which aids in the removal of products of cell damage<sup>45</sup>. The idea of self-recognition is further invalidated by the observation that rabbits injected repeatedly with large amounts of bovine serum albumin fail to form anti-bovine serum albumin so long as bovine serum albumin circulates in their blood<sup>46</sup>. Their inability of forming anti-bovine serum albumin parallels their inability of forming antibodies against their own serum albumin. Both phenomena can be attributed to the continued presence of these proteins in the blood plasma.

Summarizing, it may be said that the prolonged antibody formation and the intensity of the anamnestic reaction are explained by the template theory of antibody formation just as well as by the selective theories, and that the concept of self-recognition has lost its validity as a general principle, and therefore cannot be used as a criterion for the validity of any theory of antibody formation.

### Selective Theories of Antibody Formation

Ehrlich's ingenious theory of preformed receptors was advanced at a time when nothing was known of the nature of antibodies. In 1931 Browning<sup>47</sup> still warned that the term antibody "implies merely the acquisition . . . of properties, . . . not . . . a chemically defined substance". The idea of preformed receptors was satisfactory at a time when only a small number of antigens, mostly pathogenic bacteria or animal proteins, were known. The number of potential antigens were enormously increased by Landsteiner's<sup>30</sup> discovery that the specificity of antibodies is directed against definite chemical groups of the antigen molecules, and that numerous synthetic groups such as aromatic sulphonates, arsonates or quaternary bases are excellent determinants; antibodies against an almost unlimited number of these synthetic products can be produced by coupling the synthetic determinants to proteins and injecting the conjugated proteins into the animal organism. It is difficult to believe that the organism should contain preformed receptors against thousands of these artefacts of the chemical laboratories.

The same objection can be raised against modifications of Ehrlich's theory, particularly the natural selection theory proposed in 1955 by Jerne<sup>48</sup>, and the clonal selection theory advanced in 1959 by Burnet<sup>49</sup>. The role of Ehrlich's receptors was in these theories assigned to 'competent' cells, that is, lymphoid cells, each of them endowed with the ability of forming only one type of antibody. It was further assumed that this ability, like other genetic characteristics, is transmitted to the daughter cells and that antibody production can occur in the absence of antigen once the multiplication of the respective cell type has been stimulated by the antigen. Since multiplication of somatic cells is an asexual process, the descendants of each of the stimulated mother cell form a clone. The antigen would select that clone which produces the homologous antibody. The intensity of the antibody

response in re-injected animals was attributed to a large number of descendants of the cells stimulated by the first injection of antigen.

The original clonal selection theory was abandoned when it was found that single cells isolated from an animal sensitized with two different antigens formed occasionally two different antibody types<sup>50,51</sup>, that the bulk of the cells which formed only one or the other type of antibody were randomly intermingled and did not appear in separate clusters as would be expected in the formation of clones<sup>52</sup>, and that a pure clone of mouse cells injected into irradiated mice was able to form antibodies against four different antigens<sup>53</sup>.

In considering difficulties of the clonal selection theory, Burnet<sup>42</sup> stated that "the only escape would be to postulate a rate of random mutation of pattern as high as . . . in early embryonic life". Indeed, Burnet<sup>54</sup> has recently proposed a new theory which might be designated as 'random mutants theory'. In this theory the postulate of re-adapted cells has been abandoned and replaced by the claim that rapid random somatic mutation is the only source of immunological pattern and that "clonal selection, positive or negative, can mould the population of mutants to provide the . . . adapted structure . . .". Several objections can be raised against this new theory: (a) Rapid mutations in adult animals have not been observed; the rapid mutations in developing embryos occur unidirectionally toward differentiation, but not randomly. (b) If the cells endowed with the ability of antibody formation would continuously undergo rapid mutations, they would also rapidly lose their specificity and it would be difficult to explain the intense anamnestic reaction unless the antigen persisted in the cells as postulated by the template theory, but denied by the selective theories. (c) It is difficult to reconcile the assumption of continued rapid mutations of the  $\gamma$ -globulin producing cells with the fact that the peptide chains of the antibodies of an individual have the same genetic markers as the analogous chains of his normal  $\gamma$ -globulins.

Most of the objections raised against cellular and clonal selection have been avoided by Jerne<sup>55</sup>, who has withdrawn his original postulate<sup>48</sup> of cellular selection and has replaced it by the assumption that the genetic apparatus of each competent cell contains "a complete dictionary against all foreign antigens" and that selection occurs intracellularly. A mechanism of this type had been suggested earlier by Szilard<sup>56</sup>, who assumed that repressor molecules interfere with the production of specific proteins (antibodies) at the genetic level, and that diffusion of the antigen into the cell causes derepression. Similarly, Jerne<sup>56</sup> suggests that specific protein synthesis is accomplished by the repression of RNA-messages arriving from uninhibited genes. The diagram in Fig. 1 makes it unnecessary to invoke the presence of specific repressors since the antigenic determinant modifies directly that part of the antibody molecule which forms the combining site. However, if there were individual differences between the ribosomes of a cell, and if the antigenic fragment could combine with only some but not with other ribosomes, a selective factor would be introduced into the template model depicted in Fig. 1. At present we have no information about any specific differences within the ribosome population of a cell.

### Conclusion

The purpose of this article is to demonstrate that the template theory of antibody formation is reconcilable with the present views on coding and protein biosynthesis. The finding of the injected antigen in the microsomal fraction of spleen and liver indicates that the antigenic determinants are close to the ribosomal sites of amino-acid incorporation. It is assumed that intermolecular forces between the rigid, polar antigenic determinant and the nascent peptide chain lead to: (1) the rejection of

amino-acyl residues which would not allow the peptide chain to fold complementarily over the template; (2) their replacement by amino-acyl residues which enable the peptide chain to form the complementary combining site of the antibody molecule. It is assumed that in this manner the amino-acid sequence dictated by the messenger-RNA is modified in that part of the antibody molecule which combines specifically with the antigenic determinant. Template action of this type may also be involved in the biosynthesis of enzymes, protein hormones or other protein molecules.

The template theory of antibody formation is supported by the persistence of the antigen over long periods of time, and by the demonstration that antibody formation is possible only in the presence of antigenic determinants and ceases after their elimination. I believe I have shown that the template action of the antigen satisfactorily explains antibody formation, and that it is not necessary to invoke random mutation of lymphoid cells and selection of suitable mutants by the antigen as responsible for antibody formation. Nor is it necessary to postulate the presence of 'memory cells' in order to explain the anamnestic reaction. Finally, serious doubts have been raised whether the organism has the ability of differentiating quite generally foreign proteins from autologous proteins.

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## FOOD PREFERENCE AND SENSITIVITY OF TASTE FOR BITTER COMPOUNDS

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THE acceptance of one type of food and the rejection of another depend on a variety of factors, including the physiological state of the body as well as social custom and experience. The relative sensitivity of taste perception has also been shown to be important. Fischer *et al.*<sup>1</sup> have found that in a group of 'college'-age subjects the percentage of foods disliked from a list of 118 was significantly correlated with the taste sensitivity for quinine and probably also with that for 6-n-propylthiouracil (PROP), but not for sucrose, sodium chloride or hydrochloric acid.

The present investigation resembled the earlier one in that taste sensitivities to quinine and PROP were determined, but differed in the use of a larger and older population and in the method of estimating food preferences. A

questionnaire was used which was designed to reduce, so far as possible, the influence of social custom by using a list of a few carefully selected foods all of which are in wide use locally and which are commonly prepared in a variety of forms. Subjects were rated according to their preference for which of three alternate ways they preferred a given food to be prepared. In effect, a choice was offered between mild, moderate and strongly tasting methods of preparing certain foods.

The sample consisted of 187 adult individuals, 51 males and 136 females. All were of European descent and their ages ranged from 22 to 66 with an average of 38. Contact was made with the majority of subjects through the Cleveland Area Twin Registry. The remaining subjects were associated with the staff at the Cleveland Psychiatric



Institute, and were selected on the basis of availability. The sample included 39 husband-wife pairs, 16 pairs of monozygotic twins, and 10 pairs of dizygotic twins.

Taste thresholds (the lowest concentration of the test solutions which could be distinguished from water) were determined by the method of Harris and Kalmus<sup>2</sup> with several modifications<sup>3</sup>. The method involved separating by taste randomized sets of eight cups, four of which contained 5 ml. of test solution and four distilled water placebo. Relatively dilute solutions were tasted first and the concentration was increased gradually until the subject could distinguish correctly between the two sets of cups. The modifications included the use of distilled water as the solvent and a mouth rinse before tasting each cup.

Solutions were made up as follows: *PROP*, 1.0212 g 6-*n*-propylthiouracil (Mann Chemicals) dissolved in 1 l. water gave solution number 14. This was an almost saturated solution and had a concentration of  $6.0 \times 10^{-3}$  M. Serial doubling solutions then yielded the lower solution numbers down to 1 ( $7.32 \times 10^{-7}$  M). *Quinine*, 1.1744 g 1-quinine sulphate (U.S.P.) dissolved in 1 l. water gave solution number 13. Dilutions were then made as for *PROP*. A solution of a given number of either compound had the same molar concentration. Subjects were tested once and the threshold for quinine was determined before that of *PROP*. This method of determining thresholds has been shown to be reliable and repeated tests show little intra-test variation<sup>3</sup>.

Food preferences were determined by means of a questionnaire divided into two parts:

(A) A list of widely used foods which are commonly prepared in a variety of ways, so as to provide a graded series ranging from mild, through intermediate to strongly tasting. Subjects were instructed that "several common foods and drinks are listed below. Each may be prepared in a number of different ways. Please indicate on the list below which of the three alternate choices you prefer". The list with the alternative choices was as follows, except that the choices were randomized: (1) Coffee: with more than 1 spoon of cream/with 1 spoon of cream/black, no cream. (2) Coffee: with more than 1 spoon of sugar/with 1 spoon of sugar/no sugar. (3) Cheese: American/Longhorn or Swiss/blue cheese. (4) Cheddar cheese: mild/medium/sharp. (5) Salad dressing: mild/oil and vinegar/roquefort or blue cheese.

Scores were determined as follows: questions 1 and 2 were treated as a single question as both referred to coffee. One point was given for a choice of either more than 1 spoon of cream or sugar or 1 spoon of cream and 1 spoon of sugar. One spoon of cream or 1 spoon of sugar was given 2 points, and black coffee without sugar 3 points. For questions 3, 4 and 5, 1 point was given for the first choice, 2 points for the second and 3 for the third. The minimum score was therefore 4 points (mildest choice) and the maximum 12 (strongest tasting choice).

(B) A list of 5 foods. Subjects were asked to answer 'yes' or 'no' to whether they liked: grapefruit juice, lemon juice, sauerkraut, vinegar and horse-radish.

Verbal explanations of the questionnaire were given if required and, if a food was not customarily consumed, the subject was requested to indicate his preference if he would take it.

The entire food-preference questionnaire was given to 181 subjects. Of this number 15 could not complete Part B because they were unfamiliar with one or more of the foods listed. Incomplete forms were not counted, leaving a total of 166 complete scores on Part B. Six subjects could not complete Part A for the same reason, but an additional 12 subjects were given Part A only, as Part B was added after the experiment had begun. The total number to complete Part A was therefore 187.

Results relating to Part A of the food questionnaire will be considered first. The mean taste thresholds with their standard errors for quinine and *PROP* for subjects who preferred the mild, medium and strongly tasting

Table 1. MEAN TASTE THRESHOLDS WITH THEIR STANDARD ERRORS FOR SUBJECTS SELECTING MILD, MEDIUM AND STRONG TASTING CHOICES IN QUESTIONS 1-5 ON THE FOOD QUESTIONNAIRE

Question No.		Choice of foods tasting		
		Mild	Medium	Strong
Quinine	1 and 2	(100) 5.87 ± 0.19	(42) 5.65 ± 0.32	(45) 5.82 ± 0.27
	3	(78) 5.21 ± 0.19	(82) 6.17 ± 0.19	(27) 6.33 ± 0.48
	4	(84) 5.23 ± 0.20	(46) 5.96 ± 0.25	(57) 6.49 ± 0.24
	5	(52) 5.58 ± 0.29	(84) 5.66 ± 0.20	(51) 6.24 ± 0.24
	1 and 2	(100) 9.29 ± 0.25	(42) 9.28 ± 0.41	(45) 10.24 ± 0.37
<i>PROP</i>	3	(78) 8.68 ± 0.26	(82) 10.20 ± 0.27	(27) 10.11 ± 0.54
	4	(84) 8.74 ± 0.25	(46) 9.52 ± 0.32	(57) 10.77 ± 0.26
	5	(52) 8.84 ± 0.37	(84) 9.68 ± 0.25	(51) 10.06 ± 0.38

No. in each group are shown within parentheses.

choices in questions 1-5 are shown in Table 1. It is apparent that taste sensitivity for quinine and *PROP* is more important in determining preference in questions 3, 4 and 5 than in determining choice of the ways coffee may be prepared (questions 1 and 2).

The total scores on Part A of the food questionnaire for the total sample have been tabulated with the taste thresholds for quinine in Table 2 and for *PROP* in Table 3. The correlation coefficients between the thresholds for quinine and *PROP* and the food-preference scores are shown in the first column of Table 4. There are highly significant positive correlations between high scores on the food questionnaire (that is, a preference for strongly tasting foods) and high taste thresholds (that is, insensitive taste) of both quinine and *PROP*. Table 4 also shows that the thresholds for quinine and *PROP* are themselves correlated. The partial correlation coefficients for the three comparisons were therefore calculated, and are shown in the second column of Table 4. The correlation between food preference score and quinine threshold was then no longer significant, but that between food score and *PROP* threshold remained significant.

Results from Part B of the food-preference questionnaire were analysed by calculation of the linear regression coefficients of taste thresholds on number of foods disliked. The coefficient with quinine was found to be  $0.319 \pm 0.114$

Table 2. TASTE THRESHOLDS FOR QUININE AND SCORES ON PART A OF FOOD-PREFERENCE QUESTIONNAIRE

Quinine threshold	Questionnaire score (Part A)										Total
	4	5	6	7	8	9	10	11	12		
18						1					1
12							1				1
11											
10		1			1	1					3
9					5	1			1		7
8		1	1	4	2	2			2		15
7	1	5	2	9	6	6	1	3	3		36
6	3	9	7	10	9	6	3		1		48
5	4	4	4	5	12	3	2	1			35
4		5	1	7	3			2			18
3	5	3	1	2	2	2	1				16
2		1	2	1	1				1		6
1	1										1
Total	14	20	21	36	42	20	10	9	6		187

Table 3. TASTE THRESHOLDS FOR PROP AND SCORES ON PART A OF FOOD-PREFERENCE QUESTIONNAIRE

PROP threshold	Questionnaire score (Part A)										Total
	4	5	6	7	8	9	10	11	12		
> 14					5			1	3		9
14		2			3	1		1			7
13					2	3			1		6
12	2		1	5	4	4	1	1			18
11		3	2	4	4	1	2				16
10		1	4	7	5	4	2		2		25
9	1	6	3	8	5	1	3				29
8	2	12	6	7	7	4	1	1			40
7	5	1	2	3	4	1	1	3			20
6	2	3	3		2	1					11
5	1				1						2
4		1									1
3											
2											
1	1										1
Total	14	29	21	36	42	20	10	9	6		187

Table 4. TOTAL AND PARTIAL CORRELATION COEFFICIENTS WITH THEIR STANDARD ERRORS BETWEEN THRESHOLD FOR QUININE AND *PROP* AND THE SCORE ON PART A OF THE FOOD-PREFERENCE QUESTIONNAIRE. (Data from Tables 2 and 3)

	Correlation coefficients	
	Total	Partial
Quinine/food score	+0.262 ± 0.068	+0.082 ± 0.073
<i>PROP</i> /food score	+0.366 ± 0.064	+0.277 ± 0.068
Quinine/ <i>PROP</i>	+0.540 ± 0.052	+0.497 ± 0.055

and with PROP  $0.480 \pm 0.146$ . Both these values are significantly different from zero, and support the conclusion<sup>1</sup> that there is a positive association between increasing number of foods disliked and increasing taste sensitivity.

The sample included 39 husband-wife pairs. The members of these pairs are not closely related genetically and there is no significant correlation between their scores for quinine or PROP in spite of the fact that they have shared a common environment for varying lengths of time. A significant positive correlation was observed between the scores on the food questionnaire of husbands and wives. On Part A of the questionnaire the correlation coefficient was found to be 0.482 (significant for  $N=39$  at the 0.01 level). The 16 pairs of monozygotic twins in the sample were all married and had lived apart for varying lengths of time. Their scores on Part A of the food-preference form were found to be highly correlated (0.70, significant for  $N=16$  at the 0.01 level). The scores on Part A of the questionnaire for 10 pairs of dizygotic twins, 8 pairs of whom lived apart, showed an insignificant correlation of 0.18.

Both males and females showed a positive correlation between preference for mild-tasting foods and relatively sensitive perception of the taste of quinine and PROP. The males showed a somewhat higher correlation than the females, but the difference was not statistically significant.

Taste sensitivity for PROP is closely associated with that for phenylthiourea (PTC)<sup>2</sup>, which in turn is associated with that for a variety of thiourea and thioamide compounds<sup>3</sup>. Taste thresholds for all these compounds appear to be largely determined by the same pair of genes. The relative proportion of the two genes varies in different populations and the system provides one of the classic examples of polymorphism. The equilibrium between the genes must be of great antiquity as the polymorphism has been identified among apes<sup>4</sup>. The factors which maintain the balance are not clear. Many thiourea and thioamide compounds have anti-thyroid properties and some of them occur naturally in vegetables, particularly in members of the cabbage family<sup>5</sup>. It has been suggested that the existence of the PTC-PROP tasting genes in human populations is associated with the ability to taste these toxic products in food<sup>6</sup>.

The flavour of the majority of foods is the result of a medley of tastes and odours, and it may at first seem surprising that the choice of certain foods is correlated with taste sensitivity to a single compound such as PROP. However, sensitivity to PROP has been shown to be correlated with taste sensitivity to a wide variety of

other compounds, including those tasting bitter, salty, sour and sweet<sup>7</sup>. The association between taste and odour sensitivities, if any, has received little investigation.

Repeated determinations of the taste threshold of an individual have shown that under controlled conditions the thresholds for PROP and quinine are relatively stable characteristics<sup>8</sup>. However, a number of factors are known to effect thresholds for these compounds. For example, there is a gradual decline in sensitivity with increasing age<sup>9</sup>. Heavy smoking habits are associated with relative taste insensitivity<sup>9</sup> and duodenal ulcer is associated with increased sensitivity to PROP<sup>10</sup>. Increased sensitivities in the four taste modalities, salt, sweet, sour and bitter, have been reported for patients suffering from cystic fibrosis<sup>11</sup> and adrenal insufficiency<sup>12</sup>. In some women, taste sensitivity for PROP and quinine fluctuates with the phases of the menstrual cycle<sup>13</sup>.

Our investigation has revealed social influences on food preference as well as demonstrating the importance of perceptive factors. A correlation in food preference between husband and wife was apparent in spite of the absence of correlation between their taste thresholds. We may note, in passing, that for our 39 husband-wife pairs the correlation between PROP threshold and food preference score was 0.495 for husbands but only 0.335 for wives. This is not a statistically significant difference, though one may surmise jestingly that the woman who stirs the pot moulds her preferences to cater for the tastes of her husband rather than for her own.

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## OBITUARIES

### Dr. F. J. Richards, F.R.S.

WITH the death, after prolonged illness, of Dr. F. J. Richards on January 2, 1965, Britain has lost an outstanding plant physiologist, and his colleagues a much-valued friend. He was educated at Burton-on-Trent Grammar School and then entered the University of Birmingham, where he read botany and biochemistry, graduating with honours in 1924. Two years later, after a period of research on fungal respiration at Birmingham, he joined the staff of the Institute of Plant Physiology of the Imperial College of Science and Technology, London, under the directorship of Prof. V. H. Blackman. Located at Rothamsted from this time, he began his close collaboration with the late Prof. F. G. Gregory in the field of mineral nutrition of plants. With his extraordinary thoroughness, Richards examined the syndromes of deficiency in the barley plant (especially of phosphorus and potassium) in all its aspects: growth analysis, rates

of respiration and photosynthesis, water content, carbohydrate and nitrogen metabolism, the effects of other cations, in particular the partial replacement of the essential role of potassium by rubidium, etc. These investigations established his international reputation and continued, in collaboration with his students, until his death. More recently, the discovery that the amide putrescine is accumulated under potassium deficiency conditions has led to further important metabolic and enzymatic investigations.

In 1930, still in connexion with his Birmingham interests, and with the assistance of his wife, Richards followed up R. H. Yapp's investigations on the rates of accretion in the salt marshes of the Dovey Estuary. In the analysis of the data, he used the then new statistical techniques devised by R. A. Fisher, and became one of the first to introduce such methods into ecological problems. His interest in statistics led him to devise a method for the graphic representation of the results of factorial

Dr. R. F. A. Dean

experiments, which enables the immediate visual appreciation of their main effects and their interactions.

Richards's considerable mathematical ability found another outlet in the investigation of phyllotaxis. What was initially a hobby developed into a serious investigation. Taking into account the radial as well as the tangential spacing of leaf primordia at the apex, he was able to derive a function describing phyllotaxis uniquely in terms of a single index which grades smoothly from one order of phyllotaxis to the next and is applicable to all systems. A simple transformation allows the index to be applied to a conical surface when the apical angle is known (equivalent phyllotaxis index). Application of these techniques to data derived from serial sections of growing points enabled detailed analysis to be made of rates of growth of the various parts of the apex, its changing shape at different developmental stages of the plant, etc. These developments have placed the quantitative measurement of phyllotaxis on a completely new basis and represent the greatest advance in this field for many years. Quite recently, Richards developed a flexible, generalized growth function of great utility which is suitable for a considerable diversity of data.

In 1958, when the late Prof. F. G. Gregory retired from the chair of plant physiology and the directorship of the Institute of Plant Physiology, the Institute was dissolved, and Richards took over the research group located at Rothamsted as director of the new Agricultural Research Council Unit of Plant Morphogenesis and Nutrition formed for this purpose, which was later moved to Wye College.

Richards was one of the most modest of men and of a rather shy disposition; thus it was a particular pleasure to his friends when his achievements were recognized by his election to the Royal Society in 1954. He had a delightful, gentle sense of humour which enlivened many a tea-time discussion in the laboratory. In spite of his usual reticence, he could be very firm in any controversial matter where scientific principle was concerned. With his acutely critical mind he would at once see the essential core of any problem, and a friendly discussion between him and F. G. Gregory, who was as usual bubbling with ideas, was an intellectual joy to witness.

Richards served as executive editor of *Plant and Soil* and also on the editorial board of the *Journal of Experimental Botany*. Although he never held a teaching post, his influence on younger research workers was very considerable. In his unhurried, quiet, helpful and completely unselfish manner he would stimulate their enthusiasm as well as their critical faculties and bring out the best in all who had contact with him, and many a scientific paper which did not carry his name or even an acknowledgment was the better for his meticulous attention.

The interest in natural history in his parental home found its reflexion in Richards's scientific hobbies. He built his own reflector telescope, made a collection of most of the commoner macro-lepidoptera; a keen photographer, he specialized in stereo-photography, and above all, he enjoyed his mathematical interests: number theory, the Fibonacci series and numerous geometric and algebraic variations he derived therefrom. During his holidays in Wales, Dartmoor and elsewhere, he pursued his archaeological interests, visiting prehistoric sites and collecting artefacts. With his keen eye he even discovered and made a collection of palaeolithic flint tools on the classical fields of Rothamsted. All these hobbies were followed with the same meticulous thoroughness as was displayed in his work, and his students and colleagues were privileged indeed to share in them. When he died at the early age of sixty-three there can have been few people whose retirement would have held greater promise of useful and enjoyable activity.

He will be remembered with the greatest affection by his many friends, students and colleagues, and his wife and two daughters who survive have our sincerest sympathy in their loss.

W. W. SCHWABE

WHEN Dr. Rex Dean died on December 2, 1964, Britain lost an exceptional character.

After he left school, Dean went straight into the Bank of England and worked there for ten years. Here he found time for his artistic interests and revelled in the art galleries and musical opportunities of London. One of his characteristics was a desire for knowledge. Books were good, but better still was the chance of talking to an expert. He was not interested in money, and his restless ambition and enthusiasm found no satisfaction in a banking career. Comparatively late in life, therefore, he began to study medicine at St. Thomas's Hospital, where he qualified in the shortest possible time and took up a house appointment at Addenbrooke's Hospital, Cambridge, in 1943. By this time he was an accomplished pianist with a profound knowledge of the great musical masters past and present. He was also a painter of no mean skill.

Dean at once began to show his devotion to clinical medicine. He loved to examine all his patients himself, never took anything for granted and always saw that everything possible was done for their present and future welfare. He joined the Department of Medicine (later the Department of Experimental Medicine), Cambridge, in 1944 and thus started a career in medical research which he was to pursue for the rest of his life.

Dean went to Germany in 1946 with the Medical Research Council team that set out to investigate the effects of undernutrition on the German population. Here he took an active part in all the studies that were being made, and we quickly began to value his ability, not only as a clinician but also as an administrator. As an investigator he was at first almost a beginner, but he quickly grasped the essentials of organizing a controlled experiment involving a large number of people, and he was a tower of strength throughout the three years we were in Wuppertal. Dean worked very hard, and from the very beginning set high standards for himself and those working under him. His relaxations were painting and music. He delighted in the Bechstein grand piano which was in the 'Herrenzimmer' of the old German house where we lived, and he often went on playing long after everyone else had gone to bed.

The first investigation of which Dean took sole charge was one designed to find out if plant proteins could substitute effectively for milk proteins in the diet of infants and young children. It was possible to do this under controlled conditions in Wuppertal at that time, for the City and even the orphanages were desperately short of milk. He first tried soy bean proteins and at once ran into difficulties over the trypsin inhibitor associated with them, and made numerous journeys to visit experts, manufacturers and others who might help him and at the same time kept the nutritional work going and, even more important, the German staff at the orphanage and the public health authorities solidly behind him. The results of this investigation were published as a *Medical Research Council Special Report*, and on the basis of this work Dean was awarded the Ph.D. (Cambridge) degree.

Dean's success with this investigation made him anxious to apply the results on a large scale where they were clearly needed and likely to be so for some time. His chance came when he was invited by the Medical Research Council to set up a Unit for the study of infantile malnutrition at Kampala. From this he never looked back and, in spite of great difficulties and misunderstandings at times, he gradually built up an organization centred on the small research hospital and laboratories which he himself designed. The money for this was provided, largely through his own persuasive efforts, by the Uganda Government, the World Health Organization and the International Children's Centre in Paris, but he was always supported by the Medical Research Council. Here



Dean was in his element. He explored the possibilities of a number of plant proteins, but never lost sight of the importance of getting at the fundamental biochemistry of kwashiorkor, and his own work and that of his colleagues has thrown a flood of light on the disturbances in enzyme function and amino-acid metabolism which are such a feature of it. Through it all Dean always remained the clinician. He set up the highest standards of diagnosis and investigation, but always put the welfare of his children first. He knew how to deal sympathetically with those in charge of the child, and how to explain to them in the simplest possible way the child's future needs. When Dean first went to Uganda, severe kwashiorkor was quite common, but now it is rarely seen. This is largely due to his work and his influence, and 'Dr. Dean' is a household word among the people living in a wide area around Kampala. On the strength of his work in Wuppertal and Uganda he was elected a Member and later a Fellow of the Royal College of Physicians, honours which he much prized.

Dean was part author with Profs. H. C. Trowell and J. N. P. Davies of a book entitled *Kwashiorkor*, which was published in 1954 and is now the standard work on the subject. He also published many scientific papers describing his observations in Uganda.

At one period of his career Dean travelled a great deal for the World Health Organization, and one of these journeys in 1955 led him to Malaya. There he was asked to see a child in a jungle village suffering from an obscure disease. It was characteristic of Dean to accept such an opportunity to acquire experience and at the same time to be of help, but from this child he contracted an unknown virus infection which led to an ascending paralysis from which he never fully recovered, although some of his finest work was done thereafter. No longer able to take exercise on his feet, he took up swimming to keep himself fit and used to make a habit of this whenever he could afford the time. He remained in full charge of his Unit at Kampala until a few days before his death and, although he had become increasingly aware that his own future was becoming only too short, he continued to make plans enthusiastically for the future of his Unit and his staff.

Dean married in 1931, and is survived by his wife and daughter, who has taken up music as her career. He will be missed by many people throughout the world, but not least perhaps by the unborn babies of Uganda.

E. M. WIDDOWSON  
R. A. McCANCE

## NEWS and VIEWS

### General Physiology in the University of Leicester :

Prof. R. Whittam

DR. R. WHITTAM, who has been appointed to the new chair of general physiology in the University of Leicester, read chemistry at the University of Manchester after service in the Royal Air Force and in 1951 joined the Department of Biochemistry in the University of Sheffield, under Prof. H. A. Krebs, first as a Ph.D. student and later as a Stokes Research Fellow. He worked as a Beit Memorial Research Fellow in the Physiological Laboratory, Cambridge, from 1955 until 1958 under Prof. A. L. Hodgkin and then moved to Oxford, first as a member of the Medical Research Council Unit for Research in Cell Metabolism, and later as a University lecturer in biochemistry. In 1959 he spent 6 months in Prof. D. Nachmansohn's laboratory at Columbia University, New York. His research work has been mainly concerned with the chemistry of physiological functions and especially with those processes which involve the migration of ions across cell membranes. Together with a number of collaborators he has investigated in particular the problem of how the chemical energy of foodstuffs supports such activities as secretion, mechanical contraction and electrical conduction. He has recently published a book *Transport and Diffusion in Red Cells*, one of the monographs sponsored by the Physiological Society. Dr. Whittam will bring valuable experience to the University of Leicester and further strengthen the School of Biology which was recently extended by the creation of a Department of Biochemistry (Prof. H. L. Kornberg) and a Department of Genetics (Prof. R. M. Pritchard).

### Biochemistry in the University of Newcastle upon Tyne :

Prof. Kenneth Burton

DR. KENNETH BURTON, who is at present on the staff of the Medical Research Council's Cell Metabolism Research Unit in the Department of Biochemistry, University of Oxford, has been appointed to the newly-created chair of biochemistry in the University of Newcastle upon Tyne. Dr. Burton, a scholar of King's College, Cambridge, graduated in 1946. After a period of research in Cambridge he moved to Sheffield and later spent two years in the Department of Biochemistry in the University of

Chicago. His especial interest lies in the field of molecular biology, particularly the biochemistry of nucleic acids. The establishment of a new chair in biochemistry is part of a plan of expansion in biological sciences at Newcastle. It is expected that the teaching of biochemistry as a separate subject will begin in October 1966.

### The Oersted Medal :

Prof. Philip Morrison

PROF. PHILIP MORRISON, of Cornell University, has been awarded the twenty-ninth annual medal conferred by the American Association of Physics Teachers. Prof. Morrison has contributed many research papers, and collaborated with H. Bethe in the production of a well-known text-book on nuclear theory. His teaching experience ranges "from kindergarten to postgraduate institutes". He originated and directed a course in physics for non-science students at Cornell, and he has pioneered the teaching of science in the elementary school, especially light, mechanics, and applied mathematics. He has prepared a book on elementary science education, but is at present working in the Massachusetts Institute of Technology on a quantum mechanics text-book for first-year university students. Prof. Morrison has been a faculty member of Cornell University since 1946, and a full professor since 1956; he has visited, and taught in, London, Israel, India and Japan.

### Professional Civil Servants

IN commenting on the debate in the House of Lords on December 2, 1964, in which several speakers referred to the serious shortage of engineers, Mr. J. Lyons, writing in the February issue of *State Science*, the journal of the Institution of Professional Civil Servants, continues the Institution's criticism of the Government's failure to provide satisfactory conditions of service for its own professional staff. By the end of 1965 the pay research report on the Works Group should be available and negotiations on the pay of engineers and other professional staff in the Civil Service should commence. By that time, Mr. Lyons concludes, the Government should demonstrate very clearly, by the way it improves the salaries and status of its own professional staff, the importance it attaches to raising the status of technology and technologists throughout Britain.

## Student Numbers at U.K. Colleges of Advanced Technology

IN a written answer in the House of Commons on February 9, the Secretary of State for Education and Science, Mr. A. Crosland, stated that of the 13,811 full-time and sandwich students who had enrolled at the ten colleges of advanced technology in October 1964, 10,192 were in technology, 2,461 in science, 824 in social studies, and 334 in liberal studies. For October 1963 the corresponding figures were: 11,557, 8,925, 1,862, 559, and 211; and for October 1962: 10,089, 8,052, 1,500, 357, and 180. Of the 135,186 full-time university students (undergraduate and postgraduate) enrolled in October 1964, 19,964 were in technology, 35,799 in science and mathematics, 19,794 in medicine, dentistry, veterinary science, agriculture and forestry, and 17,671 in social studies. For October 1963 the corresponding figures were: 124,002, 18,101, 33,212, 19,042, and 15,405; and for October 1962: 116,610, 16,652, 30,791, 19,230, and 13,273. In a further written answer on February 11, Mr. Crosland added that in October 1964 universities in Great Britain, excluding Oxford and Cambridge, planned to admit 5,610 students to study technology, but 420 fewer were admitted.

## Transport Planning

IN reply to a question in the House of Commons on February 8, the Minister of Transport, Mr. T. Fraser, announced that Lord Hinton was temporarily to join his Ministry as a special adviser on transport planning. He would be responsible for enquiring into the means whereby, and the extent to which, the transport of goods and passengers in Britain could best be co-ordinated and developed in the national interest. The study, which was expected to be completed before the end of the year, would, in particular, examine: (1) the pattern of long-distance transport services likely to be required in the future, with particular reference to the co-ordination of investment policies for highways and railways; (2) the methods of achieving a properly co-ordinated use of the main trunk route transport systems, and especially the right balance between the use of road and rail; (3) how operational co-ordination could be improved between different forms of transport. Mr. Fraser said he was also appointing a small Transport Advisory Council, with himself as chairman, and with Lord Hinton as a member, to assist him on transport policy matters generally. The other members would be: Prof. A. Day, Lord Holford, Prof. E. F. Jackson, Alderman H. Walton, Messrs. J. F. H. Davies, W. J. P. Weber and E. G. Whitaker. In answer to further questions as to the functions of this Council, Mr. Fraser said that it would consider the whole question of the implementation of the Buchanan Report and the very large problems connected with intra-conurbation transport. While admitting that the co-ordination of air services did not come under the Ministry of Transport, he wanted Lord Hinton to advise on the co-ordination of rail, air, road and sea transport so that a complete picture could be obtained for determining the best way to co-ordinate the whole of Britain's internal transport services.

## The Atomic Energy Authority: Trading Fund

IN a written answer in the House of Commons on February 9, the Minister of Technology, Mr. F. Cousins, announced that from the beginning of the next financial year the commercial operations of the Atomic Energy Authority would be separated from the Authority's other activities, which are financed directly from the Atomic Energy Vote, and organized in a Trading Fund. These production activities had grown substantially in recent years, particularly the manufacture of fuel elements for sale to electricity generating authorities and for export, and sales for the next few years were likely to be: reactor fuel elements, £20 million; electricity, £7 million; radio-

active isotopes, £2 million. No change was involved in the organization and responsibilities of the Authority, nor was legislation required. The new arrangements would provide a suitable financial basis for the Authority's fuel element business, based on the Springfields plant, and would also cover the reactors at Calder Hall and Chapelcross, and the Radiochemical Centre at Amersham. The diffusion plant for the production of uranium-235 at Capenhurst and the plutonium separation plant at Windscale would not initially be brought within the Fund, but their inclusion would be considered when appropriate. The former was running at a low level of output pending decisions on the production of uranium-235 for such purposes, and trading activities at Windscale were relatively small.

The operations of the Fund would be subject to examination and audit by the Comptroller and Auditor General, and its establishment would have no effect on Defence costs. A target surplus had been agreed for the Fund, after meeting interest on capital, depreciation and other normal charges, including research and development costs, which envisaged earnings over five years to yield a surplus of £3.9 million. The Fund would retain depreciation provisions and trading surplus and would be enabled to build up its own reserves to meet contingencies and normal capital investment requirements. Its initial capital would suffice to cover the book values of both the fixed and working capital of the plants concerned, at present estimated at about £37 million, and the Exchequer would receive from the Fund interest on the initial capital and also a contribution in lieu of income tax where tax would be payable under the normal scales. Introduction of the Trading Fund would increase the existing net Atomic Energy Vote by eliminating receipts from trading operations, but this increase would be partly offset by an increase in the Exchequer Extra Receipts of interest, and the Exchequer would not in future have to provide for the Authority's capital needs that amount which could be met from reserves.

## Atomic Energy Board in South Africa

THE 3-MeV Van de Graaff particle accelerator is now fully operational at Pelindaba, the National Nuclear Research Centre. Costing R175,000, the accelerator is housed in its own building on the Pelindaba site, and since its commissioning in the past few weeks it has begun to play a significant part in the Atomic Energy Board's research activities. Its completion has preceded that of the Board's research reactor, *Safari I*, which will 'go critical' later this year. While there are two more powerful accelerators already operating in South Africa—the 16-MeV cyclotron at the Council for Scientific and Industrial Research and the 5.5-MeV Van de Graaff accelerator at the Southern Universities' Nuclear Institute—the Pelindaba machine possesses certain advantages for the specific research on which it will be used. First, it provides a more stable particle beam than the cyclotron, and secondly, it has a higher beam intensity than its counterpart at the Southern Universities' Nuclear Institute. It is also a pulsed accelerator enabling it to produce neutrons in short bursts and so permit time-of-flight experiments which are especially useful in fast neutron physics studies. The Pelindaba equipment will be available to universities for pure fundamental physics research.

## Photonuclear Reactions Series

No. 10 in the *Bibliographical Series* of publications of the International Atomic Energy Agency deals with photonuclear reactions—the interactions of electromagnetic waves with nuclei. The most important aspects of the theoretical and experimental investigations of the photo-disintegration of light are covered, as well as intermediate and heavy nuclei, photofission, electrodisintegration, and the absorption and scattering of protons. A special chapter is devoted to the photoproduction of elementary

particles. There are 2,254 references to articles in English, French, Russian, German, Italian, Japanese and other languages, published during January 1948–August 1963. The authors, English title and periodical reference are given, together with an abstract in English when possible, and the bibliographical sources used were *Nuclear Science Abstracts*, *Referativnyi Zhurnal Fizika*, *Physics Abstracts*, and journals and reports submitted by member States of the Agency. The references are classified into fifteen categories: general theory; photodisintegration of the deuteron, of tritium and helium, of light nuclei, of intermediate nuclei, and of heavy nuclei; photofission; electrodisintegration; photoproduction of mesons; books and survey articles; bremsstrahlung and X-rays; resonant scattering; elastic and inelastic scattering; absorption of photons; and miscellaneous. A comprehensive author index and a subject index complete an extremely valuable bibliography. The print, though small, is extremely clear and sharp, and the presentation of the text is in many respects an improvement on that of the original sources.

### Galileo Galilei

GALILEO GALILEI was born on February 15, 1564, and to celebrate the quadricentennial of his birth, Lehigh University, Bethlehem, Pennsylvania, held an exhibition, during October 1–December 31, 1964, of books and manuscripts by, and relating to, Galileo. A descriptive brochure has been prepared by J. D. Mack, the librarian of the University (*Brochure on an Exhibition of Books and Manuscripts by and Relating to Galileo Galilei upon the Quadricentennial of his Birth, 1564–1964*. Pp. 32. Bethlehem, Pennsylvania: Lehigh University Library, 1964). Galileo's father was a Florentine cloth merchant who was adept at drawing and who wrote extensively on music. Galileo was sent by his father to study medicine at the University of Pisa, but Galileo's main interest was in mathematics, and in 1589 he was appointed professor of mathematics at Pisa. He later moved to the University of Padua. In his comments on the 29 exhibits, Mr. Mack traces Galileo's contributions to mechanics, his astronomical observations and his arguments in favour of the Copernican cosmology, which led him into conflict with the Roman Catholic Church and finally to the judgment of the Inquisition. In addition to the works of Galileo the exhibition also contained 17 works more or less directly related to his life and writing, including a copy of Aristotle's treatise *On the Heavens*; a first edition of the *Dialogue on Music* by Galileo's father; the first English translation of Galileo's *Dialogue Concerning the Two Chief World Systems* by Thomas Salisbury—a very rare edition of 1661; and Newton's *Principia*. Mr. Mack prefaces his comments on the exhibits by a brief analysis and appreciation of Galileo's contributions to science. He emphasizes that throughout his career Galileo argued that only by means of mathematics and experiment was science possible and that their introduction made it impossible for science to remain static. Galileo first achieved a European reputation through his use of the telescope, and this is indicative of the dependence of Galileo's science on technology and of his view that science should be useful.

### National Lending Library for Science and Technology

A REPRINTED brochure describing the National Lending Library for Science and Technology, issued by the Department of Scientific and Industrial Research in August 1963, has now received further slight amendment. In November 1964 the Library was receiving 6,800 requests for loans weekly, of which 81 per cent were met from its own stock, about 8 per cent being passed to the Science Museum Library. Telex requests amount to about 250 per week, and photocopying requests to 550. Chemists (28.6 per cent), engineers (21.5 per cent) and physicists (11.9 per cent) are the principal borrowers, engineering

(22 per cent), chemistry (19 per cent), physics (15 per cent), biology (13 per cent) and applied chemistry (8 per cent) being the chief subjects of requests for loans. Approved borrowers now number about 2,240, and industrial firms are prominent among institutional users, accounting for just under half the weekly requests. The translating service provides current cover-to-cover translations of 16 periodicals, besides about 300 translations of articles, at a cost of £150,000 a year net or £50,000 gross. Expenditure on literature is at present about £146,000 a year, and 20,500 current titles of serial publications are being received from more than 100 centres. Of the 122 non-industrial staff, 27 are scientific and experimental grades. After English (72 per cent), German (9.8 per cent) and French (4.6 per cent) are the most frequent languages of requests, and educational establishments (29 per cent) and Government departments and establishments (12 per cent) follow private firms (44 per cent) in the proportion of requests for loans. Besides the course for postgraduate research students in January 1964, two courses were run for university library staff in April and July 1964, and a third course is being arranged for Easter 1965. Nearly 15,000 books in the English language and about 24,000 in the Russian language are held, the monthly intake being about 250 and 400, respectively, and microfilms of translations and reports equivalent to 96,000 volumes are also held, but this intake is expected to be replaced by microfiche.

### Institute of Biology

ON January 14, the Institute of Biology celebrated its fifteenth anniversary by a dinner at the Imperial College of Science and Technology. After the dinner, Prof. G. E. Blackburn, retiring president, presented to his successor, Prof. O. E. Lowenstein, a silver-gilt badge of office to be worn by future presidents. At the preceding annual general meeting, the honorary secretary, Dr. L. Broadbent, had reported that membership of the Institute had reached 3,550 and that with a continuing growth at its present rate membership would reach 4,000 in one year's time: this would be about half the number of qualified biologists employed in the United Kingdom. The Institute has just published regulations for the award of M.I.Biol. by examination, and part-time courses are expected to start at some of the larger technical colleges in September 1965. The ferment in biological education in schools, technical colleges and universities and the Institute's own increasing responsibility for training in applied biology have led to the appointing of an education officer, Mr. L. J. J. Brandon, who will commence work in April 1965.

### Symposium on Crustacea, Cochin

AN international Symposium on Crustacea, organized by the Marine Biological Association of India in conjunction with the University of Kerala, was held in the Oceanographic Laboratory, University of Kerala, Cochin-Ernakulam, during January 12–15, 1965. The symposium was under the chairmanship of Dr. S. Jones, president of the Association and head of the Central Marine Fisheries Institute, Mandapam Camp, South India. More than 100 participants registered, including a number from Europe and the United States. The symposium was inaugurated by Prof. Samuel Mathai, vice-chancellor of the University of Kerala, Trivandrum, and the accompanying exhibition on biology and fishery of crustacea was opened by Mr. Per Sandven, director of the Indo-Norwegian Fishery Project, Ernakulam. Abstracts of 144 papers were received before the meeting. Only those of authors present were read and discussed, these constituting a very full programme for the three days set aside for the reading of papers. Many aspects of biology, ecology and fishery for crustacea in various parts of the world were discussed. The progress of work on



Indian species of importance as food or as a cause of marine deterioration was fully covered. An invited lecture by an expert from the United States or the United Kingdom was given on each of the four evenings of the meeting. Delegates are indebted to the Marine Biological Association of India, the director, Indo-Norwegian Project, the University Department of Marine Biology and Oceanography, and the Seafood Canners and Freezers Association of India for hospitality during the meeting. It is expected that the *Proceedings* of the symposium will be published towards the end of 1965. Enquiries regarding this publication should be addressed to the Managing Editor, Marine Biological Association of India, Marine Fisheries P.O., Ramanathapuram District, South India.

#### University News:

#### Leeds

THE University of Leeds, for many years ranked among the bigger provincial universities, has now become the second largest of the 'civic' universities in England, outside London. In 1938-39 the total of full-time students was 1,757; in 1963-64 it was 6,233. Under development plans now in hand, the student population is expected to reach 8,300 in 1967, eventually to exceed 10,000. In November 1964 the University of Leeds published a documented and well-illustrated brochure entitled *University Studies in Applied Science and Technology*, being in fact an introduction to the degree courses in the Faculty of Technology, now so much in the forefront of higher educational and political planning (Pp. 88. Leeds: The University, 1964). While the academic side of the various faculties of learning in this University is by no means subordinated to present-day demands for trained technologists as such, the emphasis in this excellent guide to what is offered to "parent, potential student or teacher—to the schemes of study in applied science and technology at the University of Leeds" (to quote from the Vice-chancellor's foreword), is on practical application of the subjects of study available. These include agriculture; applied mineral sciences; ceramics; colour chemistry and dyeing; chemical, civil, electrical and mechanical engineering; food science; fuel science; leather science; metallurgy; and textile industries. Full information is given on degree schemes, careers, postgraduate courses and research in the several subjects of study; apart from this, the illustrations of the University precinct and environment, of its buildings, and particularly of its many finely designed and equipped laboratories, are convincing enough to attract widespread attention from those to whom this official publication was meant to appeal. "The University, only one mile from the city centre, and yet within such easy reach of the moors and dales, is thus fortunate in being able to offer its students all the attractions of a large modern city, and at the same time unique opportunities to escape from the fumes and noise of urban life to the beauty and peace of unspoilt rural surroundings." Those who know the University to-day would certainly agree that this, compared with less fortunate centres of learning in Britain, is no over-statement.

#### Mullard Research Fellowships

MULLARD RESEARCH LABORATORIES have established a limited number of research fellowships (to be awarded on a basis of personal invitation) to enable visiting scientists to spend a period of about one year at the Mullard Laboratories, Redhill, Surrey. The Fellowships will enable scientists engaged in academic, government or industrial laboratories to work with well-established teams in the Research Laboratories. Joining a 'ready made' research team, it is hoped, will facilitate individual contributions with the minimum of non-productive work. Fellows will normally have had experience in a sphere in which the Mullard Laboratories are active and will come from Britain and overseas. Stipends "appropriate to the status and seniority" of the Fellows will be paid.

#### Research Fellowships in Fisheries Science

THE Fisheries Division of the Irish Department of Lands, in co-operation with the constituent Colleges of the National University of Ireland, Trinity College, Dublin, Queen's University, Belfast, the Irish Sugar Co., Ltd., and the Institute for Industrial Research and Standards, Dublin, is to award a research fellowship in fisheries science, tenable for at least three years. The fellowship will be awarded to a man or woman with an honours degree, preferably in biology, for full-time research into water purity and the various conditions affecting fishery productivity in selected Irish rivers. Further information can be obtained from the Secretary, Department of Lands, Fisheries Division, 3 Cathal Brugha Street, Dublin 1.

#### Announcements

DR. D. M. KEMP has been appointed chief chemist of the Atomic Energy Board of South Africa as from January 1. Dr. Kemp joined the Board in 1962, and since the middle of that year has headed the Chemistry Division. Dr. J. W. L. de Villiers has been appointed senior principal research officer. Dr. de Villiers joined the Reactor Engineering Division of the Board in October 1959, and spent more than two years on extensive training at several overseas institutions, notably the Argonne National Laboratory in the United States.

DR. F. BLOCH, professor of physics at Stanford University, became president of the American Physical Society on January 30. Dr. Bloch succeeded Dr. R. F. Bacher, Provost of the California Institute of Technology. Dr. J. Wheeler, of Princeton University, became vice-president and Dr. C. H. Townes, Provost of the Massachusetts Institute of Technology, became vice-president-elect of the Society.

THE Helen B. Warner Prize of the American Astronomical Society has been awarded to Prof. M. Schmidt, professor of astronomy at the California Institute of Technology, for his spectroscopic studies of radio galaxies and quasi-stellar radio sources.

THE Smithsonian Astrophysical Observatory, Cambridge, Massachusetts, has announced that it has been requested by the International Union of Geodesy and Geophysics and the International Committee on Space Research to establish and operate a Central Bureau for Satellite Geodesy.

THE seventy-first annual conference of the Museums Association will be held in Dublin during June 21-26. Further information can be obtained from the Secretary, Museums Association, 87 Charlotte Street, London, W.1.

A JOINT conference of the Aeronautical and Technical Translation Group of Aslib will be held at the College of Aeronautics, Cranfield, during April 1-3. Further information can be obtained from the conference secretary, Mr. E. J. MacAdam, 74 Stancliffe Road, Bedford.

A CONFERENCE on "The Effects of Sunlight in Buildings, with Special Reference to the Visual Effects", arranged under the International Commission on Illumination, will be held at the University of Newcastle upon Tyne during April 5-9. The programme will include papers and discussions on: psychophysical aspects of the admission and exclusion of sunlight in buildings—visual and thermal comfort; physics of sunlight and the radiation distribution of the clear sky; sun position diagrams and lighting calculations; design of sun control devices; design criteria for sunlight in buildings. Further information and forms of registration can be obtained from Mr. J. Longmore, Building Research Station, Garston, Watford, Hertfordshire.

## SCIENTIFIC RESEARCH IN AUSTRALIA

THE sixteenth annual report of the Commonwealth of Australia Scientific and Industrial Research Organization\*, covering the year ending June 30, 1964, includes lists of published papers and of the members of the Advisory Council, State Committees and staff. Of the total income of £14.87 million, £10.7 million was by Treasury appropriation, with a further £583,910 for capital works and £2.4 million from the Wool Research Trust Fund. Of the £10.8 million expended on investigations, 36.6 per cent was on agricultural research, 9.7 per cent on processing of agricultural products, 11.6 per cent on physical research of industrial interest, 8.1 per cent on chemical research of industrial interest, 7.3 per cent on general physical research, 6.8 per cent on processing, recovery and use of minerals and coal, 4.5 per cent on general industrial research, 2.8 per cent on fisheries and 5.5 per cent on research services. The shortage of laboratory accommodation remained acute.

Among the research developments emphasized in the survey are the studies by the Controlled Environment Research Laboratory in the Division of Plant Industry. These were concerned with the distributions of temperature, humidity and solar radiation in the canopy of two contrasting plant communities, a wheat field and a pine forest, and with the corresponding distribution of evaporation sources and the movement of sensible heat. Recent work at Katherine has shown that Townsville lucerne pastures without the perennial grass are of greater feeding value in the dry season than grass/Townsville lucerne pastures, and the results point towards more productive grazing systems for beef cattle in the far north. Research on the nutrition of tropical legumes has provided information which will be invaluable in establishing and managing them in pastures and has also shown that their responses to fertilizers often differ from those of temperate legumes. Studies on the Northern Tablelands have indicated that, although there are very broad relations between the appearance of the soil and its general fertility status, the pattern of the relationship varies with different elements.

Genetic and clinical work on the enteric bacillus *Escherichia coli* indicates at least five ways in which a bacterium, and probably any other organism, can become resistant to a poison.

The tenth survey of land resources in Papua and New Guinea was completed in 1963, and about one-sixth of the area of these territories has now been surveyed. Generally surveys have revealed the existence of larger areas with a potential for development than was expected. Studies of the drying of grapes used mainly for production of sultanas or raisins showed that the rate of drying is determined by the wax constituents, which control the loss of water, and the rate can be increased by a brief immersion of the grapes in an emulsion of oil and potassium carbonate before drying. A systematic investigation being carried out on the ensiling characteristics of tropical pasture species should facilitate development of more suitable ensilage techniques and even indicate features to the plant breeder which could be introduced into otherwise unsuitable strains.

Field and laboratory studies associated with more than fifty failures of earth dams have revealed two main patterns, apart from seepage losses through the floor of the dam; chemical studies on the water and the clay fraction of soils from dams which failed when it was first filled with water showed that these 'sus-

ceptible' soils are dominated by monovalent cations, such as sodium, in the exchange complex of the clay. Research programmes are also in progress which are aimed at evaluating the pedological and terrain systems of classification as a basis for obtaining and recording such data for engineering purposes.

The report points out that, so far, the Animal Health Laboratory at Parkville has succeeded in isolating viruses of some six bovine diseases from Australian livestock which cause economic losses in herds, especially in younger animals through their effects on the brain and associated nervous tissues, the respiratory tract, the genital tract and the intestines. Some of the results of the investigations which have established the critical importance of the cobalt-containing complex vitamin B<sub>12</sub> and of a continuous supply of cobalt to protect sheep against *phalaris* staggers are reviewed. Other investigations have indicated that the supply of protein available for digestion and absorption in the small intestine is an important nutritional factor limiting the rate of growth of wool—its content of *S*-amino-acids being particularly important. Cystine, labelled with carbon-14 or sulphur-35, is being used to measure the rate of growth of length of wool fibres on experimental sheep, and besides investigations into contagious bovine pleuropneumonia, there is a concurrent programme of fundamental research into the biology of the pleuropneumonia organism and related organisms. Studies of the inheritance of tick resistance in cattle indicate that tick resistance is highly heritable and it should be possible to breed for this character.

Studies on the ecology of *Sirex noctilio*, a serious pest of *Pinus radiata* in Australia, and of two parasites *Ibalia leucospoides* and *Rhyssa*, now well established in Tasmania, are in progress and, although no dramatic control by these parasites is expected, the *Sirex* population should be reduced. The main features of the distribution of blue-fin tuna in Australian waters have now been determined, and factors influencing reproduction in the rabbit are being studied in climatic and pastoral conditions from sub-tropical through semi-arid to sub-alpine.

A special Fabry-Pérot interferometer has been constructed to measure the width of the green oxygen line in the high atmosphere and its variations from night to night. The electron microscope and a 'field-ion' microscope are being used in investigations of the creep of metals and have detected cavities at grain boundaries at a very early stage of development. Proton magnetic resonance spectrometry has now shown conclusively that dimethyl aluminium chloride exists as such in solution in ether. Other investigations have been concerned with the chemical properties of water at very high pressures, and the structure of casein micelles; a new chromatographic procedure for determining lanthionine, and a radiochemical method for thiol analysis, have been used to evaluate the relative roles of these groups in the setting process of wool. A new enzyme, protein disulphide reductase, which is involved in the reactivation of triode phosphate dehydrogenase, has been identified, and it would seem that it may be partly responsible for the Pasteur effect in intact plant tissue. The polyphenols in the leaves of the *Eucalyptus* genus are being surveyed and most of the genus has been examined. A new type of high-intensity atomic spectral lamp has been developed and considerable progress has been made towards deriving a mathematical model to account for the stages of breakage of selected size ranges in ball-grinding. An investigation of load distribution in wooden floors has indicated that nailing floors to joists and joints to bearers has little effect. Results with a solar air-heater and rockpile have

\* Australia: Commonwealth Scientific and Industrial Research Organization. Sixteenth Annual Report, 1963-64. Pp. 187. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1964.)

led to the installation of a full-scale prototype air-heater and thermal storage system to heat part of a laboratory. Studies of molybdenum disulphide have demonstrated its outstanding adherence to metal surfaces. A new

machine has been designed for shelling peas, while new fibre-board containers have been designed for transporting apples and pears. A prototype machine for the continuous dyeing of wool slubbing has been constructed.

## THE RESEARCH COUNCIL OF ALBERTA

**T**HE forty-fourth annual report of the Research Council of Alberta\*, which covers the year 1963, includes the usual reports of branches and divisions, and lists of publications (1959-63), and members of the technical and other advisory committees. About one-quarter of the total effort is now devoted to mapping and classifying natural resources, about 10 per cent to investigations undertaken jointly with other Government agencies including the highway research, hail studies and watershed research programmes.

Almost half the total activity of the Council is devoted to long-range research programmes directly or indirectly related to the natural resources of the Province. In recent years, forest projects of direct industrial significance have become more prominent in the Council's programme. Several of the longer-range studies have reached a stage when they are leading to developments of commercial significance. Particular reference is made in this connexion to the development of a process for producing iron from the low-grade ores in the Peace River area, to work on the pipeline transport of solids, and to the development of several new products from coal.

In the Coal Division, interest during the year continued to centre on the study of the 'paste-slug' technique for pipeline transport of granular solids, while work on coal chemicals was chiefly directed to investigations of humic acids and their application.

The Petroleum Division is nearing the completion of a massive analysis, based on data from several thousand wells, of the regional distribution of oil, gas and water in the various formations of the Alberta sedimentary basin. Several possible means of transporting sulphur by pipeline were explored during the year, while investigations of the kinetics of hydrocarbon reactions were concerned with the decomposition of ethylene oxide and of ethylene imine, under both photochemical and thermal activation. The technique of electron-spin resonance spectroscopy is being applied to questions relating to coal and carbon structure in the in-

vestigation of certain charge-transfer complexes which are similar in spectral behaviour to coals and chars. Another investigation involved a series of 10 methylated anthra-semiquinones, and demonstrated the stearic hindrance of a methyl group in the one position in anthraquinone.

In the Earth sciences, a regional survey of the ground water resources of the Peace River block was completed, and pilot plant investigations were continued into a process for producing high-grade iron powders from the iron deposits in the Clear Hills. An evaluation of the ground water resources of some 17,000 square miles of East Central Alberta was published. Stratigraphical studies had their main emphasis on the Cretaceous rocks of Alberta, but research is also in progress on Precambrian, Mississippian, Tertiary and Pleistocene strata. A detailed field study was made of the Marmot Creek Basin near Kananaskis, west of Calgary, and, during soil surveys, the presence of a permanent icepan in organic soils in parts of Alberta was noticed; the chemical forms of sulphur occurring in Alberta soils are also being studied.

Under the hail studies programme, analysis of small-scale pressure patterns over the project area indicates an interesting relation between the small high-pressure areas and the rainfall. Physical research into problems relating to highway design, undertaken within a co-operative programme sponsored by the Department of Highways, the Department of Civil Engineering of the University of Alberta and the Research Council, included investigations into the properties and behaviour of soils and paving materials, the structural design and evaluation of pavements and problems in the hydraulics of highway bridges and related structures. Work continued on the problem of scour around bridge foundations in alluvial streams. Industrial and engineering services included investigations aimed at the development of techniques for producing foamed asphalt and foamed sulphur. Studies of the ten-year biological cycle have been primarily devoted to the ecology and population dynamics of the Snowshoe hare which, as the dominant herbivore and basic prey species, holds the key to the cycle.

\* Research Council of Alberta. Forty-fourth Annual Report, 1963. Pp. 71. (Edmonton: Research Council of Alberta, 1964.)

## INTERNATIONAL COLLABORATION IN PULP AND PAPER PRODUCTION

**F**ROM time to time, the Food and Agriculture Organization of the United Nations, the headquarters of which are in the Via delle Terme di Caracalla, Rome, in collaboration with Unesco or a similar body, organizes elaborate conferences having the objective of exploring ways and means by which the fibrous natural resources of developing countries can be used to the best advantage to produce pulp and paper. Normally the regional areas covered by a symposium are quite large. Thus, in 1954 and 1959 Latin America and South-east Asia and the Far East were covered, respectively; and in 1965 Africa will come under review. A similar regional symposium, but on a rather smaller scale, was also held in Beirut, Lebanon, in December 1962, having as its subject pulp and paper research and technology in the Middle East and North Africa\*. On this occasion the Food and Agriculture

Organization and Unesco acted jointly. The papers read were published by Unesco several months ago (somewhat belatedly, one feels).

These symposia follow a generally similar pattern, the usual procedure being to receive papers of three different types. First are those prepared by the Food and Agriculture Organization Secretariat in Rome; they usually summarize the position in the region as a whole and do so mainly from an economic angle. Official representatives of the individual countries in the region then summarize the present position in their respective countries, and their hopes and plans for the future. Finally, papers also come from invited consultants, who present appropriate case studies or specific technical papers of relevant interest. Ample opportunity is available for discussion of the papers presented, and although this was used to the fullest advantage in the present example in Beirut, and proved extremely valuable, the discussions are unfortunately not reproduced in the published volume.

\* *Proceedings of the UNESCO/FAO Regional Symposium on Pulp and Paper Research and Technology in the Middle East and North Africa, 10-14 December, 1962, Beirut, Lebanon.* Pp. ix+479. (Cairo: Unesco Middle East Science Co-operation Office, 1963.).



An element of incompleteness also arises, partly from the strange geographical frontiers imposed by the organization of these conferences, and partly by the fact that some nations in the region did not participate. Ten nations were directly represented, but countries in the area not directly represented were: Algeria, Afghanistan, Cyprus, Israel, Saudi Arabia, Somalia, Sudan and Yemen.

Specialized papers presented dealt with eucalyptus (San-  
yer); poplar (Bersano); bagasse (Atchison); straw (Muller);  
reeds (Chivu); esparto (Grant); and cotton linters (Howell).

The published version of these papers provides, therefore, authoritative information on a wide variety of specialized aspects of the utilization of fibrous materials. Experience based on the Latin America and Tokyo symposia has demonstrated their value in initiating pulp and paper projects in the region concerned; and the Beirut symposium as recorded in this volume is a further example of the great value of this little-publicized activity sponsored indirectly by the United Nations Organization.

## ART AND SCIENCE OF PACKAGING

THE opening of the new engineering research buildings of the Printing, Packaging and Allied Trades Research Association ('Patra') at its headquarters at Leatherhead, Surrey, on November 4, 1964, directed renewed attention to the important work of this progressive organization. The Printing Industry Research Association, as it was first called, was formed as the result of a lecture, "Application of Science to Printing", given by Dr. G. L. Riddell in the Stationers' Hall, London, on February 8, 1929. A meeting of printing trade organizations was convened which decided to set up "a technical research bureau for the pooling of technical information and to conduct scientific investigation of technical problems". The Stationers' Company at first provided free accommodation, but in 1932 the Association moved to St. Bride's Institute. Laboratory facilities were at this time provided mainly by the London School of Printing (London County Council). By 1936 the Association was renamed the Printing and Allied Trades Research Association and became a grant-aided research unit; it was then moved to premises in Robin Hood Court, off Fleet Street, London. In 1941 these premises were completely destroyed during one of the major fire raids on London, but with generous help from several member firms the Association was re-housed in temporary accommodation in various parts of London and the Home Counties, and so its work continued. By 1942 the packaging problem of service equipment badly damaged on arrival in the Middle East and Far East combat areas had arisen, and with the co-operation of the Department of Scientific and Industrial Research, manufacturers and users of packages, and with an increased grant, the Packaging Division was formed in 1943. This expansion virtually doubled the Association's income, and thus the foundations of the larger organization, as we now know it, were laid and its future was fully secured. Leatherhead has for many years been

a favoured locality for research associations sponsored by industry; this Association moved into new laboratories there in 1948. But within ten years enlarged premises were required and a new wing was added (opened in 1959) to accommodate the Packaging Division. The latest extension, built in 1963 to house the new engineering research buildings, brings the total floor area available at this centre to 42,000 square feet. This growth record in 35 years is one of which the Association may be justly proud. Apart from fundamental research projects based on problems in both printing and packaging techniques, 'Patra' has established some important routine day-to-day technical services, among which are printing enquiries, critical appraisals, machine testing, air-conditioning, high-speed camera service, package testing and advisory service, library, abstracting and translation facilities.

The work of the Association, particularly in the modern field of packaging, has been quite outstanding, although perhaps not as well known as it should be. In this connexion, the division concerned "... assesses products for packaging, recommends methods, materials and containers, tells where to obtain supplies, advises on specifications, tests materials or packs, indicates the standard of package performance and guards against over-packaging by ensuring economic pack design". The package testing laboratory is fully equipped for what in other industries are generally known as 'accelerated breakdown or weathering tests'; both mechanical and climatic hazards that a package may meet in transit can be simulated in a variety of apparatus and specially constructed environments; the scope includes tests applicable to standards of packages and packaging materials recommended in British and American Society for Testing Materials Standards, and in United States and British Government specifications.

## GALACTIC FLARES AND QUASI-STEELAR RADIO SOURCES

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AT the present time there is considerable interest in explosions which occur in objects of galactic dimensions. Oort<sup>1</sup> has suggested that such an explosion has occurred in the nucleus of our own Galaxy, and there is convincing evidence of such explosions in certain galaxies<sup>2</sup> which are strong radio emitters, and also in the quasi-stellar objects associated with certain very strong radio sources<sup>3</sup>. In such an explosion, a great quantity of energy is suddenly released in the form of relativistic electrons (which produce radio and optical synchrotron radiation), kinetic energy of ejected gas clouds, and possibly other forms. This energy can be so large that it is at present believed that, for the very strong radio sources at least, it must be derived from gravitational energy<sup>4,5</sup>. The mechanism for release of gravitational energy which has been mostly widely investigated is that of 'gravitational

collapse'<sup>6-8</sup>. However, this theory does not yet give a convincing interpretation of the phenomena it sets out to explain. Gravitational collapse is a runaway process, whereas there is evidence that explosions can recur<sup>9</sup>, indicating that one needs an instability. Moreover, the complicated theories of gravitational collapse have not been integrated into a larger theory showing how the initial conditions of the theory come about, and how the theoretical consequences lead to the observed characteristics of exploding galactic objects—such as strong radio emission. This suggests that alternative processes should be sought for the release of gravitational energy, and it is the purpose of this article to propose one.

Data concerning galactic explosions suggested the following requirements: (1) it should be possible for the mechanism to occur over a wide range of energy; (2) the

mechanism should involve the gradual accumulation of energy and its sudden release; (3) the explosion should be such as to convert a large fraction of the released energy into high-energy particles and mass-motion; (4) it should be possible for the mechanism to repeat itself.

These requirements of a theory of galactic explosions are also requirements of a theory of solar flares<sup>10</sup>. Further considerations show that there is indeed a strong similarity between these two phenomena, which prompts one to enquire whether the mechanism of solar flares might occur on a galactic scale. A preliminary investigation indicates that this is indeed possible, leading to the view that the galactic phenomena referred to may prove to be 'galactic flares'.

Until recently, it was generally believed that the energy released in a solar flare is stored entirely in magnetic form<sup>11</sup>. This point of view would discourage an attempt at identifying galactic explosions as flares. However, a recent investigation of the flare problem by Dr. B. Coppi and myself<sup>12</sup> has led to the conclusion that the energy released in a solar flare is stored partly in magnetic and partly in gravitational form. This new interpretation of solar flares provides strong encouragement for investigating the possible occurrence of galactic flares.

The pre-flare state of a solar flare consists of a mass of gas supported against gravity by a compressed magnetic field. The Coppi resistive instability<sup>13</sup> permits relative motion of the plasma and field, when a certain criterion is satisfied, so that the gas falls and the field expands upwards; this process releases gravitational and magnetic energy in the form of Joule heating, particle acceleration, and kinetic energy of mass motion. A large fraction of the energy goes into Joule heating; a substantial fraction into mass motion; and a fraction above 1 per cent into particle acceleration. This is probably the most efficient mechanism in Nature for converting gravitational energy into relativistic-particle energy.

An analogous pre-flare state on a galactic scale would comprise a large mass of gas which is prevented from collapsing by the pressure of a trapped magnetic field. It is desirable to have a non-committal name for such an object, so as to leave as an open question its identification with a protogalaxy, a compact elliptical galaxy, the nucleus of a diffuse galaxy, or a quasi-stellar object: the term 'galaxoid' will therefore be used—hopefully without prejudice.

Once again, both gravitational and magnetic energies will be released if the magnetic field and the gas can be de-coupled. This will not happen in magnetohydrodynamic theory, since the diffusion rate is extremely slow (in excess of 10<sup>20</sup> years even if the scale is only 1 pc.). Magnetohydrodynamic instabilities do not effect or permit de-coupling of the gas and magnetic field and so, presumably, will not provide an efficient acceleration mechanism. If the galaxoid contains a density gradient which is opposite in sign to that in a body supported by gas pressure, it will be unstable against the Rayleigh–Taylor<sup>14</sup> magnetohydrodynamic instability, unless the magnetic field exhibits a sufficient degree of shear. If there is sufficient shear (and some shear must always be present), the relevant instability will be the Coppi instability, which is resistive. This instability causes the plasma to break up into fine filaments and so permits rapid relative motion of the field and plasma, the growth time of the instability being the time of propagation of an Alfvén wave across the region of anomalous density gradient.

If gas pressure, radiation pressure and centrifugal force are all negligible in comparison with gravitational and magnetic forces, the magnetic energy and (negative) gravitational energy will be approximately equal. We assume that the galaxoid has a shape which may be adequately characterized by a single dimension  $R$ , an equivalent radius, and write:

$$W = G M^2 R^{-1} \approx 10^{8.8} M^2 R^{-1} \text{ erg} \quad (1)$$

and

$$W = \frac{1}{6} R^3 B^2 \approx 10^{1.2} R^3 B^2 \text{ erg} \quad (2)$$

where  $M$  is the total mass and  $B$  is the average intensity of the magnetic field.

Observations of the optical and radio emission associated with an explosion make it possible to set a lower limit to  $\Delta W$ , the change in  $W$  due to the explosion, and hence to  $W$ . In order to determine both parameters  $M$  and  $R$ , one further item of information is needed. For this reason, we shall now restrict our attention to the radio source 3C273 since the component 3C273B, which we identify with the galaxoid, exhibits a periodicity in its optical radiation of period 13 years (10<sup>8.6</sup> sec)<sup>3</sup>. Since the Alfvén speed has the mean value:

$$v_A = B(4\pi\rho)^{-1/2} \approx 10^{4.6} M^{1/2} R^{-1/2} \text{ cm sec}^{-1} \quad (3)$$

(the escape speed has approximately the same value), the oscillation period may be estimated to be:

$$\tau = R v_A^{-1} \approx 10^{3.4} M^{-1/2} R^{3/2} \text{ sec} \quad (4)$$

In order to estimate  $W$  for 3C273, it is necessary to estimate  $\Delta W$ . At the present rate of radiation, the jet 3C273A would have emitted 10<sup>57</sup> ergs or more since the flare. The energy of the magnetic field of the jet will not be counted since it is not assumed that the magnetic field is ejected with the particles. Moreover, the optical and radio emission of the  $B$  component is not regarded as a product of the flare. If the foregoing energy is only 10 per cent of the energy originally present in accelerated particles directly following the flare, and if this energy was only 1 per cent of the total energy released,  $\Delta W = 10^{60}$  ergs. If this were a substantial fraction of  $W$ , say 10 per cent, we would be led to the choice  $W = 10^{61}$  ergs. It is possible that  $\Delta W$  was a smaller fraction of  $W$ , but it is also possible that the energy in high-energy particles represents more than 1 per cent of the released energy. For example, it is possible that particles are accelerated in the shock front which must form ahead of the ejected gas; this process occurs in connexion with solar flares, giving rise to Type II radio bursts<sup>15</sup>. However, a factor of ten is not very important in the present exploratory calculation, so we adopt  $W = 10^{61}$  ergs.

The foregoing values for  $\tau$  and  $W$  lead to the following set of parameters for the galaxoid:  $M = 10^{43} \text{ g} = 10^{9.8} M_\odot$ ;  $R = 10^{17.8} \text{ cm} = 10^{1.3} \text{ pc.}$ ;  $B = 10^{4.3} \text{ gauss}$ ;  $v_A = 10^{9.2} \text{ cm sec}^{-1}$ . If the gas were predominantly hydrogen, the mean number density  $n$  would be 10<sup>13</sup> cm<sup>-3</sup>. The trapped magnetic flux  $\Phi = \pi R^2 B$  is found to be 10<sup>40.2</sup> gauss cm<sup>2</sup>. The question whether (general) relativistic effects are important cannot be answered without a detailed account of the way in which the mass and magnetic field distributions change with a change of linear scale. This question is complicated by the facts that the magnetic field which we associate with a galaxoid is open rather than closed, and that there is an exchange of dynamical and magnetic angular momentum during the process of contraction or expansion. Since the Schwarzschild radius for the foregoing mass is 10<sup>14.7</sup> cm, relativistic effects will show up only if there is a close approximation in the dependence of gravitational and magnetic energies on radius, and this seems to be unlikely. The Alfvén velocity and escape velocity are small enough to render relativistic effects unimportant in the plasma instability process, but large enough to permit the inferred turbulent velocities<sup>3</sup> of 10<sup>8.5</sup> cm sec<sup>-1</sup>.

In order to give a more detailed picture of a galaxoid, it is necessary to consider the process by which it is formed. If one takes the view that the magnetic field is created by the galaxoid, one must determine what remarkable mechanism can generate, rapidly enough, a magnetic field strong enough to balance gravitational attraction in a condensing body. If, on the other hand, one assumes that the gas condenses from a region permeated by a magnetic field, the magnetic field will be

compressed with the gas. If, at the very beginning of the condensation process, gravitational and magnetic forces are comparable in magnitude, then they will always be comparable, although which of the two is stronger may change during the evolution of the condensation. The latter hypothesis, which we now adopt, leads us to picture the galaxoid as a mass of gas trapping and compressing a large magnetic flux which, at large distances, connects with a weak intergalactic magnetic field. We may note that the stored magnetic energy has been derived from gravitational energy during the condensation process.

It is noteworthy that such a magnetic field configuration would form an 'hour-glass' configuration, comprising a pair of funnels which channel the inflowing gas directly into the galaxoid. If the gas from which the galaxoid condenses has significant angular momentum, this will lead to a distortion of the magnetic field into helical form; this will lead to shear in the magnetic field, and it also provides a mechanism for reducing the angular velocity of the central core, transferring the angular momentum of the core material to the surrounding shells and possibly to the intergalactic gas.

We now enquire whether this hypothesis could lead to a galaxoid with the parameters suggested for 3C273. We suppose this object to be formed from a region of space in which the number density of hydrogen atoms is  $n_0$ , the temperature is  $T_0$  and the magnetic field strength is  $B_0$ . We may determine approximately the radius of the boundary between the region in which gas is flowing into the galaxoid and the region in which gas is undisturbed by the galaxoid by equating the mean thermal energy of a hydrogen atom to its gravitational energy in the field of the galaxoid. Hence:

$$R_c = GMm/\chi T_0 \approx 10^{16.9} M T_0^{-1} \text{ cm} \quad (5)$$

At this boundary, the inflow velocity will be approximately  $(2GM/R_c)^{1/2}$ , so that the influx of mass is given by:

$$J = 4\pi R_c^2 n_0 m (2GM/R_c)^{1/2} \approx 10^{49.1} M^2 n_0 T_0^{-3/2} \text{ g sec}^{-1} \quad (6)$$

This represents an inflow of energy into the galaxoid of amount:

$$S = JGMR^{-1} \approx 10^{-56} M^3 R^{-1} n_0 T_0^{-3/2} \text{ erg sec}^{-1} \quad (7)$$

Following Sciamia<sup>14</sup>, we adopt  $n_0 = 10^{5.7} \text{ cm}^{-3}$ , but we choose  $T_0$  to be such as would lead to the formation of the galaxoid in a few times  $10^9$  years. The value  $T_0 = 10^4$  deg. gives  $R_c = 10^{23.9} \text{ cm} = 10^{5.4} \text{ pc.}$ ;  $J = 10^{26.8} \text{ g sec}^{-1} = 101.1 M_\odot/\text{year}$ ; and  $S = 10^{44.9} \text{ ergs sec}^{-1}$ . (If the mass of the inflowing gas is taken into account,  $R_c$ ,  $J$  and  $S$  are all increased.) Hence the rate of increase of mass of the galaxoid has the time scale  $10^{8.7}$  years. We now find that the flux trapped in the galaxoid can be explained by assuming that the strength of the intergalactic magnetic field is  $10^{8.1}$  gauss. This is considerably less than the value  $10^{7.7}$  gauss which Sciamia<sup>14</sup> estimates to be typical of small galactic clusters. It leads to a ratio of gas pressure to magnetic pressure of about 10:1.

It is interesting to see that the energy input is close in magnitude to the radio and optical output of the component 3C273B. This suggests that the radio and optical emission of this component is due to bremsstrahlung: the flat spectrum can be explained by a suitable distribution of density and temperature with radius, and the energy radiated can be made up by the energy flux of the inflowing gas.

The foregoing model of the magnetic field associated with 3C273 would lead one to expect that the strength of the field at the position of the emitting cloud 3C273A is about  $10^{-6}$  gauss. However, the value is likely to be higher, following the flare, for the following reason: In a body of the dimensions given, in which the magnetic field is as high as that quoted, synchrotron radiation of both electrons and protons is so high in intensity that charged particles will have no transverse energy when they leave the accelerating region. For this reason, one

would expect the jet to be directed along the local magnetic field; there will be no transverse pressure tending to expand the magnetic field in which the cloud is moving but, on the other hand, there will be a high longitudinal momentum tending to straighten the magnetic field lines and so to increase the strength of the field along the path of the cloud. If the accelerating process occurred in a region involving about 10 per cent of the flux of the galaxoid, one would infer from the diameter of the cloud ( $10^{22.3} \text{ cm}$ ) that the magnetic field strength is now about  $10^{-5}$  gauss. With this value, one is led to estimate that the total energy of relativistic electrons is about  $10^{58}$  ergs, in agreement with our earlier hypothesis. Since the acceleration mechanism is electromagnetic, and since the energies are relativistic, there is no reason to suppose that there is a great deal more energy in protons than in electrons. The maximum energy must be at least  $10^{12.4} \text{ eV}$ . The radiation lifetime of these electrons will be  $10^{4.6}$  years. It should, however, be emphasized that electrons will not radiate until they are scattered into transverse orbits by strong magnetic-field inhomogeneities. It seems that these inhomogeneities are most likely to arise at the shock front which must form near the interface of the expanding cloud and the inflowing gas.

Since the flare which gave rise to the jet associated with 3C273A released a substantial fraction of the stored energy of the galaxoid, the dimensions of the flare layer must have been comparable with the radius of the galaxoid. This indicates that the growth time of the instability must have been about  $10^{8.6} \text{ sec}$  (the same as the present period of oscillation). Since micro-flares are much more common than intense flares on the Sun, we should expect that the galaxoid would exhibit low-energy explosions more frequently than catastrophic explosions. This suggests that we should identify the 'flashes' which occur in 3C273B as 'microflares'. Since the time scale is  $10^{6.3} \text{ sec}$ , the dimensions of the associated flare layer must be about  $10^{15.5} \text{ cm}$ . The energy of the microflare, which is about  $10^{52.3} \text{ ergs}$ , must be derived from the change in gravitational energy of a mass of gas moving towards the central mass by a distance comparable with the thickness of the flare layer, together with the corresponding change in magnetic-field energy. Hence the mass must be about  $10^{36.3} \text{ g}$ ,  $10^{7.3}$  times the total mass. The 'horizontal' dimension may be larger, say  $10^{17} \text{ cm}$ , which is still considerably less than one light-month: the gas density in this region would then be  $10^{10.6} \text{ cm}^{-3}$ , a reasonable value for an outer layer of the galaxoid.

The picture which we have given of a galaxoid, and the way in which it is formed, suggests that it should be a common stage in the early development of a galaxy. The flare process provides a mechanism for the protogalaxy to disentangle itself from the magnetic flux in which it was formed, and so proceed with normal galactic evolution. The remnant (or remnants) of the original galaxoid would presumably constitute the galactic nucleus (or nuclei).

Since quasi-stellar galactic objects are rare, there must be something unusual about their origin. The galaxoid hypothesis provides a possible answer to this question: the maximum mass and energy attained by a galaxoid depend on its age at the time it suffers a 'fatal' galactic flare. A very symmetrical galaxoid should live to an old age, but an unsymmetrical galaxoid will die young. We may now interpret quasi-stellar objects as galaxoids which have condensed from an unusually orderly region of intergalactic space, such as a region of fairly uniform density, temperature, vorticity and magnetic field, possibly with the additional favourable circumstance that the directions of the vorticity and magnetic field vectors are almost parallel.

Confirmation of the following observations on quasi-stellar objects would favour the model here described: (1) These objects rarely if ever occur in galactic clusters. (2) The quiescent optical spectrum indicates a filamentary



structure associated with the ducting of inflowing gas clouds along magnetic flux tubes. (3) The quiescent radio emission (by the star-like component) exhibits some degree of circular polarization, indicating bremsstrahlung radiation from a plasma in a magnetic field. (4) Flares occur over a wide range of energy, the frequency decreasing as the energy increases. (5) The optical emission associated with a flare indicates a fine filamentary structure<sup>13,17,18</sup> (of scale 10<sup>8</sup> cm, more or less).

A more complete account of this theory will be published at a later date.

*Note added in proof.* The recent discovery of 'interlopers' (*New York Times*, Jan. 27, 1965), which appear to be quasi-stellar objects with little or no radio emission, fits neatly into the foregoing scheme; an 'interloper' may be simply interpreted as a galaxoid which has not yet suffered a significant galactic flare.

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<sup>13</sup> Coppi, B., *Annal. Phys.*, New York, **30**, 178 (1964).  
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## HYDRATION KINETICS OF CALCIUM SULPHATE HEMIHYDRATE

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THE most conspicuous feature of the hydration kinetics of calcium sulphate hemihydrate is the pronounced maximum in the rate which occurs near the mid-point of the reaction. It is generally agreed that the precipitation of gypsum controls the rate until the maximum, and thereafter the control passes to the dissolution of hemihydrate. Consequently the exact position of the maximum is of great interest because it indicates the relative importance of the two processes in determining the rate.

Previous workers have chosen to fit experimental data to integrated forms of their rate equations. This is a very insensitive test for the rate equations. For example, provided the rate maximum is sufficiently close to the mid-point, plots of rate versus  $F$  (the fraction reacted) may be of quite different shapes (Fig. 1a) and still give an approximately linear plot of  $F$  versus time on arithmetic probability paper (Fig. 1b).

Polak<sup>1</sup> and Schiller<sup>2</sup> have derived a kinetic model based on sets of uniform spheres. By absorbing the concentration difference and the numbers of particles into two empirical rate constants (this kind of rate constant has been discussed before<sup>3</sup>; empirical rate constant is a better description than experimental rate constant since the latter usually means the numerical value of a rate constant obtained by treating experimental data) the rate equation simplifies to:

$$1/R = 1/gF^{2/3} + 1/h(1-F)^{2/3} \quad (1)$$

where  $R$  is the rate  $= dF/dt$ ,  $F$  is the fraction reacted,  $g$  is an empirical rate constant for the precipitation of gypsum,  $h$  is an empirical rate constant for the dissolution of hemihydrate.

However, since the gypsum crystals are not a set of spheres of uniform size, the exponent of  $F$  is not 2/3 unless all the following conditions are met: (1) Each crystal of gypsum maintains a constant shape as it grows. (2) Nucleation of gypsum is confined to a short period near the beginning of the reaction. (3) Crystal growth is not appreciably affected by crystal-to-crystal contacts. (4) The number of growth defects on a given crystal face does not change with age or crystal size, that is, the linear growth rate of a given crystal face depends on the supersaturation only.

Similarly the hemihydrate particles are not of uniform size or spherical, and the exponent of  $(1-F)$  is unlikely to be 2/3 unless all the following conditions are met: (5) The hemihydrate particles are of uniform size.

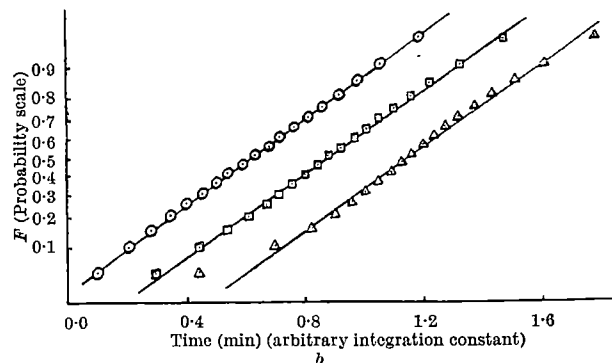
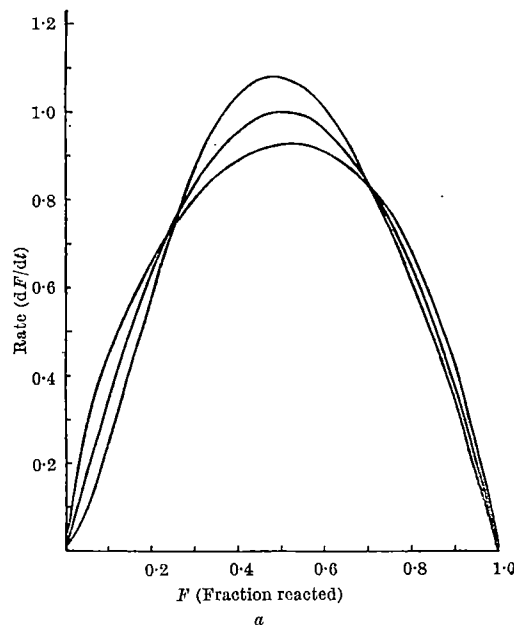


Fig. 1. Three rate equations of the form:  $1/R = 1/gF^{2/3} + 1/h(1-F)^{2/3}$  with  $Y = 1.0$ ;  $X = 2/3, 1.0, 1.5$ ;  $g = 2.31, 4.00, 8.52$  and  $h = 5.13, 4.00, 3.37$ , respectively. a, Rate versus fraction reacted. The curves are easily distinguished; b, plots of fraction reacted versus time on arithmetic probability paper. The plots are approximately linear.

(6) The particles are not elongated or flattened. (7) All particles have the same reactivity.

Since all these conditions are not met, and since an exact model would require a quantitative description of the changing geometry of the system which is so far unattained, the following more general rate equation is advocated as a more realistic and accurate method of describing kinetic experiments in this system:

$$1/R = 1/gF^X + 1/h(1-F)^Y \quad (2)$$

where  $X$  and  $Y$  are parameters which depend on conditions (1) to (7) and which can be adjusted to give the best fit for any particular data. This equation will be applied to some data from an experimental investigation of the hydration kinetics of a gypsum plaster in order to illustrate the utility and the limitations of this approach.

The hydration kinetics of a commercial casting plaster (without retarder) were investigated by means of a conduction calorimeter. The copper base of the calorimeter formed part of the heat conduction path and was 4.75 cm in diameter. The calorimeter was calibrated with a dummy sample containing an electrical heater. The rate of hydration was determined from an equation:

$$dH/dt = k_1\Delta T + k_2(d\Delta T/dt) \quad (3)$$

where  $dH/dt$  is the rate of heat evolution,  $\Delta T$  is the difference in temperature between the calorimeter sample holder base and the water bath,  $k_1$  is a calibration constant determined from a steady-state calibration,  $k_2$  is a calibration constant determined from a cooling rate calibration.

The temperature rise in the calorimeter base was 2°–3° C and, since there was a temperature difference across the specimen, the average maximum rise in temperature in the specimen was about 4°–7° C. The data to be presented here have not been corrected for this temperature rise, although for this plaster I found a larger temperature co-efficient than reported by other workers<sup>4</sup>.

The following six experiments illustrate a variety of kinetic effects. *B 32*, a control; *B 44*, acceleration by addition of 1 per cent potassium sulphate; *B 48*, retardation by addition of 1 per cent calcium acetate; *B 46*, acceleration by addition of 20 per cent of gypsum; *B 27*, acceleration by grinding the hemihydrate with pestle and mortar; *B 74*, retardation by lack of stirring.

The ambient temperatures were between 20° and 23° C and the water to calcium sulphate ratio was unity. Specimens contained 2–9 g of plaster. The water and plaster were stirred with a glass rod in a small beaker for 60 sec before pouring into the calorimeter except that in experiment *B 74* the dry plaster was placed in the calorimeter and the water added from a pipette without mechanical stirring.

The temperature difference across the calorimeter was recorded automatically and the reaction rate found from equation (3). The fraction reacted was found by numerical integration. Equation (2) in the cross-multiplied form was fitted to the data by the method of averages. In order to fit the control experiments,  $X$  was taken as 0.8 and  $Y$  as 1.3. The empirical rate constants are shown in Table 1. Using these rate constants and the integrated

Table 1. THE EMPIRICAL RATE CONSTANTS OBTAINED BY FITTING A RATE EQUATION  $1/R = 1/gF^{0.8} + 1/h(1-F)^{1.3}$  TO EXPERIMENTAL DATA

Experiment	No.	Empirical rate constants ( $\times 10^3$ )	
		$g(\text{min}^{-1})$	$h(\text{min}^{-1})$
1 per cent potassium sulphate solution	<i>B 44</i>	48	132
Control	<i>B 32</i>	14	94
1 per cent calcium acetate solution	<i>B 48</i>	5.3	50
Addition of 20 per cent A.R. gypsum	<i>B 46</i>	68	68
Re-ground plaster	<i>B 27</i>	41	99
No stirring	<i>B 74</i>	11	54

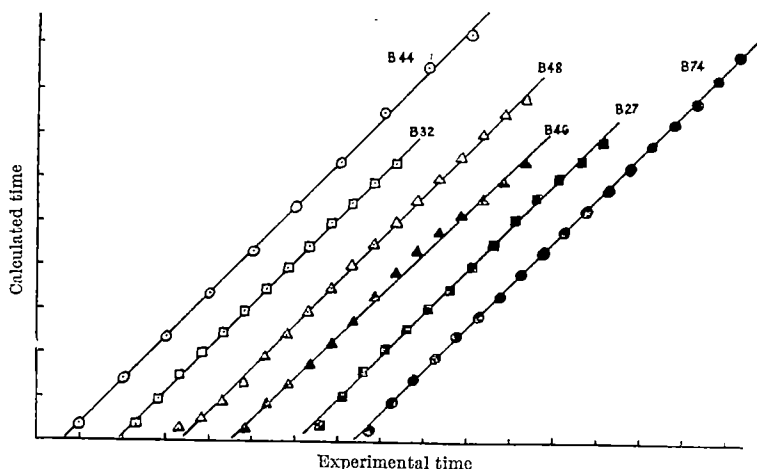


Fig. 2. Calculated times for the reaction to reach successive values of  $F$ , the fraction reacted, plotted against the corresponding experimental times. The empirical rate constants from Table 1 and the integrated form of the rate equation were used to obtain the calculated times. The time intervals marked on the co-ordinates are: *B 44*, 1 min; *B 46* and *B 27*, 2 min; *B 32* and *B 74*, 4 min; *B 48*, 8 min

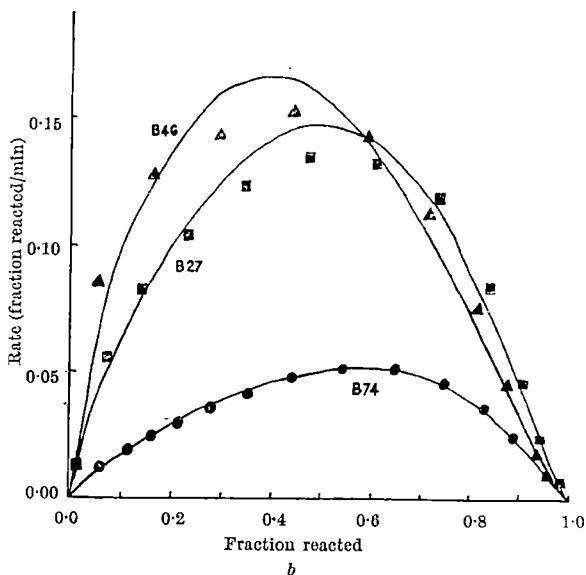
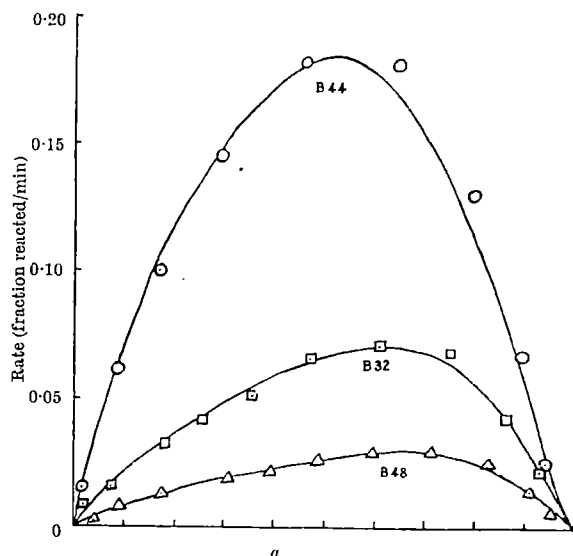


Fig. 3. Rate versus fraction reacted. The points are experimental data. The curves are drawn from the equation:  $1/R = 1/gF^{0.8} + 1/h(1-F)^{1.3}$ , where  $g$  and  $h$  are the appropriate empirical rate constants from Table 1

form of the rate equation, the time needed for the reaction to reach each experimental value of  $F$  was calculated and plotted against experimental time (Fig. 2). At least 91.5 per cent of the reaction is covered by the points in each plot. No data for  $F < 0.02$  or  $> 0.98$  were used.

The description of the experimental data by this equation leads to a simple interpretation of the experiments as follows:

(i) Neither grinding the hemihydrate nor adding gypsum greatly affected the rate of dissolution of hemihydrate, but the rate of precipitation of gypsum increased three and five times respectively.

(ii) Complete lack of stirring nearly halved the rate of dissolution but scarcely affected the rate of precipitation. (Increased stirring, on the other hand, increases the rate of precipitation but has no further effect on the rate of dissolution.)

(iii) A 1 per cent solution of potassium sulphate increased the rate of precipitation three and a half times and the rate of dissolution only one and a half times.

(iv) A 1 per cent solution of calcium acetate reduced the rate constants for precipitation and dissolution to one-third and one-half respectively.

Plots of rate versus fraction reacted (Fig. 3) show the extent to which the equations (continuous lines) fit the data (points). Since  $X$  and  $Y$  were chosen to fit the control experiments it is not surprising that the same equation does not fit too well for large additions of gypsum or for plaster which has been re-ground. If  $X$  and  $Y$  are varied from run to run a much better fit can be obtained but at the expense of the simple quantitative description since empirical

rate constants can be compared directly only when defined by the same function. The ascending arm of the rate curve is often approximately linear, but since the line extrapolates above the origin (especially for longer periods of stirring) it suggests that  $g$ , the empirical rate constant for precipitation, should be defined by the function  $(F + \alpha)^X$  where  $\alpha \approx 0.10$  and  $X \approx 1.5$ .

Comparing Figs. 2 and 3, it is seen that a plot of reaction rate versus fraction reacted is a more critical graphical test of a rate equation than is a plot of fraction reacted versus time.

There is little point in describing the kinetics of calcium sulphate hemihydrate by a rate equation with only one empirical rate constant or, if there are two, of assuming that they have a fixed ratio. The major interest in the system from the point of view of kinetics is in deciding the extent to which a given effect is due to a change in the rate of precipitation of gypsum, or in the rate of dissolution of hemihydrate (or perhaps in the rate of diffusion if this becomes rate controlling).

The equation:

$$1/R = 1/gFX + 1/h(1 - F)^Y$$

(where  $X$  and  $Y$  are adjusted for each batch of hemihydrate) is suggested as an improvement on the uniform spheres model (with  $X = Y = 2/3$ ) since the latter is unconvincing and fits experimental data only poorly.

<sup>1</sup> Polak, A. F., *Kolloid. Zhuv.*, **22** (6), 689 (1960).

<sup>2</sup> Schiller, K., *J. Appl. Chem.*, **12**, 135 (1962).

<sup>3</sup> Taplin, J. H., *Nature*, **194**, 471 (1962).

<sup>4</sup> Ridge, M. J., *Nature*, **184**, 47 (1959).

## SODIUM-22 AND CAESIUM-134 IN FOODS, MAN AND AIR

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THE radionuclides sodium-22 and caesium-134 are present in the atmosphere in very low concentrations, but because of their relatively high biological uptake they are concentrated in food products and thus find their way to man. The radionuclide sodium-22 (2.6 yr.) is a naturally occurring isotope, being produced by cosmic ray spallation of argon in the atmosphere. Its presence in the atmosphere was first reported by Marquez<sup>1</sup>, who found 0.017 d.p.m./l. in rainwater at Rio de Janeiro. Its potential value as a tracer of atmospheric circulation has been recognized, and measurements for this purpose by Bhandari and Rama<sup>2</sup> have shown air concentrations ranging from 1 d.p.m./10<sup>6</sup> ft.<sup>3</sup> below the tropopause to 460 d.p.m./10<sup>6</sup> ft.<sup>3</sup> above the tropopause. Its production rate in the atmosphere is a function of the effective cosmic ray flux, and it increases by about an order of magnitude with latitude between the equator and the geomagnetic poles at elevations above the tropopause. However, its production rates at elevations below 10,000 ft. vary only slightly with latitude. An additional source of sodium-22 is from nuclear weapons testing, and it is possible that large amounts have been added to the atmosphere during the past atomic test series. The caesium-134 content of the atmosphere has resulted from atomic weapons testing and it appears that its main source is the  $n, \gamma$  reaction on inert caesium-133 with smaller amounts resulting from fission<sup>3</sup>.

The radionuclides sodium-22 and caesium-134 have been measured in air filter samples and other materials which were collected during the past few years as part of a comprehensive investigation of fall-out materials. Air filtration is performed with membrane filters<sup>4</sup> and the concentrations of 9-12 of the long-lived radionuclides are measured by direct counting in an anticoincidence shielded  $\gamma$ -coincidence spectrometer<sup>5</sup>. This instrument surveys the sample with two detectors and simultaneously meas-

Table 1. AIR CONCENTRATION OF CAESIUM-137, CAESIUM-134 AND SODIUM-22 (D.P.M./10<sup>6</sup> FT.<sup>3</sup>)

Date	<sup>137</sup> Cs	<sup>134</sup> Cs	<sup>22</sup> Na	<sup>134</sup> Cs/ <sup>137</sup> Cs × 100	<sup>22</sup> Na/ <sup>137</sup> Cs × 100
1-62*	2,730	6.8	11.9	0.25	0.44
2-62*	4,260	24.7	41.6	0.58	0.98
3-62*	1,470	2.5	0.9	0.17	0.06
4-62	4,430	17.8	13.5	0.40	0.31
5-62	3,900	9.1	8.9	0.23	0.23
6-62	4,540	11.6	15.8	0.26	0.35
7-62	3,710	10.7	11.5	0.29	0.31
8-62	1,810	5.7	7.5	0.31	0.41
9-62	2,750	17.0	12.7	0.62	0.46
10-62	1,290	9.4	4.9	0.73	0.38
11-62	1,450	10.6	5.9	0.73	0.41
12-62	1,030	2.9	4.0	0.28	0.39
1-63A†	1,490	9.8	4.3	0.66	0.29
1-63B	4,410	21.5	10.8	0.49	0.25
2-63A	2,610	9.1	10.4	0.35	0.40
2-63B	1,730	4.7	5.0	0.27	0.29
3-63A	3,090	8.3	12.1	0.27	0.39
3-63B	2,960	7.5	9.4	0.25	0.32
4-63A	5,130	8.9	13.9	0.17	0.27
4-63B	5,080	12.0	22.7	0.24	0.45
5-63A	6,550	17.1	27.8	0.26	0.42
5-63B	13,400	43.2	55.0	0.32	0.41
6-63A	5,010	14.6	21.3	0.29	0.43
6-63B	7,390	20.8	27.8	0.28	0.38
7-63A	5,990	18.9	34.0	0.32	0.57
7-63B	6,910	20.7	39.5	0.30	0.57
8-63A	6,980	29.2	39.5	0.42	0.57
8-63B	5,030	13.5	24.7	0.27	0.49
9-63A	3,820	15.4	16.4	0.40	0.43
9-63B	3,800	10.3	15.2	0.26	0.39
10-63A	3,010	11.5	17.9	0.38	0.60
10-63B	1,820	5.8	5.8	0.36	0.36
11-63A	2,060	21.5	6.4	1.04	0.31
11-63B	2,170	8.9	6.2	0.41	0.29
12-63A	1,030	5.8	5.8	0.56	0.56
12-63B	1,700	3.1	5.6	0.48	0.33

\* Only small samples were available. Caesium-134 and sodium-22 value give only the order of magnitude increase.

† A and B signify approximately the first and second half of each month respectively.



Table 2. SODIUM-22, CAESIUM-134 AND CAESIUM-137 IN FOODS, BIOASSAY SAMPLES AND MAN

Sample	Location	Date obtained	d.p.m./kg <sup>22</sup> Na	d.p.m./kg <sup>134</sup> Cs	d.p.m./kg <sup>137</sup> Cs	<sup>22</sup> Na/ <sup>137</sup> Cs × 100	<sup>134</sup> Cs/ <sup>137</sup> Cs × 100
Elk	Quinault, Wn.	5/64	74.4	9.19	6,770	1.10	0.14
Bass	Columbia River	4/64	8.3				
Milk	Washington	3/64	6.2	0.76	396	1.57	0.19
Wheat	Washington	8/63	3.31	1.86	261	1.27	0.71
Beef	Local food store,	5/64	2.47	1.34	1,070	0.23	~0.13
Beef	Washington	3/64	0.52	1.00	255	0.20	0.39
Corn	Oregon	4/64	~0.04		3.8	~1.07	
Lettuce	California	3/64	0.16		43.6	0.37	
Carrots	California	4/64	~0.07		13.1	~0.53	
Potatoes	Idaho	4/64	0.68		57.6	1.18	
Green beans	Oregon	4/64	~0.20		12.7	~1.57	
Urine sample*	Washington		1.45	0.56	144	1.01	0.39
Man†	Washington	11/63	39.5†	160†	48,000†	0.082	0.34
Caribou meat		7/63	180	27.6	8,320	2.16	0.33
Caribou meat		7/63	89.1	20.9	6,000	1.49	0.35
Moose meat		3/64	58.7		2,846	2.06	
Moose meat	Near Kotzebue, Alaska	3/64	80.6	~2.3	3,670	2.20	~0.06
Caribou meat		8/5/61	9.13	33.9	2,520	0.36	1.34
Caribou meat		10/9/61	23.0	287	20,600	0.11	1.39
Reindeer meat		8/63	15.6	215	36,000	0.043	0.60
Urine‡	Anaktuvuk Pass, Alaska	1/64	126	16.2	5,840	2.16	0.28
Man (Eskimo)†	Anaktuvuk Pass, Alaska		3,427†	4,620†	1,950,000†	0.18	0.24

\* Richland, Washington, composite from several individuals.

† These values are estimated whole-body burdens for sodium-22, caesium-134 and caesium-137.

‡ Composite from two Alaskan Eskimos.

ures and records the coincidence  $\gamma$ -ray energies in its 4096 channel multiparameter memory. The observed concentrations of sodium-22, caesium-134 and caesium-137 as measured at Richland, Washington, at a sampling point 15 ft. above the ground are recorded in Table 1. During 1962, relatively low volume sampling equipment was used to provide samples so small as to result in counting uncertainties of 10–20 per cent for sodium-22 and caesium-134 (and larger uncertainties during the period January–March, see Table 1); however, during 1963 the large composite samples ( $2 \times 10^6$  ft.<sup>3</sup>) permitted more precise measurements. It is of interest that the air concentrations of each of the radionuclides increased in 1963 by about a factor of 2 over the 1962 rates. The 1963 sodium-22 levels are about an order of magnitude greater than those reported for troposphere air<sup>2</sup> prior to the 1961 atomic tests. A small increase could result from the higher cosmic-ray production owing to the normal sunspot cycle, but the main contribution appears to be the past atomic weapons test series.

During late 1962 and January 1963, caesium radionuclide analyses were performed on the dust collected on the air filters of our laboratory. These are high-volume fibre-glass filters and are not designed for removal of very small particles. The observed caesium-134 to caesium-137 ratio on these filters was 0.016–0.017 (refs. 3 and 6). It is very significant that the relative amount of caesium-134 was 2–4 times higher than that found on membrane air filters collected during the same period of time. It is evident that the caesium-134 is in a greater isotopic abundance on large atmospheric dust particles which are preferentially held by the fibre-glass filters than on small particles which are all removed by the membrane filters<sup>4</sup>. This observation is in accordance with the methods of formation of the caesium isotopes. Caesium-137 is the daughter of xenon-137 (half-life, 3.8 min), while caesium-134 is formed directly either by the reaction caesium-133 ( $n, \gamma$ ) caesium-134, or as a direct fission product.

*Concentrations in foods and man.* Of particular interest in our work is the route by which sodium-22 finds its way to man. On the basis of chemical similarity one might suspect the same foods which carry the caesium radionuclides. The concentrations measured in several of these foods together with measurements from bioassays and estimated body burdens based on these bioassay measurements are recorded in Table 2. In addition to the samples obtained locally, Alaskan caribou, moose and

reindeer meat, and bioassay samples from Alaskan Eskimos were analysed and are included in Table 2. The whole-body concentrations of sodium-22 and the caesium radionuclides in Table 2 were estimated using biological half-lives<sup>7</sup> of 11 days and 140 days, respectively. It was assumed in this estimate that 90 per cent of the sodium and caesium excreted was in the urine and that the daily urinary excretion rate was 1.5 l. The major sources of sodium-22 in the foods considered are meat, milk, wheat and fresh-water fish, with smaller contributions coming from vegetables. The few food samples considered here are certainly not sufficient to give a complete picture of man's sodium-22 intake. However, they indicate the relative importance of different foods and suggest that the foods which carry caesium-137 to man are also the main sources of sodium-22. It is interesting to note that the elk tissue from Quinault, Washington, has sodium-22, caesium-134 and caesium-137 concentrations which are comparable with those in Alaskan caribou. Obviously, a small amount of this meat in the diet could provide the major source of these radionuclides.

The ratios of sodium-22 and caesium-134 to caesium-137 in the samples of Alaskan caribou meat taken just before and just after the 1961–62 test series show a considerable difference. The sodium-22 to caesium-137 ratio in samples taken after the tests (July 1963) was about ten times greater than in samples taken just before the tests, while the caesium-134 to caesium-137 ratio was lower by a factor of 4. This would suggest that during the past test series considerable amounts of sodium-22 were generated. It would also indicate that the major injection of caesium-134 into the atmosphere occurred in tests prior to the 1961–62 series.

We thank D. G. Watson, W. C. Hansen and H. E. Palmer for making the Alaskan and Quinault samples available for investigation. The work was carried out under contract AT(45-1)-1350 for the U.S. Atomic Energy Commission.

<sup>1</sup> Marquez, L., Costa, N. L., and Almeida, I. G., *Nuovo Cimento*, **6**, 1292 (1957).<sup>2</sup> Bhandari, N., and Rama, J., *Geophys. Res.*, **68**, 1959 (1963).<sup>3</sup> Palmer, H. E., and Perkins, R. W., *Science*, **142**, 66 (1963).<sup>4</sup> Perkins, R. W., *Health Physics*, **9**, 1113–22 (1963).<sup>5</sup> Perkins, R. W., *HW-81746* (in the press).<sup>6</sup> Perkins, R. W., *HW-77609*, January, 1963.<sup>7</sup> International Commission on Radiological Protection, *Report of Committee II on Permissible Dose for Internal Radiation* (Pergamon, London, 1959).

# POSTULATED CHEMICAL BASIS FOR OBSERVED DIFFERENCES IN THE ENZYMIC BEHAVIOUR OF CHYMOTRYPSIN AND TRYPSIN

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CHYMOTRYPSIN and trypsin are members of a group of hydrolytic enzymes with an activated serine residue which apparently participates in the catalytic mechanism<sup>1</sup>. The two enzymes share a number of other characteristics as well, some of which are as follows: Both are inactivated by organophosphorus compounds<sup>2</sup>; both can be inactivated by diphenylcarbamyl chloride, the rate of inactivation of  $\alpha$ -chymotrypsin being more rapid<sup>3</sup>; each contains the sequence Gly-Asp-Ser-Gly at its active centre<sup>1</sup>; both are amidases as well as esterases and, although their overall specificities differ, some overlap exists, for example, acetyl-L-tyrosine ethyl ester, a 'specific' substrate of chymotrypsin, is also hydrolysed by trypsin at about 1/13th the rate<sup>4</sup>; evidence for a similar catalytic pathway (including an acyl enzyme intermediate) has been obtained for both enzymes<sup>5</sup>; histidine residues have been implicated in their catalytic mechanism, and in both enzymes the two histidine residues are in close proximity<sup>6</sup>.

There is thus a remarkable similarity between the two enzymes, and Bender and Kaiser have suggested that "in all important mechanistic aspects, catalyses by trypsin and  $\alpha$ -chymotrypsin are similar"<sup>5</sup>. During investigations on the reactivation of the diethylphosphoryl (DEP) and diphenylcarbamyl (DPC) derivatives of  $\alpha$ -chymotrypsin and trypsin<sup>3,7</sup>, however, differences between the two enzymes became apparent. We wish to report on those and other differences and to show that they can be explained if it is postulated that the active centre of  $\alpha$ -chymotrypsin has a general acid not present at the active centre of trypsin.

In 1954, during investigations on the reactivation of DEP-chymotrypsin by hydroxylamine, it was reported by Cunningham<sup>8</sup> and later confirmed in this laboratory<sup>7</sup> that it was not possible to effect more than a 35-40 per cent return of activity regardless of the pH of the medium and the time allowed for completion of the process. We found, on the other hand, that complete reactivation could be obtained using formohydroxamic acid as the reactivator<sup>7</sup>. DEP-trypsin could be completely reactivated by either hydroxylamine or formohydroxamic acid<sup>7</sup> as could DPC-chymotrypsin and DPC-trypsin<sup>3</sup>. Thus, of all the derivatives examined, only DEP-chymotrypsin could not be completely reactivated by hydroxylamine. It was decided that this observation merited further investigation.

The object of the first series of experiments was to determine whether formohydroxamic acid would release the 60-65 per cent of the potential activity remaining after hydroxylamine treatment of DEP-chymotrypsin. DEP-chymotrypsin (0.4 mg/ml.), which was dissolved in 0.25 M *tris*-maleate buffer containing CaCl<sub>2</sub>, 0.01 M, pH 7.0, 1.5 M with respect to hydroxylamine, was incubated for 48 h at 25° C. Maximal reactivation was obtained after this period of incubation. One volume of the reactivated enzyme solution was then mixed with an equal volume of buffer containing hydroxylamine, 1.5 M, and formohydroxamic acid, 1.0 M. Parallel experiments were carried out with chymotrypsin. As shown in Table 1, addition of formohydroxamic acid after incubation with hydroxylamine was without effect.

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Table 1. REACTIVATION OF DEP-CHYMOTRYPSIN BY HYDROXYLAMINE FOLLOWED BY FORMOHYDROXAMIC ACID

	Activity* after treatment with 1.5 M NH <sub>2</sub> OH		Activity after additional treatment with 1.0 M formohydroxamic acid	
	0 h	48 h	Additional 24 h	Additional 48 h
DEP-chymotrypsin	0.2	27.5	21.4	24.4
Chymotrypsin	100.0	94.0	83.0	74.0

\* Assayed with N-acetyl DL-phenylalanine  $\beta$ -naphthyl ester<sup>7</sup>.

Apparently, therefore, treatment of DEP-chymotrypsin with hydroxylamine resulted in the formation of two products: active enzyme and a product incapable of reactivation by either hydroxylamine or formohydroxamic acid.

It occurred to us that the foregoing phenomenon might be related to the process of 'ageing', which had caused di-isopropylphosphoryl (DIP)-pseudocholinesterase, on standing for some time in solution, to be incapable of complete reactivation<sup>9</sup>. In this case it was found that some mono-isopropylphosphoryl (MIP)-pseudocholinesterase, a derivative refractory to nucleophilic attack, had been formed spontaneously from the DIP derivative. We therefore wished to determine whether our unreactivable chymotrypsin fraction was, analogously, mono-ethylphosphoryl (MEP)-chymotrypsin. Twenty-five mg of radioactive DEP chymotrypsin was incubated with 4 ml. of 2 M NH<sub>2</sub>OH in 0.05 M *tris*-chloride buffer, pH 7.0, for 48 h at 25° C. Following dialysis and lyophilization, the product was hydrolysed at pH 12.6, 100° C for 10 min, neutralized to pH 4 with concentrated hydrochloric acid, chromatographed on Whatman No. 1 paper in the solvent system *n*-butanol/acetic acid/water, 120:30:50, and then exposed to X-ray film for autoradiography. This procedure, as was shown by Berends *et al.*<sup>3</sup>, should have yielded diethyl phosphate and monoethyl phosphate from DEP-chymotrypsin and MEP-chymotrypsin, respectively. As shown in Fig. 1, treatment with 2 M NH<sub>2</sub>OH did indeed convert DEP-chymotrypsin to MEP-chymotrypsin in addition to yielding active chymotrypsin. *I*<sub>1</sub> and *I*<sub>2</sub> represent control experiments in which hydroxylamine was omitted from the solution. The major spots (*R*<sub>F</sub> 0.60)

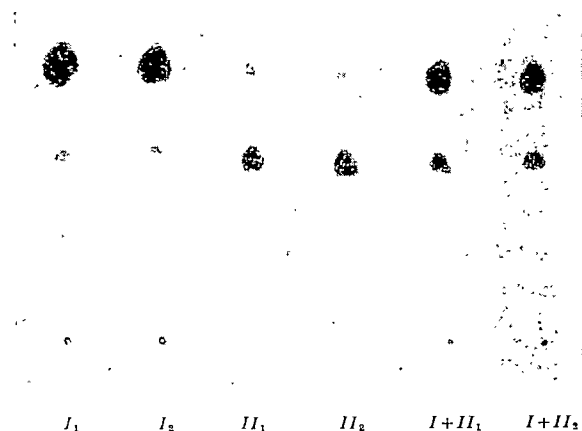
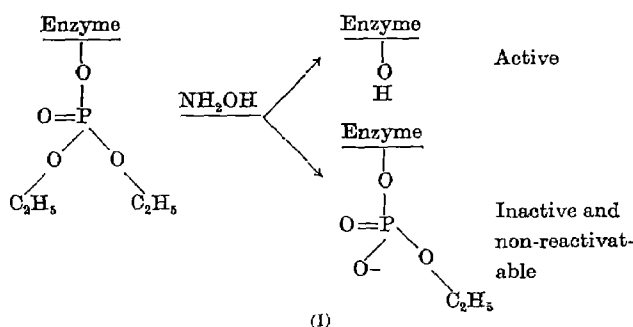


Fig. 1. Autoradiograph of experiments designed to show whether hydroxylamine converted DEP<sup>32</sup> chymotrypsin to MEP<sup>32</sup> chymotrypsin

are diethyl phosphate.  $II_1$  and  $II_2$  represent experiments in which DEP-chymotrypsin was exposed to 2 M  $NH_2OH$ . Traces of diethyl phosphate are present, but the major spots with  $R_F$  0.37 are monoethyl phosphate. It should be noted that traces of monoethyl phosphate exist also in  $I_1$  and  $I_2$ , indicating that some spontaneous 'ageing' had occurred in the absence of hydroxylamine.

The following reaction, therefore, must have occurred:



Thus it was demonstrated that the partial conversion of DEP-chymotrypsin to MEP-chymotrypsin by  $NH_2OH$  is the side reaction responsible for the incomplete reactivation of DEP-chymotrypsin. It cannot occur to DPC-chymotrypsin or DPC-trypsin since there is only one susceptible ester bond—that involving the active serine residue. Hence, the diphenylcarbamyl derivatives are completely reactivated by hydroxylamine<sup>9</sup>.

As the side reaction does not occur during the reactivation of DEP-trypsin, we can postulate from the above that the functional groups at or near the active serine are not the same in  $\alpha$ -chymotrypsin and in trypsin. Although we cannot state with certainty that any difference is relevant to the catalytic mechanism of the enzyme, there is evidence that this is so. The evidence favours the hypothesis that  $\alpha$ -chymotrypsin has an additional electrophilic group which is favourably located to interact with the ethoxy groups of DEP as shown in scheme II. In this scheme, the fate of the hydroxylamine derivative is as described for another system by Jandorf<sup>10</sup>.

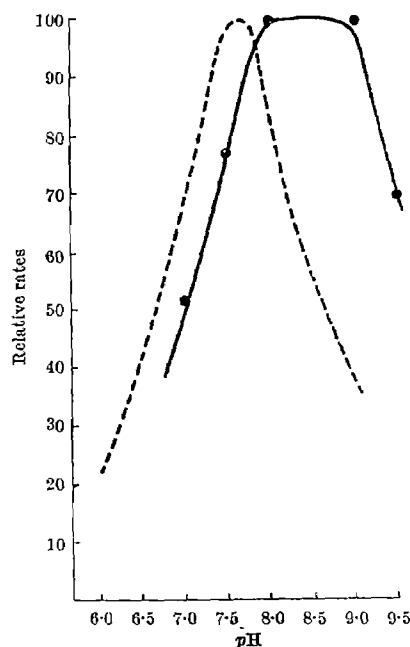
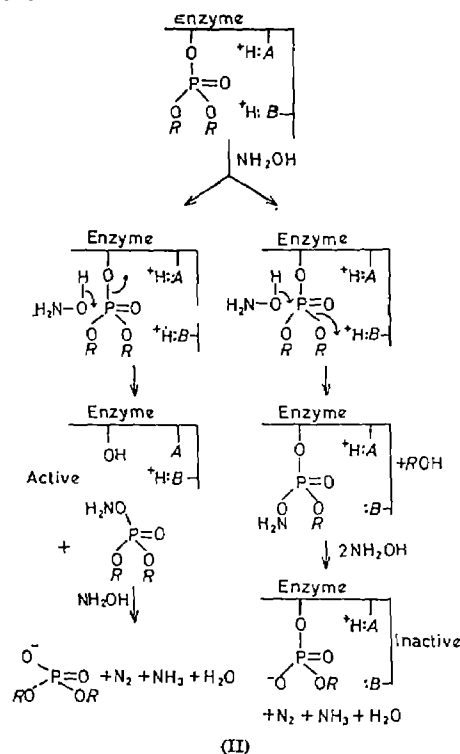


Fig. 2. Effect of pH on rate of reaction of diphenylcarbamyl chloride with trypsin (solid line).  $[E]_0 = 0.6 \times 10^{-4}$  M;  $[I]_0 = 1.2 \times 10^{-4}$  M; buffer, 0.08 M borate-maleate-acetate containing 0.056 M  $CaCl_2$  and 5 per cent acetone. Residual tryptic activity measured with  $\alpha$ -N-benzoyl-DL-arginine *p*-nitroanilide hydrochloride (BAPA)<sup>12</sup>. Dashed line taken from ref. 3.

Assuming the existence of this additional group (data will be presented below), the possibility that it might be part of the binding site of chymotrypsin was considered. If it were, it might be possible, by blocking this site with indole, to obtain complete reactivation with hydroxylamine. However, incubation of DEP-chymotrypsin with M  $NH_2OH$  in the presence of  $4 \times 10^{-3}$  M indole at pH 7 (0.057 M *tris* buffer) for 48 h did not increase the yield of active enzyme.

There is further evidence of the existence of the additional group in chymotrypsin. An unexpected difference between chymotrypsin and trypsin was revealed during an investigation of their reaction with diphenylcarbamyl chloride. We reported elsewhere<sup>3</sup> that a plot of pH against rate of inactivation of chymotrypsin gave a bell-shaped curve with a maximum at pH 7.8. Similar experiments with diphenylcarbamyl chloride and trypsin gave the curve shown in Fig. 2. For comparison, the curve for the reaction with  $\alpha$ -chymotrypsin is also shown. As can be seen, the curve for the reaction with trypsin starts to descend at pH 9-9.5 instead of having a maximum at 7.8. If the chymotrypsin curve can be considered to be a reflexion of a reaction involving two groups, one with a  $pK_a$  of 6.6 and the other with a  $pK_a$  of 8.7<sup>3</sup>, we can conclude that trypsin lacks the latter group. This would be in accord with the previous results and with the hypothesis that chymotrypsin has an additional group at its active site. On the other hand, the curve in Fig. 2 is compatible with the participation of a group with a  $pK_a$  near 10.

It has recently been shown by Trowbridge and collaborators<sup>11</sup> that the tryptic hydrolysis of the specific substrate  $\alpha$ -N-*p*-toluenesulphonyl-L-arginine methyl ester hydrochloride (TAME) is activated by the substrate. In investigations in this laboratory, we found no substrate activation of the chymotryptic hydrolysis of the specific substrate glutaryl-L-phenylalanine *p*-nitroanilide (GPANA) (Erlanger and Cooper, in preparation). Since substrates of trypsin contain ammonium or guanidinium groups, we also investigated the effect of ethylamine on the chymotrypsin hydrolysis of GPANA; neither inhibition nor activation was observed. Thus, again, we find a difference between the manner in which the two enzymes attack their respective specific substrates.



A clue as to the reason for this difference lies in the finding of Inagami and Murachi<sup>12</sup>, and the additional investigations of Erlanger and Castleman<sup>13</sup>, that the tryptic hydrolysis of acetyl glycine ethyl ester (AGEE) is enhanced by a large number of amines, among them ethylamine and cyclopropylamine. We now find that the hydrolysis of AGEE by chymotrypsin is not affected in any way by 0.05 M ethylamine or cyclopropylamine under conditions similar to those used with trypsin<sup>13</sup>. Our recent examinations show, furthermore, that the rate-determining step in the tryptic hydrolysis of AGEE is the formation of the acyl enzyme intermediate. Thus the amines increase the rate of this step of the reaction.

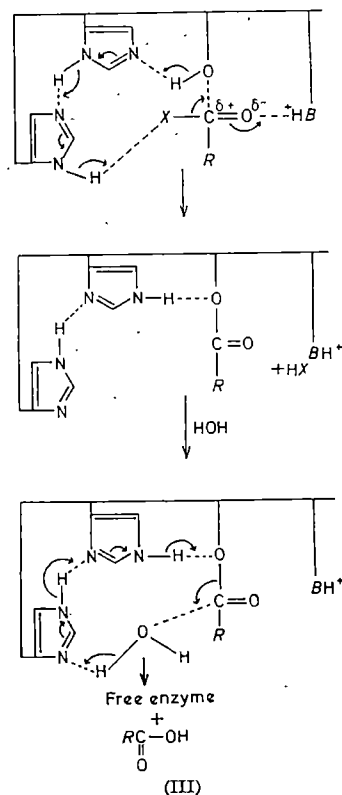
All the foregoing findings can be reconciled if we postulate that trypsin uses an ammonium or guanidinium group of the substrate or activator as the general acid that it lacks at its active centre. In other words, its hydrolysis of a specific substrate occurs via the same mechanism as does the chymotryptic hydrolysis of specific substrates except that trypsin makes use of part of the substrate in its catalytic mechanism.

It might follow from these suggestions that reactivation of DEP-trypsin by hydroxylamine would be incomplete in the presence of ethylamine or cyclopropylamine, since, by analogy with the results obtained with DEP-chymotrypsin, some MEP-trypsin would be formed. This possibility was investigated using 0.1 M ethylamine and 0.1 M cyclopropylamine. No decrease in the extent of reactivation could be detected with either amine. However, in <sup>32</sup>P experiments analogous to those run with radioactive DEP chymotrypsin (the results of which were presented in Fig. 1), small amounts of a substance with the *R<sub>F</sub>* of monoethyl phosphate were found when either of the two amines were present with 2 M NH<sub>2</sub>OH but not when they were absent. Perhaps suitable conditions can be found to increase the yield of MEP-trypsin.

To summarize, the following differences between chymotrypsin and trypsin have been found: (a) Hydroxylamine can completely reactivate DEP-trypsin but not DEP-chymotrypsin. Reaction with the latter leads to the formation of some MEP-chymotrypsin which cannot be converted to active enzyme; (b) MEP-trypsin apparently can result from the reaction of DEP-trypsin with hydroxylamine only if ethylamine or cyclopropylamine is present; (c) ethylamine and cyclopropylamine can accelerate the tryptic hydrolysis of AGEE but have no effect on its chymotryptic hydrolysis; (d) the tryptic hydrolysis of specific substrates is subject to substrate activation. The hydrolysis of specific substrates by chymotrypsin is not activated by substrates, nor is it affected by ethyl or cyclopropylamine; (e) the curve relating pH to the rate of reaction of  $\alpha$ -chymotrypsin with diphenylcarbamyl chloride has a maximum at pH 7.8. That of trypsin with diphenylcarbamyl chloride is essentially 'S'-shaped up to pH 9.0-9.5.

The foregoing findings agree with the hypothesis that chymotrypsin has an additional general acid at its active centre. This group, although not absolutely necessary for the catalytic mechanism, may increase the efficiency of the enzyme. Trypsin lacks this group but can utilize the ammonium or guanidinium group of its specific substrate for the same purpose. If absent from the substrate, these groups, in certain circumstances, can be added as aliphatic amines or alkyl guanidines. The postulated catalytic mechanism of chymotrypsin may be schematically represented as is shown in III.

BH<sup>+</sup> represents the general acid present at its active centre but not at that of trypsin. Trypsin, on the other hand, can use the amine or guanidinium groups of specific substrates or of a large number of aliphatic compounds for the same purpose. The designation of two histidines as participants in the catalytic mechanism is an extension of the findings and suggestions of Neurath and his collaborators<sup>6</sup> and of Hartley<sup>14</sup>. In this scheme they function alternately as general acids and general bases.



The scheme postulates that BH<sup>+</sup> by polarizing the C=O bond increases the net positive charge on the carbon and facilitates a nucleophilic attack on the substrate by the oxygen atom of serine. It is not absolute necessary to the catalytic mechanism but serves to increase the rate of the acylation step. Kupcham *et al.*<sup>15</sup> have shown that this type of polarization can increase the rate of solvolysis of strophanthidin-3-acetate to four times that of coprostane-3 $\beta$ -5 $\beta$ -diol 3-monoacetate in which the additional C=O polarization cannot take place. Thus, finding that the hydrolysis of AGEE (which lacks an NH<sub>3</sub><sup>+</sup> group) by chymotrypsin is about 3  $\times$  faster than its tryptic hydrolysis<sup>12</sup> accords with our hypothesis. The nine-fold increase in the rate of the tryptic hydrolysis of AGEE when ethylamine is present<sup>3,12</sup> is also in reasonable agreement with the postulated role of RNH<sub>3</sub><sup>+</sup> as the BH<sup>+</sup> group in the suggested mechanism. Apparently the BH<sup>+</sup> group does not participate in the deacylation step since the pH-rate profile of this step is sigmoidal for both trypsin and chymotrypsin<sup>16,17</sup>. It is possible that a conformational change accompanying acylation of the enzyme displaces this group. In the reaction scheme, water is shown as the attacking group; the nucleophile may also be NH<sub>2</sub>OH or the unionized species of a hydroxamic acid (cf. ref. 7).

As another consequence of the suggested function of the BH<sup>+</sup> group, the pH-rate profile for the acylation step of chymotryptic catalysis should not be truly bell-shaped. Rather than approaching zero on the basic side of the curve, it should tend towards a value consistent with the rate of the reaction in the absence of the polarizing BH<sup>+</sup> group. A search of the literature reveals that, in those cases examined (for example, see ref. 18), although bell-shaped curves are drawn, the actual data, in fact, indicate a levelling-off of the rate at a pH in the neighbourhood of 9.5-10.0. With respect to the acylation of trypsin, the shape of the curve would depend on the pK<sub>a</sub> of the amine or guanidine derivative when bound to the enzyme. In most cases it should be broader than the chymotrypsin curve.

The identity of the additional electrophile in chymotrypsin cannot be ascertained from these investigations. Presumably it may be an amino group. Although man-

investigations (see, for example, ref. 19) have shown that amino groups are not necessary to the catalytic mechanism of chymotrypsin, they do not rule out an auxiliary function, as postulated. In fact, it has been reported<sup>19</sup> that, while extensive acetylation of chymotrypsin caused a loss of specific activity, the specific activity of acetylated trypsin was the same as that of native trypsin. This could have been predicted from the hypothesis advanced here. Earlier experiments in this laboratory<sup>20</sup> yielded evidence for an additional conformation-sensitive group (other than serine) at or near the active centre of chymotrypsin. The imidazole function of histidine and the hydroxyl group of tyrosine were eliminated as possibilities. On the other hand, the data were consistent with the involvement of an amino group, perhaps the same group postulated as participating as the general acid in the scheme described in this article.

The very interesting findings of Hess and his collaborators<sup>21</sup> implicate the  $\alpha$ -amino group of the N-terminal isoleucine in the catalytic mechanism. This group, however, exists in trypsin also<sup>22</sup> and thus is probably not that postulated in our reaction scheme.

Of course, it is not the purpose of this article to suggest that there exists only one difference between the active centres of trypsin and chymotrypsin. Structural investigations<sup>6,14</sup> have shown otherwise. Moreover, the rates at which trypsin splits substrates containing arginine and lysine are several orders of magnitude higher<sup>4</sup> than the rate at which it hydrolyses AGEE, even in the presence of amines<sup>3</sup>. Differences therefore must exist in those portions of the active centre responsible for orientation and 'freezing' of the substrate into its 'correct configuration'<sup>23</sup>, that is, in the portions responsible for specificity. A discussion of this aspect of the problem is beyond the scope of this article.

One of the questions that remain concerns the ability of formohydroxamic acid to reactivate DEP-chymotrypsin completely. Why does it not, like hydroxylamine, cause the concomitant formation of MEP-chymotrypsin? This, however, is only one of a number of instances in which hydroxylamine behaves anomalously. For example, its pH-rate profile for the reactivation of DEP-trypsin and

DEP-chymotrypsin is bell-shaped<sup>7</sup>; by analogy with its reaction with other acyl enzymes (including DPC-trypsin and DPC-chymotrypsin) it should be sigmoidal. On the other hand, the pH-rate profile for the reactivation of DEP-trypsin and DEP-chymotrypsin by hydroxamic acids is sigmoidal if one assumes that the nucleophilic attack is made by the acid rather than the ionized species<sup>7</sup>. It is hoped that further investigations will clear up these discrepancies.

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## CHEMICAL CARCINOGEN AND ONCOGENIC VIRUS: A POSSIBLE INTERACTION MECHANISM

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**M**ANY diverse agents, chemical, physical or viral in nature, are now known to cause tumours in particular conditions. Several authors have reported synergism between chemical carcinogens and viruses<sup>1</sup>, but so far no common step in the various processes by which these factors act has emerged.

Many chemical carcinogens bind to cytoplasmic protein; thus, following the demonstration of the binding of hepatocarcinogenic aminoazo dyes to cytoplasmic liver proteins<sup>2</sup>, similar findings have been made with acetylaminofluorene in rat liver<sup>3</sup> and aromatic polycyclic hydrocarbons to mouse skin<sup>4,5</sup>. In each of these, there was a quantitative relationship between carcinogenicity and binding to a soluble cytoplasmic protein with characteristic electrophoretic properties. In almost all cases this protein is deleted from the tumours afterwards induced<sup>6-7</sup>.

These and other investigations have yielded an impressive amount of correlative data which point strongly to a causal association between protein binding and carcino-

genesis. In contrast to the large volume of work associating protein-binding with carcinogenesis, there have been few reports of the interaction of carcinogens with DNA<sup>8,9</sup>. However, it must be realized that present technical limitations could preclude the demonstration of carcinogen-DNA complexes involving hydrogen bonding or transient anion-cation complexes which could be of critical importance in carcinogenesis.

If protein-binding is of causal significance in carcinogenesis, and since all malignant change is heritable, carcinogens must bind to a protein having some genetic function. Interaction of a carcinogen with soluble cytoplasmic protein could produce a heritable change in a cell by way of: (a) induction of a 'somatic mutation'; (b) inactivation of a repressor protein; or (c) activation of a latent oncogenic viral infection.

(a) 'Somatic mutation' hypothesis. Protein-binding of carcinogens may effect changes in cellular DNA function if the protein concerned is a gene stabilizer, a gene suppressor or part of the protein moiety of the mitotic spindle

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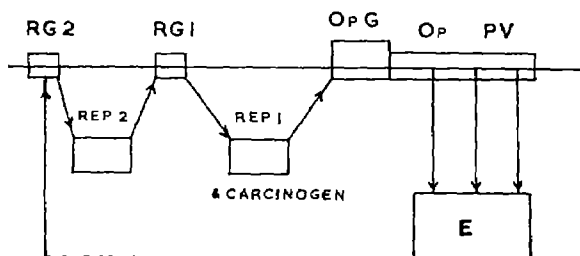


Fig. 1. Diagram illustrating postulated site of action of carcinogen on cellular repressor and means of perpetuating the effect

apparatus. I myself<sup>10</sup>, from a study of the localization of protein-bound tritiated carcinogen in regenerating rat liver, could find no evidence in support of any of these hypotheses.

(b) *Binding to repressor proteins.* It has been suggested<sup>11</sup> that carcinogens may act by binding to repressor proteins. In Fig. 1 the regulator gene (RG1) produces a repressor protein (REP1) which represses the operator gene (OPG) governing the operon (OP). If the carcinogen inactivated the repressor protein (REP1), then the operon (OP) could produce product E. If product E stimulated another regulator gene (RG2) to produce another repressor (REP2) which, in turn, repressed RG1, then the carcinogen would only have to be present for sufficient time to enable enough of product E to accumulate to stop production of REP1 for the process to become self-perpetuating.

Such a scheme would require the appearance of two new cytoplasmic proteins, E and REP2, in pre-neoplastic tissues. The finding<sup>12</sup> that carcinogenic azo-dyes and 2-acetylaminofluorene, but not the non-carcinogenic 2-methyl-4-dimethylaminoazobenzene, increase slow-*h*<sub>2</sub> proteins may be relevant.

(c) *Activation of a latent viral infection.* Many viruses, like herpes simplex, cytomegalovirus and adenovirus, often produce latent infection in mammalian cells. The number of viruses known to have oncogenic potential is steadily increasing. Some of these, like SV40 and adenovirus, were not previously suspected to be oncogenic. Even viruses with no known oncogenic potential, such as vaccinia, can increase the incidence of chemically-induced tumours<sup>1</sup>.

If the role of a chemical is to activate a pre-existing latent oncogenic viral infection so as to make it overt, then the carcinogen would only need to act for an interval sufficient to enable the inactivation of a repressor of viral oncogenesis. Thus the point of biochemical attack on the cell by the carcinogen need not be at a genetically determinant level.

If, in Fig. 1, we associate virus (PV) with the operon, then the inactivation of repressor REP1 by carcinogen could enable PV to produce E and so initiate a malignant change. In such a scheme X-rays would be carcinogenic through the production of a mutation in one of the relevant genes, chemical carcinogens by inactivating repressor REP1, and oncogenic viruses through association with operon OP to produce, in some particular circumstances, sufficient of E to initiate a malignant change.

The importance of immune responses in protecting against polyoma oncogenesis has often been stated. However, the effects of thymectomy on polyoma carcinogenesis<sup>13,14</sup> and on the induction of tumours by 3:4-benzpyrene<sup>15</sup> need not necessarily have an immunological basis. An alternative explanation could involve a cytoplasmic repressor protein which needs a thymic hormone as a prosthetic group for repressor function. Carcinogens could compete with such a hormone at specific reactive sites and give rise to inactive repressor.

It is possible that, for a virus such as polyoma to be oncogenic in suckling mice, it must gain access to the cell before the production of REP1. REP1 may not be produced in mice in the first few days of life. Penetration

of polyoma virus into the cell, after production of REP1 has begun, would then lead to its incorporation into the host genome but with no overt oncogenic activity produced. Operon OP may have evolutionary significance being functionally active in rapid growth and differentiation during embryonic life. At or shortly after birth production of REP1 would control this process. extraneous genetic material is incorporated into the operon, subsequent activation could yield aberrant growth characteristic of neoplasia.

On this hypothesis, if polyoma virus and REP1 could be introduced simultaneously into the cells of susceptible suckling mice, the introduced REP1 could possibly preclude malignant transformation by the virus occurring in the infected cells.

Certain technical difficulties are involved in such an experiment. First, most biochemical work on carcinogen binding has been performed using rat liver, but no virus oncogenic for this organ, is available.

Since fractionation procedures for rat liver cytoplasmic proteins have been developed and this material is available in quantity, it was used, since, if REP1 is neither organ nor species-specific, it could be effective in mice.

Secondly, it is not certain that this particular protein would enter cells, but, as the dye-binding protein is one of the most basic in rat liver and the virus is negatively charged, the protein may adsorb non-specifically to virus. Virus enters cells by pinocytosis<sup>16</sup> and under these conditions protein could also enter either incidentally or adsorbed to virus.

Thirdly, it is possible that some protein may act not specifically in inhibiting the replication of viruses, and it is necessary to incorporate adequate control groups in the experiment. Thus the tumour incidence following the injection of polyoma virus plus protein prepared from normal rat liver (active REP1) either with or without *in vitro* exposure to carcinogen can be compared with the following virus plus buffer (no REP1), virus plus protein prepared from carcinogen-fed rat liver (REP1 rendered functionally inactive by covalently bound carcinogen) and virus plus protein prepared from aminoazo dye induce hepatomata (REP1 missing from tumour).

The results of these investigations are reported here.

All water used was double glass distilled.

'Carbowax MW 6,000' (Union Carbide Co., St. Kild Road, Melbourne) was dialysed against two changes of water and dried. 3'-Methyl-4-dimethylaminoazobenzene (3'MeDAB), m.p. 119°-120° C, was prepared by the method of Giese, Miller and Baumann<sup>17</sup>. Protein solution were stored at 2° C. All possible operations were carried out in a cold room 2°-4° C and completed in 5-6 days after isolation of the original material.

*Preparation of livers.* Male Sprague-Dawley rat (180-240 g) were fed a semi-synthetic diet<sup>18</sup> either unmodified (control group) or containing 0.06 per cent 3'MeDAB for 10 days. The rats were then injected intra peritoneally with 1 ml./100 g body-wt. of sunflower oil (control group) or 1 ml./100 g body-wt. of a 2 per cent solution of 3'MeDAB in sunflower oil (dye-fed group). Two days later their livers were perfused *in situ* via the portal vein with sodium chloride solution (0.15 M, 25 ml. at 37° C. The livers were removed, washed with 0.25 M sucrose at 0° C, minced with scissors and homogenized in pairs, in sucrose (0.25 M, 30 ml.) at 0° C for 2 min in a glass homogenizer. Some of the livers from rats on control diets were homogenized in sucrose solution alone while to others a solution of 3'MeDAB in sunflower oil (2 per cent, 0.5 ml.) was added before homogenization.

Aminoazo-dye-induced hepatomata were dissected free from surrounding liver tissue and homogenized in 0.25 M sucrose as above.

The homogenates were centrifuged at 28,000g for 1 h and the sediments discarded. Thus four liver protein solutions were obtained: (a) from rats on control diet; (b) from rats on control diet plus *in vitro* exposure of live;



proteins to 3'MeDAB; (c) from rats on diet containing 3'MeDAB; (d) from aminoazo-dye-induced hepatomata.

In initial experiments homogenates from rats of groups (a) and (c) were prepared; in later experiments all four groups were included each time.

**Chromatography on ion-exchange cellulose.** DEAE cellulose (Eastman Kodak Co., Rochester, New York) was suspended in pH 7.9 phosphate buffer (0.1 M) and repeatedly filtered and resuspended in starting buffer (0.005 M phosphate, 0.005 M chloride, pH 7.9). The suspension was evacuated with a water pump, poured into the column and packed with a head of buffer so that 12 g of DEAE cellulose gave a length of 20 cm column material. The final supernatant fractions prepared from two livers of rats of groups (a), (b), (c) and (d) were dialysed in a single vessel containing 6 l. of buffer solution (0.005 M phosphate, 0.005 M chloride, pH 7.9) at 2° C. Each dialysate was concentrated to 15–20 ml. by dialysis against a slurry of 'Carbowax' in the same buffer.

The protein solutions were equilibrated overnight by dialysis against 6 l. of this buffer and chromatographed on DEAE cellulose columns, using similar buffer for elution, at a rate of 30 ml./h. Fractions of 5 ml. were collected and the optical density at 280 m $\mu$  was recorded. In accordance with the findings of Whitcutt, Sutton and Nunn<sup>19</sup>, the dye-binding protein was found in the first peak of eluted material (usually fractions 8–18).

**Estimation of azo dyes associated with protein.** For dye estimations the method of Dijkstra and Joubert<sup>20</sup> was used for free dye and that of Miller and Miller<sup>2</sup> for protein-bound dye.

Dye estimations on 100-mg samples of individual protein preparations (first peak from DEAE cellulose) from dye-fed rat liver showed optical densities  $E_{520}$  between 0.740 and 0.990 for protein-bound dye and  $E_{565}$  between 0.067 and 0.180 for free dye.

Levels of free dye per 100 mg protein prepared from livers of rats on control diet but homogenized in the presence of free dye had  $E_{565}$  values from 0.098 to 0.210.

**Preparation of protein inocula.** The 'first peak' proteins from all DEAE cellulose columns were collected and concentrated by dialysis against a slurry of 'Carbowax' in buffer (0.03 M phosphate, 0.11 M chloride, pH 7.0). If any protein solution showed an undue precipitate during dialysis all the protein preparations of that group of two or four were discarded and fresh preparations made.

The concentration of protein in the solutions for inoculation into mice was adjusted so that the solutions had an optical density at 280 m $\mu$  of 27.1–29.8. Attempts to sterilize these solutions using 'Millipore' filters were unsuccessful.

Two thousand units of penicillin and 1,000  $\mu$ g of streptomycin per ml. were added to all protein inocula.

Most protein inocula were used within 5 (never more than 6) days following the killing of the rats and were not frozen at any time. Preliminary experiments show that injection of 0.1 ml. of these protein solutions into newborn mice produced no sign of toxicity.

**Virus.** Polyoma virus obtained from Dr. W. P. Rowe was grown in primary cultures of mouse embryo fibroblasts<sup>21</sup> and gathered at maximum cytopathic effect. The cultures were pooled, the virus was freed from cells by ultrasonic vibration and the preparations were snap frozen and stored at –70° C. The stock had a haemagglutination titre of 2,400 per ml. when tested against 0.4 per cent guinea-pig red cells by the technique of Rowe *et al.*<sup>21</sup>.

**Mice.** Swiss albino mice from a colony maintained in a separate building from the experimental animals were used. Random samples of 12 mice from the breeding colony were screened for polyoma antibody by the technique of Rowe, Hartley, Law and Huebner<sup>22</sup>. All sera were negative.

The mice were bred as required and used when less than 36 h old. Litter mates were distributed at random among

foster mothers and were injected with either 0.1 ml. of buffer (0.03 M phosphate, 0.11 M chloride, pH 7) containing 200 units of penicillin and 100  $\mu$ g streptomycin or with 0.1 ml. of protein preparation intraperitoneally.

Equal volumes of buffer or protein solutions were mixed with viral preparations and allowed to stand for 2 h at 0° C. Two hours after the initial buffer or protein injection the mice were inoculated with 0.1 ml. of the corresponding viral mixture. This was given in the nape of the neck by a fine-gauge needle introduced through the buttock. As soon as the injection bleb in the neck had subsided the mice were replaced with their foster mothers. For each batch of proteins randomized litter mates of all groups were injected with the same dose of virus.

Preliminary experiments showed that injection of undiluted virus produced much runting, while dilutions of 10<sup>-3</sup> (using medium 199 for dilution) gave too low an incidence of tumours to be of use. Dilutions in the range 10<sup>-0.75</sup>–10<sup>-1.5</sup> gave 25–30 per cent tumour incidence in the mice used and were used in subsequent experiments.

The mice were weaned on the 21st day and afterwards fed on 'Barastoc' and greens. No attempt was made to segregate the sexes and any litters were removed daily. The animals were examined weekly for tumours and all were killed and necropsied at 22 weeks.

Fifteen new-born mice were injected intraperitoneally with 0.1 ml. of protein solution obtained from livers of rats on control diet with *in vitro* exposure to 3'MeDAB and 0.05 ml. of the same solution subcutaneously and kept for 22 weeks in an animal house free from polyoma.

Animals were scored depending on whether or not tumours had developed within 22 weeks, no extra weight being given to those animals having multiple tumours. Mice given protein with dye adsorbed *in vitro* only showed no tumours. In all the groups of mice given polyoma virus, typical neck and breast tumours were commonly found. No significant differences in sites of tumour development were found between any of the groups and no liver tumours were found in any mouse.

The cumulative tumour incidence in various groups is shown in Table 1. Some mice died from maternal neglect, trauma or shock after injection. Among the animals surviving longer than one week after injection were several runt mice. Many of these died long before the termination of the experiment without developing tumours. In particular, those mice treated with protein from aminoazo dye-fed rat liver protein and viral dilutions of 10<sup>-0.75</sup> and 10<sup>0</sup> showed a very high incidence of runting. The percentage cumulative tumour incidence in these groups of mice, in particular those given 10<sup>0</sup> virus dilution, was lower than that in mice given corresponding protein and 10<sup>-1.5</sup> dilution of virus inoculum. It is possible that the runt mice dying early in the experiment would have been more likely to develop tumours if they had survived; this is suggested by the dose-response curves of Kraemer<sup>23</sup>.

It can be seen that there is a trend in those mice given normal liver protein to develop fewer tumours with polyoma virus than those given other proteins.

$\chi^2$  tests were applied to each of the small groups at each virus dilution, but with the numbers of animals used

Table 1. INCIDENCE OF TUMOURS IN MICE GIVEN POLYOMA VIRUS AND VARIOUS PROTEIN FRACTIONS

Treatment groups	No. of surviving mice*	No. of mice developing tumours	Percentage tumour incidence
Virus dilution Protein inoculum			
10 <sup>-1.5</sup> Buffer	50	14	28
Dye-fed rats	41	11	27
Normal rats	46	4	9
10 <sup>-0.75</sup> Buffer	56	16	29
Dye-fed rats	38	10	26†
Hepatoma	30	8	27
Normal rats + <i>in vitro</i> dye	53	6	11
Normal rats	54	7	13
10 <sup>0</sup> Buffer	54	20	37
Dye-fed rats	24	5	21†
Normal rats	37	6	17

\* Animals dying without tumours during experiment are omitted.

† These groups showed high incidence of runting.

differences significant at a 5 per cent level were only found occasionally—between buffer and normal liver protein groups, virus  $10^0$  and between buffer and *in vitro* dye protein virus  $10^{-0.75}$ .

However, when the groups at the  $10^{-0.75}$  dosage level are pooled the numbers are large enough to show significant differences. Thus the pooled groups given buffer only, hepatoma protein and aminoazo dye-fed rat liver protein are significantly different (at a 1 per cent level) from the combined groups given proteins from normal rats. The control group is not significantly different from the pooled groups given hepatoma and dye-fed liver proteins, but differs at a 5 per cent level from the combined groups given protein from normal rats. Likewise, the pooled groups of hepatoma and dye-fed liver proteins differ from the two groups given normal liver protein (< 5 per cent level). The results of the  $\chi^2$  tests are shown in Table 2.

Table 2. RESULTS OF  $\chi^2$  TESTS COMPARING NUMBER OF MICE WITH TUMOURS VERSUS (/) NUMBER WITHOUT TUMOURS

Virus	Groups compared Protein	$\chi^2$	Significance (one degree of freedom)
$10^{-0.75}$	Buffer/hepatoma	0	—
	Buffer/dye <sup>1</sup>	0	—
	Buffer/dye <sup>2</sup>	4.02	5% level
	Buffer/normal liver	3.16	—
	Hepatoma/dye <sup>1</sup>	0.06	—
	Hepatoma/dye <sup>2</sup>	2.22	—
	Hepatoma/normal liver	1.62	—
	Dye <sup>1</sup> /dye <sup>2</sup>	2.48	—
	Dye <sup>1</sup> /normal liver	1.83	—
	Dye <sup>2</sup> /normal liver	0	—
$10^{-1.5}$	Buffer/dye <sup>1</sup>	0.01	—
	Buffer/normal liver	4.66	5% level
	Dye <sup>1</sup> /normal liver	3.81	—
$10^0$	Buffer/dye <sup>1</sup>	1.83	—
	Buffer/normal liver	3.70	—
	Dye <sup>1</sup> /normal liver	0.01	—
$10^{-0.75}$	Buffer + hepatoma + dye <sup>1</sup> /dye <sup>2</sup> + normal liver	7.35	1% level
	Buffer/hepatoma + dye <sup>1</sup>	0	—
	Buffer/dye <sup>2</sup> + normal liver	5.70	5% level
	Hepatoma + dye <sup>1</sup> /dye <sup>2</sup> + normal liver	4.91	5% level

Exposures to dye: dye<sup>1</sup> = *in vivo*; dye<sup>2</sup> = *in vitro*.

The experiment was based on the hypothesis that the carcinogen-binding protein(s) is a repressor of viral oncogenesis. The results are reported for general interest and in the hope that other laboratories may repeat the experiment.

In the system used the observed differences in tumour incidence between groups are small. The individual groups at any dosage level of virus are, in general, too small to show significant differences. However, if the groups are pooled in various ways the results become significant at a

5 per cent level in combinations which support the original hypothesis. It must be remembered that the protein inoculum in every case is heterogeneous (each may be resolved into 5 or 6 peaks on carboxymethyl cellulose columns) so that the inoculum consists of mixed proteins plus hypothetical active repressor in the proteins from normal rat livers, mixed basic proteins plus hypothetical inactive repressor in the preparations from dye-fed rat liver and mixed basic proteins without repressor in the hepatoma proteins. The differences between the first of these groups and the others thus suggest that the lower tumour incidence is not simply a matter of non-specific antiviral activity on the part of proteins in the mixture. Thus the results support the hypothesis although, no doubt, other explanations may be advanced.

Further investigations are proposed to test the protective action of this protein on the development of polyoma 'microtumours' in hamster cell lines in tissue culture.

I thank Dr. D. O. White for the virus preparations and Miss A. Doig for her statistical advice.

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## BIOSYNTHESIS OF NIACIN RIBONUCLEOTIDE FROM QUINOLINIC ACID BY MYCOBACTERIA

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MYCOBACTERIA are able to grow in vitamin-free synthetic media and produce niacin. Human tubercle bacilli produce remarkably large amounts of niacin in culture compared with other mycobacteria<sup>1</sup>. The niacin test<sup>2,3</sup>, a biochemical method which differentiates human tubercle bacilli from other mycobacteria, is based on this phenomenon.

Mothes *et al.*<sup>4</sup> and Rio-Estrada *et al.*<sup>5</sup> reported that niacin was formed from aspartic acid by tubercle bacilli, but intermediates in the niacin metabolic pathway, and the nature of the differences in niacin biosynthesis among mycobacteria, are not known.

Recently, Nishizuka and Hayaishi<sup>6</sup> discovered that quinolinic acid was the key intermediate in the biosynthetic pathway of niacin adenine dinucleotide, and free

niacin was excluded as an intermediate in the pathway in rat liver. They also found that this reaction was dependent on 5-phosphoribosyl-1-pyrophosphate (PRPP). Andreoli *et al.*<sup>7</sup> investigated this enzyme activity using *E. coli*. Nakamura *et al.*<sup>8</sup> named the enzyme which catalyses quinolinic acid to niacin ribonucleotide—quinolinate transphosphoribosylase.

The aim of the present investigation was to establish quinolinic acid as a precursor of niacin ribonucleotide in mycobacteria and to define the difference in the activity of quinolinate transphosphoribosylase among mycobacteria. Ten strains of various types of mycobacteria grown on the surface of Sauton's synthetic liquid medium for 3 weeks (one week for rapid-growing and non-pathogenic mycobacteria) were filtered off, and washed three

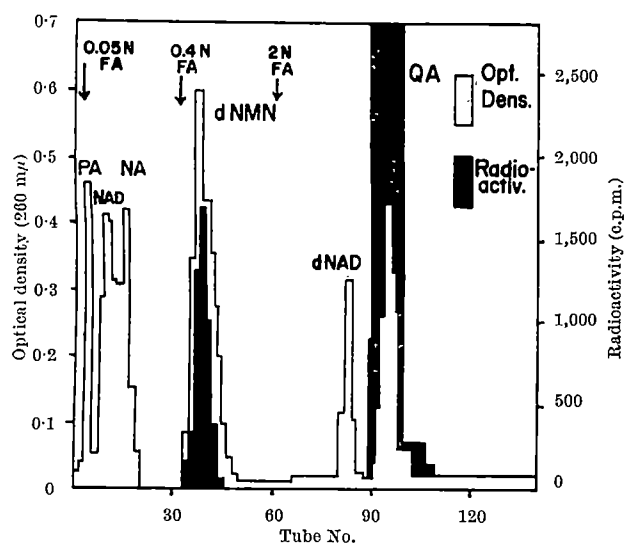


Fig. 1. Column chromatography of the reaction products of quinolinic acid- $^{14}\text{C}$  with extract of human tubercle bacilli *H37Ra*. The mixing chamber contained 300 ml. of 0.01 N formic acid, followed by 300 ml. of 0.05 N, 300 ml. of 0.4 N and 500 ml. of 2 N formic acid. PA, picolinic acid; NA, niacin; dNMN, niacin ribonucleotide; dNAD, deamido-NAD; QA, quinolinic acid. Added carriers were 2  $\mu\text{moles}$  of niacin, 2  $\mu\text{moles}$  of niacin ribonucleotide, 2  $\mu\text{moles}$  of deamido-NAD and 3  $\mu\text{moles}$  of quinolinic acid.

times with water. Cell-free extracts were prepared by grinding mechanically the washed bacteria with sea-sand and 15 per cent bacterial weight of 'Norit A' was added and centrifuged in the cold at 10,000g for 40 min. The protein content of the extracts was determined by Mehl's biuret method.

First, the quinolinate transphosphoribosylase activity of human tubercle bacilli was investigated using quinolinic acid- $^{14}\text{C}$ . 162  $\mu\text{moles}$  (39,600 c.p.m.) of quinolinic acid- $^{14}\text{C}$  (uniformly labelled except  $\beta$ -carboxyl carbon), 600  $\mu\text{moles}$  of PRPP, 400  $\mu\text{moles}$  of phosphate buffer (pH 7.0) and 100  $\mu\text{moles}$  of  $\text{MgCl}_2$  were added to 2 mg protein of charcoal-treated bacterial extract made from human tubercle bacilli *H37Ra* to give a total volume of 2 ml. which was incubated at 37° for 6 h. After the addition of 0.5 ml. of 5 N  $\text{H}_2\text{SO}_4$ , 32.2  $\mu\text{moles}$  (1,320 c.p.m.) of radioactive  $\text{CO}_2$  were recovered. The reaction mixture was neutralized with saturated  $\text{Ba}(\text{OH})_2$  solution and centrifuged to remove  $\text{BaSO}_4$ . After carriers were added, the reaction mixture was put through a 'Dowex 1'-formate, X-8 column (diameter 0.8 cm, length 29 cm) and was washed with 100 ml. of water. The column was developed by the application of a formic acid concentration gradient<sup>8,9</sup>, and 10-ml. fractions were collected. The results of chromatographic separation were shown in Fig. 1 and Table 1. As seen in Fig. 1, two peaks of radioactivity appeared, which coincided with quinolinic acid as a substrate and niacin ribonucleotide as a reaction product. As seen in Table 1, 24.9  $\mu\text{moles}$  of radioactive niacin ribonucleotide was formed from 162  $\mu\text{moles}$  (39,600 c.p.m.) of quinolinic acid- $^{14}\text{C}$ . No radioactivity was detected in the NAD, niacin and deamido-NAD fractions.

The niacin ribonucleotide fractions recovered were combined and concentrated under reduced pressure at

Table 1. DISTRIBUTION OF RADIOACTIVITY OF ENZYMIC PRODUCTS FROM QUINOLINIC ACID- $^{14}\text{C}$  BY HUMAN TUBERCLE BACILLI *H37Ra*

Product	Radioactivity c.p.m.	Amount of product $\mu\text{moles}$	Amount recovered %
$\text{CO}_2$	1,320	32.2*	19.9
Niacin	0	0	0
Niacin ribonucleotide	5,100	24.9†	15.4
Deamido-NAD	0	0	0
NAD	0	0	0
Quinolinic acid	30,160	23.1	75.9
Total	36,580		92.5

\* The value was calculated from the specific activity of 1 carbon which was one-sixth of that of original substrate.

† The value was calculated from the specific activity per 5 carbons. Five carbons out of the original  $^{14}\text{C}$ -carbons should remain in the products.

25° C. When an aliquot of the solution was subjected to paper chromatography, the radioactivity migrated with an authentic sample of niacin ribonucleotide. The reaction product, niacin ribonucleotide, was further identified by vitamin activity. *Leuconostoc mesenteroides* (ATCC 9135), which utilized only free niacin as a growth factor, was unable to utilize this product as a growth factor but did use the product after alkaline hydrolysis (0.1 N  $\text{NaOH}$ , 100° C, 30 min). Thus, human tubercle bacilli is proved to have quinolinate transphosphoribosylase activity and to convert quinolinic acid to niacin ribonucleotide. The formation of niacin ribonucleotide from quinolinic acid was shown to be PRPP-dependent because no niacin ribonucleotide was formed without PRPP.

Niacin- $^{14}\text{C}$  was tried in the same enzyme system as quinolinic acid, but no radioactive niacin ribonucleotide was formed from niacin- $^{14}\text{C}$ , as detected by chromatographic examinations.

Various strains of mycobacteria were tested for quinolinate transphosphoribosylase activity by the determination of radioactive  $\text{CO}_2$  evolution from quinolinic acid- $^{14}\text{C}$  and by the microbiological assay using *Leuconostoc mesenteroides* after hydrolysis of the reaction mixture. As shown in Tables 2 and 3, human tubercle bacilli have a remarkably high quinolinate transphosphoribosylase activity, about 9–20 times that of other mycobacteria, with the exception of BCG. The enzyme activity of the mycobacteria was proportional to the amount of niacin produced in the culture filtrate.

Table 2. EVOLUTION OF RADIOACTIVE  $\text{CO}_2$  FROM QUINOLINIC ACID- $^{14}\text{C}$  BY THE EXTRACT OF MYCOBACTERIA

Species	Strains	$^{14}\text{CO}_2$ evolved c.p.m.	$\mu\text{moles}$
<i>M. tuberculosis</i>	<i>H37Rv</i>	506	12.4
	<i>H37Ra</i>	432	10.5
<i>M. avium</i>	11755	46	1.0
Photochromogen	p-8	48	1.0
Scotochromogen	p-6	30	0.7
Non-photochromogen	100616	30	0.7

The reaction mixture contained 57.2  $\mu\text{moles}$  of quinolinic acid- $^{14}\text{C}$  (14,000 c.p.m.), 200  $\mu\text{moles}$  of PRPP, 100  $\mu\text{moles}$  of  $\text{MgCl}_2$ , 400  $\mu\text{moles}$  of phosphate buffer (pH 7.0) and 0.5 mg of charcoal-treated bacterial extract in a total volume of 1.5 ml. Incubated at 37° for 6 h.

Table 3. NIACIN RIBONUCLEOTIDE FORMATION FROM QUINOLINIC ACID BY MYCOBACTERIA

Species	Strains	Niacin ribonucleotide formed ( $\mu\text{moles}$ ) Before hydrolysis	After hydrolysis
<i>M. tuberculosis</i>	<i>H37Rv</i>	3	94
	<i>H37Ra</i>	3	91
<i>M. bovis</i>	BCG	1	42
<i>M. avium</i>	11755	0	6
Photochromogen	p-8	0	4
Scotochromogen	p-6	0	10
Non-photochromogen	100616	0	8
Rapid growers	Sato	0	5
Non-pathogenic mycobacteria	<i>M. phlei</i>	0	4
	<i>M. smeg.</i>	0	4

The reaction mixture contained 2  $\mu\text{moles}$  of quinolinic acid, 4  $\mu\text{moles}$  of PRPP, 100  $\mu\text{moles}$  of  $\text{MgCl}_2$ , 4  $\mu\text{moles}$  of phosphate buffer, pH 7.0, and 2 mg of charcoal-treated bacterial extract in a total volume of 3.0 ml. Incubated at 37° for 6 h. Free niacin was determined using *Leuconostoc mesenteroides* (ATCC 9135) before and after hydrolysis (0.1 N  $\text{NaOH}$ , 100°, 30 min) of the reaction mixture.

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Quinolinic acid- $^{14}\text{C}$  was kindly provided by Drs. O. Hayaishi and Y. Nishizuka, Department of Medical Chemistry, Kyoto University Faculty of Medicine.

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COMPARATIVE STUDIES OF AMINO-ACIDS IN SHELL STRUCTURES OF  
*Gyraulus trochiformis*, STAHL, FROM THE TERTIARY OF  
STEINHEIM, GERMANY

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Fool: Canst tell how an oyster makes his shell ?  
 Lear: No.  
 Fool: Nor I neither: but I can tell why a snail has a house.  
 Lear: Why?  
 Fool: Why, to put's head in.  
*(King Lear, Act 1, Sc. V (Shakespeare))*

SHAKESPEARE'S concept on "Why a snail has a house" has been modified recently. The present consensus on mineralization phenomena in biological systems is that certain organic tissues provide a set of highly specific templates which act as nucleation sites and appear to exercise control over the mode and orientation of the mineral phase. Little is known, however, to account for the wide variation in kind, size and orientation of minerals that are generated by organisms.

Recent biochemical investigations of the organic matrix in bone and shell structures have opened new vistas<sup>1-5</sup>; that is, the arrangement and biochemical composition of mineralized tissues seem to influence both the shell mineralogy and the morphology of the biological species. If these data fall into a pattern, comparative biochemical investigations may reveal interesting details on evolutionary trends in the Earth's history and be used for taxonomic purposes. The objective of the work recorded here is to continue along this line by investigating the development of the organic matrix in the shell structures of a series of perfectly preserved Tertiary freshwater gastropods from the Steinheim Basin, Germany.

The Steinheim *Planorbis* gastropods of the species *Gyraulus trochiformis*, Stahl, are characterized by a unique morphological development of the shell structure. Simple (small-flat) specimens gradually advance into complex (large-high) specimens in relatively short periods of time<sup>6</sup>. This type of alteration has been observed at least twice in the stratigraphical record of the Steinheim Basin sediments. The first sequence investigated starts with the sub-species *steinheimensis* and has its more advanced member in *trochiformis*, whereas the second sequence begins with *oxystoma* and ends with *supremus* (Table 1). Thus *steinheimensis* is the oldest and *supremus* the youngest sub-species of the *Planorbis* series. It should be noted that another sub-species precedes *steinheimensis*, but specimens were not available at the time of the investigation.

The amino-acid analysis involved the following steps: (1) 6 N HCl hydrolysis of the carbonate material at 105°C for 22 h in a nitrogen atmosphere; (2) desalting by ion-exchange resins and elution of amino-acids with 2 N

$\text{NH}_3$ ; (3) chromatographic separation of amino-acids on ion-exchange columns and determination by the ninhydrin reaction, both achieved by the same automatic device<sup>7</sup>: flow rate 70 ml./h and pressure 800 lb./in.<sup>2</sup>. A combined basic-acidic run is accomplished in about 4 h. The carbon dioxide for mass spectrometer analysis is obtained by treating the carbonate with 100 per cent phosphoric acid at 25° C (ref. 8). The isotope data are reported as per mil deviation relative to the PDBI, Chicago belemnite Standards.<sup>9</sup>

Alterations in shell morphology of *Gyraulus* sp. have been interpreted on geochemical grounds to be the result of environmental changes in the sense that a progressive increase or decrease in the salinity of the ancient lake water caused the snails to alter their shell structure<sup>10</sup>. Other organisms, for example fishes, that once populated the Steinheim Lake were presumably less fortunate in adapting to their new surroundings, as they became extinct. Only a few species of *Gyraulus* and some ostracods and algae could perfectly adjust to the new environment; however, judged by the millions of fossilized snails and ostracods, it would seem that the changes favoured these organisms.

The high  $^{18}\text{O}$  content of the shell carbonates reflects a somewhat unusual freshwater environment considering the general low  $\delta^{18}\text{O}$  range for freshwater shell carbonates of about  $-5$  to  $-15$  per mil.<sup>11</sup> The  $^{18}\text{O}$  enrichment in case of the Steinheim specimens must, therefore, mean that prolonged evaporation preferentially removed  $\text{H}_2^{16}\text{O}$  from the ancient Lake leaving a heavy water behind; consequently the concentration of salt must also have increased. Internal (seasonal) variations in the  $^{18}\text{O}/^{16}\text{O}$  ratio of the kind observed earlier in belemnites<sup>12</sup> are also recorded in the *Planorbis* shells; and aragonite is the only mineral form<sup>10</sup>. This attests to the perfect preservation of the fossil material investigated.

The total yield in amino-acids from the shell is rather small (Table 1). On first appearances this feature suggests diagenetic destruction of most of the original organic matrix. The striking resemblance of ancient and recent amino-acid spectra of *Planorbis* sp., however, suggests that one is still dealing with intact proteins. Inasmuch as the organic matrix of the shell structures can be recovered on decalcification as fine, almost transparent, sheets, and furthermore free amino-acids are virtually absent, the second suggestion appears to be true. In summary, it is tentatively concluded that the amino-acids given in Table 1

Table 1. AMINO-ACID COMPOSITION AND  $^{18}\text{O}/^{16}\text{O}$  AND  $^{13}\text{C}/^{12}\text{C}$  RATIO OF SHELL CARBONATES OF *Planorbis* SPECIES

	Basic			Acidic		OH + Imino			Neutral					Sulphur		Aromatic		Total	Isotopes		
	Orn	Arg	Lys	His	Glu	Asp	Thr	Ser	Pro	Gly	Ala	Val	Iso	Leu	Cys	Met	Tyr	Phe	μmoles/g	δ <sup>18</sup> O	δ <sup>13</sup> C
Recent	0	46	79	33	86	95	55	60	51	111	85	59	36	53	20	10	65	56	39.08	<i>n.d.</i>	<i>n.d.</i>
<i>supremus</i>	70	6	34	42	103	118	44	62	90	105	101	56	29	40	10	10	52	28	2.59	+1.3	-1.8
<i>supremus-revertens</i>	60	7	36	28	97	108	32	70	79	122	121	59	29	52	17	22	22	39	1.80	+0.3	-3.8
<i>revertens</i>	75	3	61	29	124	75	21	80	86	126	93	57	29	47	17	17	28	32	2.95	+2.3	-1.4
<i>oxyostoma</i>	50	29	65	22	138	75	22	57	58	147	108	49	39	61	22	11	17	32	2.31	+2.2	-0.4
<i>trochiformis</i>	78	22	37	24	104	96	20	77	66	181	157	39	16	33	11	12	9	18	2.02	+4.7	-0.9
<i>planorbiformis</i>	86	0	42	21	109	89	33	77	25	130	115	58	41	58	26	29	17	44	0.97	+3.2	-0.7
<i>sulcatus</i>	43	11	35	33	157	86	24	58	25	187	155	41	20	36	34	12	19	24	2.37	+2.8	-1.0
<i>tenuis-steinheimensis</i>	66	6	59	40	145	60	31	64	59	120	89	51	33	50	44	21	16	46	0.75	+2.4	-0.0
<i>steinheimensis</i>	52	4	58	42	150	44	16	34	45	170	161	57	31	45	16	18	25	32	1.15	<i>n.d.</i>	<i>n.d.</i>

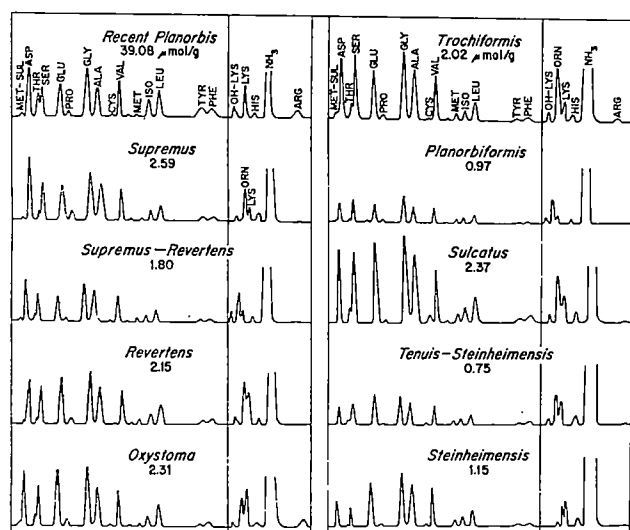


Fig. 1. The amino-acid composition of the organic matrix in shell structures of *Planorbis* sp.

and Fig. 1 represent in close approximation the unaltered building blocks of the original mineralized tissues of *Gyraulus trochiformis*, Stahl. One exception is ornithine, the presence of which appears to be related to the alteration of arginine; it is remarkable, however, that acid hydrolysis is required for the release of ornithine from the organic matrix.

In adopting the customary way of presenting data on amino-acids in mineralized tissues<sup>1,2,4</sup>, the amino-acid values originally calculated on a μmole basis are expressed in amino-acid residues per thousand total (Table 1 and Fig. 1). The isomers of OH-lysine are not included in Table 1, for the area under the peaks could not be reasonably quantitized. It should also be noted that the concentration of the amide amino-acids, that is, glutamine and asparagine, could not be estimated, since the analytical extraction techniques (NH<sub>3</sub>-elution) involved the addition of large amounts of ammonia to the sample.

The neutral amino-acids account for approximately 40 per cent of the total residues; but the ratio of Gly:Ala:Val:Iso:Leu is about the same for all specimens investigated. In contrast, the acidic and basic residues, which respectively amount to 20 and 15 per cent of the total, change in a systematic fashion. While glutamic acid and lysine decrease in abundance from *steinheimensis* to *trochiformis* and again from *oxystoma* to *supremus*, aspartic acid and to a lesser degree the remainder of the basic amino-acids show the reverse trend. The glutamic acid/aspartic acid relationships are plotted in Fig. 2. The loss or gain in residues along the *steinheimensis-trochiformis* profile can be as high as 46 in 1,000 for a single basic or acidic amino-acid. Concerning the rest of the amino-acids, it is only possible at this point to say that the ancient mineralized tissues are generally lower in threonine, tyrosine and phenylalanine than the organic matrix in recent *Planorbis* shell structures; and that proline and sulphur-containing amino-acids exhibit about the same level of concentration for most of the samples investigated.

The following tentative interpretation is offered to account for the amino-acid relationships found in the organic matrices of the gastropod shell structures. Although the primary structure, that is, the specific sequence of amino-acids in the polypeptide chain, cannot be fully delineated at present, it is suggestive that alternating peptide cores, largely consisting of serine, proline and the neutral amino-acids, constitute the molecular framework of the fibrous proteins. Most of the basic, acidic and amide amino-acids, on the other hand, represent the linking residues between one core and the

other. Because the electrical behaviour of proteins and their isoelectric points are determined by the number and location of acidic and basic amino-acids, the special importance of these groups of amino compounds for the problem at hand is well documented. It is conceivable that peptide bonds may open (hydrolysis) at a position where basic, acidic or amide amino-acids confront each other. In consequence, small peptide chains composed predominantly of neutral amino-acids that fall between two such isoelectric points may be eliminated from the matrix. Thus, holes will develop within the protein structure and the now freely exposed acidic or basic amino-acids in the organic residue may concentrate Ca<sup>++</sup> at the acidic side-chains and CO<sub>3</sub><sup>--</sup> at the basic side-chains<sup>2-4</sup>. Nucleation of a mineral phase may proceed inside the holes once an appropriate concentration of the ions has been achieved. Indeed, electronmicrographs of mineralized tissues show a variety of pore patterns that are characteristic for a certain biological species<sup>13</sup>.

In following the scheme outlined, it is finally concluded that the increase in salinity caused the organic tissues in the shell structure to develop a series of additional pores, thereby increasing the number of nucleation sites and decreasing the amount of protein per total carbonate. Thus, some calcified tissues become more effective areas of mineralization than others. It is conceivable that systematic variations in the total amount of organic matter in the shell structure are related to this phenomenon and consequently may be utilized in comparative biochemistry. Inasmuch as the secondary structure of a polypeptide chain and the spacing of the pores will definitely influence the orientation and location of the biogenic minerals, the shell morphology has to change accordingly. Perhaps the alteration of the shell structures as observed in the *Steinheim* specimens is only a consequence of the 'evolutionary' development of the organic matrix and, therefore, a secondary feature. Of particular biochemical interest is also the appearance of hydroxylysine and the absence of hydroxyproline. Both amino-acids are found in mineralized tissues of vertebrates (collagen), but are missing in a number of invertebrates<sup>2</sup>.

Comparative biochemical investigations of mineral tissues tracing, for example, the appearance, disappearance or ratio of amino-acids throughout the plant and animal kingdom may eventually help to outline evolutionary trends in biology and palaeontology, or be of assistance in taxonomic investigations. This can be of special interest in the case of the lower organized groups of organisms (bacteria, foraminifera, etc.), where classifications are frequently based solely on the morphology of the mineralized parts. The relatively high level of acidic and basic constituents in gastropods compared with most other invertebrates so far investigated may explain why they exhibit such a wide variety of shell morphology.

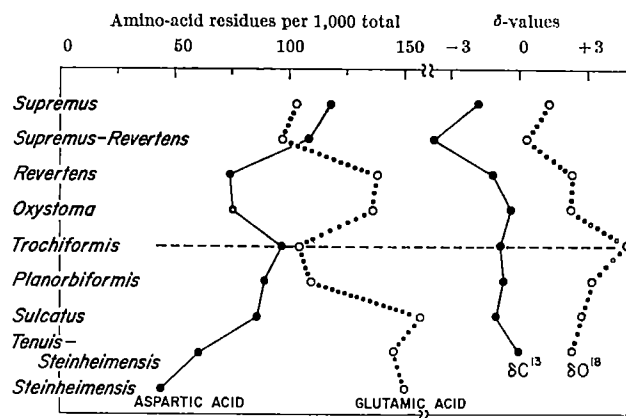


Fig. 2. The aspartic acid/glutamic acid relationships in mineralized tissues of *Gyraulus trochiformis*, Stahl, and the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of the shell carbonate

Finally, if one uses these data as analogy, one perhaps can also tell "How an oyster makes his shell" and thus answer the old Shakespearean question in a more constructive fashion.

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## GROWTH OF A PRODUCTID SHELL AND ITS IMPLICATION ON A METHOD OF STATISTICAL CORRELATION

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MARTIN, in his original usage of the word 'productus' in the combination *Anomites productus*, commented directly on the considerably greater length many productid shells have in comparison with their width. The statistical characterization of productid shells in modern investigations always includes, therefore, the measurement of this property, and an important aspect of bivariate analysis is the correlation of the length with the other primary dimensions of the shell. Because of the peculiar shape of the productid shell it is necessary to distinguish between (1) the curved length of the pedicle valve or peripheral distance from umbo to anterior margin; (2) the curved length of the brachial valve or peripheral distance from the hinge-line to anterior margin; and (3) the straight

length from the hinge-line to the anterior margin, all three being measured in the median plane of the shell.

Several authors have noticed a change in growth rate of brachiopod shells during 'old age'—for example, Parkinson in *Dielasma*<sup>1</sup> (pp. 209–213) and *Schizophoria*<sup>2</sup> (pp. 373–376), and Prentice in *Gigantoproductus*<sup>3</sup> (pp. 230–232). The present article attempts to illustrate graphically the character of this alteration in the growth pattern and to demonstrate the necessity of distinguishing the component growth curves before calculating growth ratios. The productid profile used in the figures is that of a new species of marginiferid from the upper Viséan of Scotland, a systematic account of which will be published shortly.

Consider initially a standard plot of the correlation and reduced major axis of, for example, the thickness (height) of the visceral cavity and the curved length of

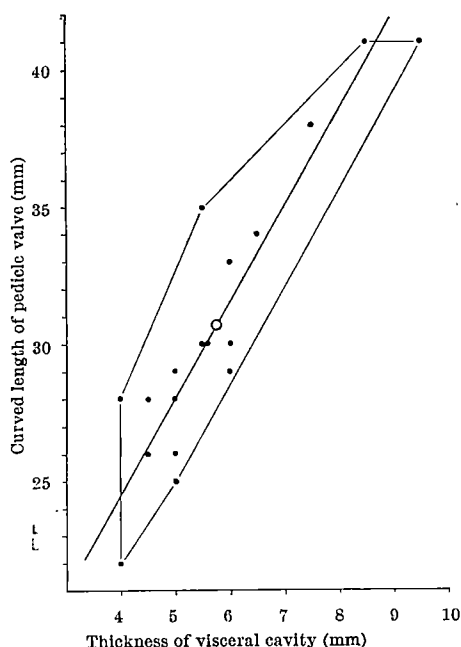


Fig. 1. Correlation of the curved length of the pedicle valve with the thickness of the visceral cavity. Observed values are shown as dots, the sample mean as an open circle. The reduced major axis gives a growth ratio of 1:3.5

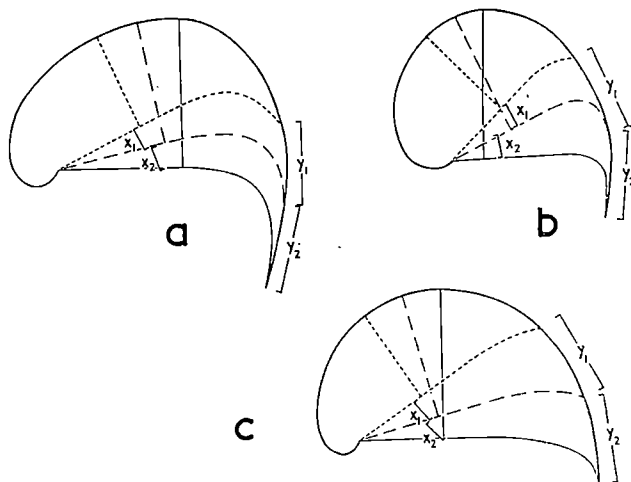


Fig. 2. a, b and c represent three productid profiles; in each, a unit increase in the thickness of the visceral cavity ( $x_1$ ,  $x_2$ ) corresponds to a unit increase in the curved length of the pedicle valve ( $y_1$ ,  $y_2$ ). The growth ratio is 1:3.5 as in Fig. 1. The scatter of the observed data on Fig. 1 would normally be interpreted as the effect of the type of variation in growth form illustrated on Fig. 2. If the growth form of the productid is stable, however, this scatter has another meaning as explained in the text



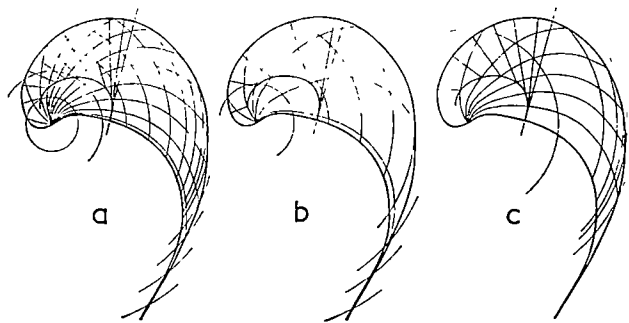


Fig. 3. The profile of the pedicle and brachial valves of a productid is reproduced identically in *a*, *b* and *c*; the heavy line between the valves is the thickness curve (see text) which is also identical on each drawing. The remaining lines represent a system of co-ordinates to illustrate the growth pattern of the shell. From the intercepts of these lines on each other, on the valves, and on the thickness curve, it is possible to measure the relative increases in the curved length of the pedicle valve, the curved length of the brachial valve, and the thickness of the visceral cavity. In *a*, the variation in the increments of the curved length of the brachial valve and the thickness of the visceral cavity are shown for unit increase in the curved length of the pedicle valve; in *b*, the variation in the increments of the curved length of the pedicle valve and the thickness of the visceral cavity are shown for unit increase in the curved length of the brachial valve; and in *c*, the variation in the increments of the curved length of the pedicle valve and the curved length of the brachial valve are shown for unit increase in the thickness of the visceral cavity.

the pedicle valve (Fig. 1); the measurements are taken from a sample of the population at a single locality. The correlation is good ( $r = 0.917$ ) and the slope of the regression line indicates a growth relationship whereby a unit increase in the thickness of the visceral cavity corresponds to an increment of the curved length of the pedicle valve 3.5 times as great. Using the method described by Prentice<sup>3</sup>, the curvature of the pedicle valve can be analysed in terms of a compound of successive logarithmic spirals. Following this procedure for the sample in question reveals this curvature to be an extremely constant feature of the shell morphology, the sample variation being less than 5 per cent. Assuming, therefore, that variation in curvature of the pedicle valve can be considered to be negligible in this context, it is then apparent that the 1:3.5 growth ratio, here, is not a measure of the average growth ratio for a sample of shells of differing growth forms (Fig. 2) within the total fossil population but a summary of the relative growth of the curved length of the pedicle valve and the thickness of the visceral cavity of any individual of the population. This conclusion enables direct comparison of the growth ratio obtained from the population sample with a growth ratio measured directly from one specimen.

Minimizing the effect of the thickness of the pedicle valve, an accurate median plane profile has been drawn as the basis of Fig. 3. This profile has three variable qualities: (1) the curved length of the pedicle valve; (2) the curved length of the brachial valve; and (3) the thickness (height) of the visceral cavity. It is assumed that the profile of the shell represents a continuous growth form which has not been modified by resorption or addition of secondary accretionary deposits. Such changes may have taken place in the umbonal region, which is consequently omitted from the investigation. From this known profile it is possible to reconstruct an infinite number of previous growth stages by rotating the brachial valve about the hinge towards the pedicle valve and recording points of intersection of the curves of the two valves.

The thickness of the visceral cavity is the maximum separation of the valves normal to tangents of the shell surfaces. As the curvature of the two valves is constant (as opposed to increases in length along these curves) it follows that there must be a third constant curve described by the locus of the point of intersection of the line of maximum thickness with the brachial valve as the brachial valve is rotated. This line is termed the thickness curve and is shown as a thick line in Fig. 3.

Figs. 3*a*, *b* and *c* represent plots of the growth increments of two of the variables for unit increase of the third. From Fig. 3*a* and the graphical representation of the data in Fig. 4*a*, it is clear that for successive unit increase in the length of the pedicle valve the amount by which the brachial valve grows in the same interval of time progressively diminishes to a minimal value approaching the trail. At this point a change in growth rate must have occurred, for subsequent increments of the brachial valve progressively increase to a point lower on the trail. At this second point the parallelism of the brachial and pedicle trails prevents continued growth of the valves at independent rates. Subsequent growth of the two valves is therefore practically equal. Similarly from Figs. 3*a* and 4*c*, it can be seen that for successive unit increase in the length of the pedicle valve the equivalent increments in the thickness of the visceral cavity rise asymptotically to a maximum value which corresponds to a position on the pedicle valve at the junction of the visceral disk and the trail. As in the previous instance a change in the growth pattern then takes place and the subsequent increments of thickness rapidly decrease until the stage is reached in which parallelism of the growth directions of the two valves is achieved. At this point no further increase in the thickness of the visceral cavity is possible without abandoning the curved surface of the trail of the brachial valve. The termination of the growth curve at the point X on Fig. 4*c*, therefore, indicates the position of onset of this ultimate condition in which the thickness remains constant regardless of the extent to which the pedicle valve may grow.

Fig. 3*b* illustrates the variation of incremental values of the curved length of the pedicle valve and the thickness of the visceral cavity corresponding to a succession of equal increases in the curved length of the brachial valve. The growth curves for Fig. 3*b* are complementary to those of Fig. 4*a* and 4*b*, with, in this case, unit increase in the length of the brachial valve plotted on the ordinate axis. Such reciprocal curves show, of course, the inflexions corresponding to the growth-rate changes. As the brachial valve increases in length the corresponding increments in the thickness of the visceral cavity follow a curve complementary to that of Fig. 4*b* but of the type of Fig. 4*c*, that is, rising asymptotically to a maximum value corresponding to the growth stage of the brachial valve where it commences to turn towards the pedicle valve. From this initial change in the growth direction of the brachial valve to the growth stage at which the two valves eventually become parallel there is a rapid decrease in successive expansions of the visceral cavity. After parallelism is reached no further increase in the height of the visceral cavity is possible, but the brachial valve may continue to grow.

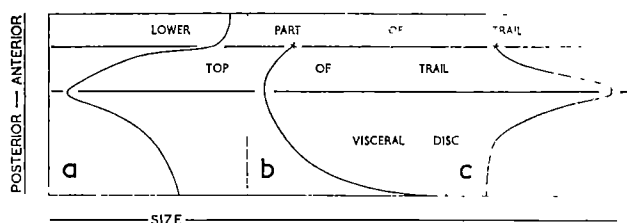


Fig. 4. The growth curves *a*, *b* and *c* are graphical representations of data obtained from Fig. 3. In each case, unit increase of one variable has been plotted on the ordinate axis and the size of the corresponding increment of another variable along the abscissa. By adjusting the ascending scales on the ordinate axes so that the bottom of the diagram approaches the origin of growth and the top margin the terminus of growth, it is possible to divide the field into three parts. The lines which thus separate the lower part of the trail, the top of the trail, and the visceral disk, connect points of abrupt change in growth. Growth curve *a* is a plot of unit increase in the curved length of the pedicle valve against the corresponding increments in the curved length of the brachial valve. Growth curve *b* is a plot of unit increase in the thickness of the visceral cavity against the corresponding increments in the curved length of the brachial valve. Growth curve *c* is a plot of unit increase in the curved length of the pedicle valve against the corresponding increments in the thickness of the visceral cavity. The point X on *b* and *c* represents the maximum thickness of the visceral cavity.

The third situation in which unit increments in the thickness of the visceral cavity are plotted against the corresponding extensions of the two valves is shown in Fig. 3c. The growth curve for the relative thickness-brachial valve increments is shown in Fig. 4b. It has an initial asymptotic form corresponding to the progressive decrease in the increments in length of the brachial valve, with a minimal value at the point where the brachial valve commences to turn towards the pedicle valve. A growth-rate change then takes place and the remaining supplements increase to the point X, the maximum thickness of the visceral cavity. The growth curve for the relative thickness of the visceral cavity and the corresponding additions to the length of the pedicle valve is complementary to Fig. 4c and of the curve type of Fig. 4b.

For those productids that do not modify the curvature of the brachial valve, of which the species used in Fig. 3 is an example, a value for the maximum thickness of the visceral cavity can be obtained. Because this value is dependent on the curvature of the two valves, which is almost identical in every individual of this population sample, the maximum thickness of the visceral cavity approximates to an absolute determinant and as such may be of considerable taxonomic importance. In those productids in which the original trail-curve of the brachial valve may be abandoned, by the secretion of overlying layers or by the growth of a diaphragm and new brachial trail, the thickness of the visceral cavity may continue to increase throughout life. In Fig. 5, for example, it can be seen that, if the gap between the trails of the brachial and pedicle valves is widened by supplements to the brachial valve, this has the effect of rotating one valve about the

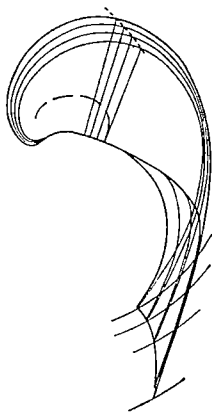


Fig. 5. Hypothetical profiles illustrating how progressive thickening of the trail of the brachial valve will permit a continual increase in the thickness of the visceral cavity. When the curve of the brachial valve is modified by the first additional layer the thickness curve (long-dashed line) is forsaken; successive increases mark out a 'curve of abandonment' (short-dashed line)

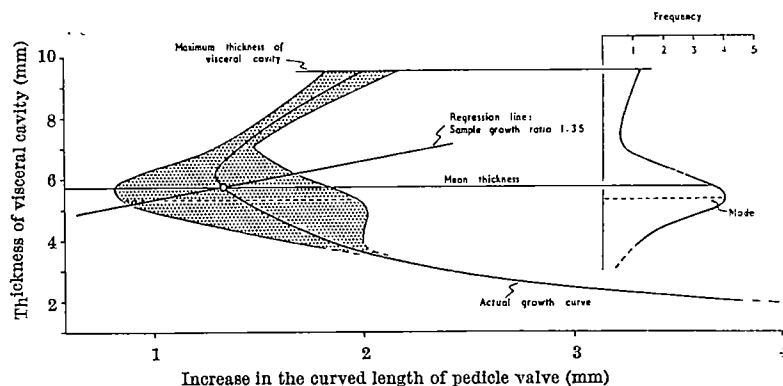


Fig. 6. Part of a growth curve (of the same type as Fig. 4b, but actually complementary to Fig. 4c) showing the variation of increments to the curved length of the pedicle valve for unit increase in the thickness of the visceral cavity. On the right is the frequency distribution of the thickness of the visceral cavity for the sample used to construct Fig. 1. The true relationship between the growth pattern and the sample is shown by the sample area (stippled), a plot of the sample frequency about the growth curve. The regression line from Fig. 1 indicating a growth ratio of 1:3.5 'averages out' the effect of the true change in growth and is therefore not a particularly meaningful construction

hinge relative to the other, thus increasing the thickness of the visceral cavity. The trace of the intersection between the line of maximum thickness and the brachial valve abandons the original thickness curve (Fig. 5, long-dashed line) and as the thickness increases the intersection of the line of maximum thickness with the pedicle valve can be plotted along a 'curve of abandonment' (Fig. 5, short-dashed line). For unit increase in the separation of the original brachial valve from the pedicle valve there is a progressive increase in the corresponding increments in the curved length of the two valves.

It is now clear (Fig. 1) that the correlation based on the total sample data is spurious in that the simple linear function represented by the regression line merely 'averages out' a much more complex growth history. The data of Fig. 1 and Fig. 3c are shown together in Fig. 6. The frequency distribution of the sample variation in thickness of the visceral cavity is plotted about the particular growth curve which illustrates the size of the increments in the curve length of the pedicle valve corresponding to unit increase in the thickness of the visceral cavity. The sample area (stippled) approximates to a dilated parabola and is bisected by the reduced major axis determined on Fig. 1. Thus the value of the growth ratio obtained by the method used to produce Fig. 1 will depend on the shape of the sample distribution curve and the coincidence of this curve with the growth curve. To make an accurate evaluation of the shell growth from sample data it is therefore necessary to reduce the compound growth curve to its component parts and to subject each of these to the standard statistical procedures.

I thank Dr. W. D. I. Rolfe and Prof. T. Neville George for advice.

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## INDUCTION OF AN ALPHA-GLUCOSIDASE BY GLUCOSE

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THE fermentation of melezitose by *Saccharomyces* is controlled by the gene-system *MZ*, which generates a single adaptive enzyme, melezitase. This enzyme is induced by exposure of cells carrying *MZ* to certain

specific alpha-glucosides, namely, turanose (*T*), maltose (*M*), sucrose (*S*), alpha-methyl-glucoside (*G*), melezitose (*Z*) and palatinose (*P*). The enzyme is capable of hydrolysing these same alpha-glucosides<sup>1-3</sup>. A series of multiple

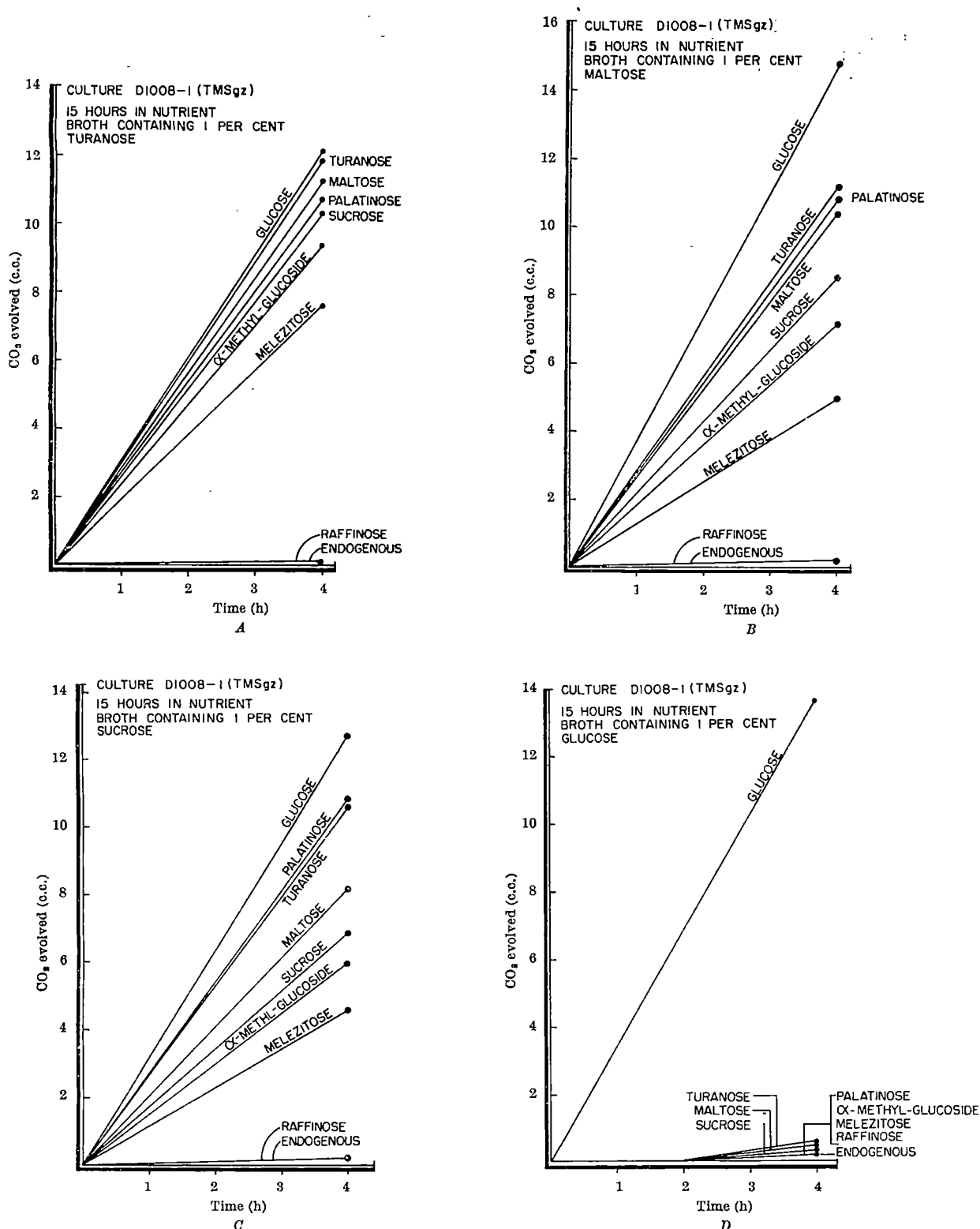


Fig. 1. Culture D1008-1 (*TMSGz*) was grown in 2 per cent glucose nutrient broth for 24 h, adapted for 15 h and tested for CO<sub>2</sub> production on 4 per cent glucose, turanose, maltose, sucrose, α-methyl-glucoside, melezitose, palatinose and raffinose by the syringe gasometer (8 × 10<sup>3</sup> cells in 4 ml. phosphate buffer, pH 4.5 at 30° C)

alleles has been discovered at the *MZ* locus<sup>4</sup>. The allele which elicits enzyme on exposure to all α-glucosides listed here is called 'totipotent', and has been designated *MZTMSGz*, abbreviated to *TMSGz*. Other alleles of the series which differ from one another by their ability to respond adaptively to different α-glucosides have been designated *TMSGz*, *TMSGz*, *TMSGz*, *TMSGz* and

*tmsgz*. (The small letters indicate the inability of a culture to respond adaptively to the respective substrates.)

Cells carrying the *MZ* gene exhibit the phenomena of 'heterologous adaptation' and 'de-adaptation'. In heterologous adaptation, cells incapable of fermenting melezitose become able to do so without lag after exposure, not to melezitose, but to maltose; that is, exposure to maltose



can adapt the culture for the fermentation of melezitose. Adapted cells undergo de-adaptation when the inductor is removed, and de-adapted cells cannot ferment the alpha-glucosides without further adaptation. Cells of the genotype *TMSGZ* (D1008-1) were grown for 24 h under aerobic conditions in broth containing 1 g of  $MgSO_4$ , 2 g of  $KH_2PO_4$ , 2 g of  $(NH_4)_2SO_4$ , 5 g of yeast extract powder, 3.5 g of bacto-peptone and 20 g of glucose in 1,000 ml. of distilled water. The cells were collected by centrifugation. Approximately  $5 \times 10^8$  cells were inoculated into 50 ml. broth containing the components listed here except that it contained 10 g of glucose (control) or 10 g of alpha-glucoside (adaptation) per 1,000 ml. distilled water. The

medium containing the cells was incubated for 15 h on a shaker at 30° C. Both 'control' and 'adapted' cells were collected, and washed three times with  $M/15 KH_2PO_4$ . A total of  $8 \times 10^8$  cells were re-suspended into 4 ml. of the same menstruum containing 4 per cent Seitz-filtered carbohydrate (either turanose, maltose, sucrose, alpha-methyl-glucoside, melezitose, palatinose, raffinose or glucose). The measurement of gas production was made with a syringe gasometer at 30° C (ref. 5). The absence of a source of nitrogen ensured that vegetative growth (budding) did not occur during the measurement of fermentative activity and, hence, that the occurrence of adaptation as indicated by  $CO_2$  production, after a lag

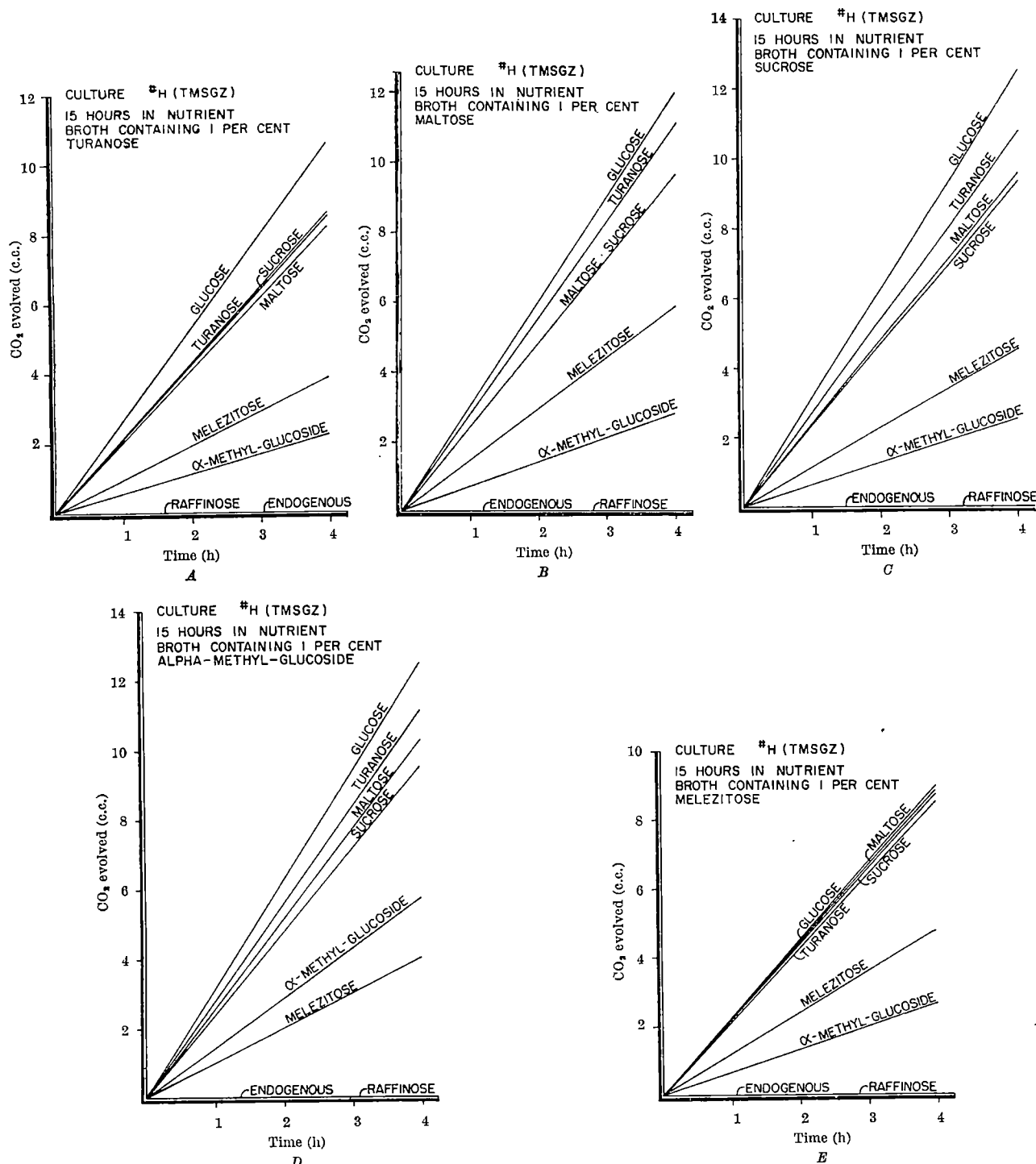


Fig. 2. Culture 'H' (*TMSGZ*) was grown in 2 per cent glucose nutrient broth for 24 h, adapted for 15 h and tested for  $CO_2$  production on 4 per cent carbohydrate by the syringe gasometer under the conditions specified in Fig. 1

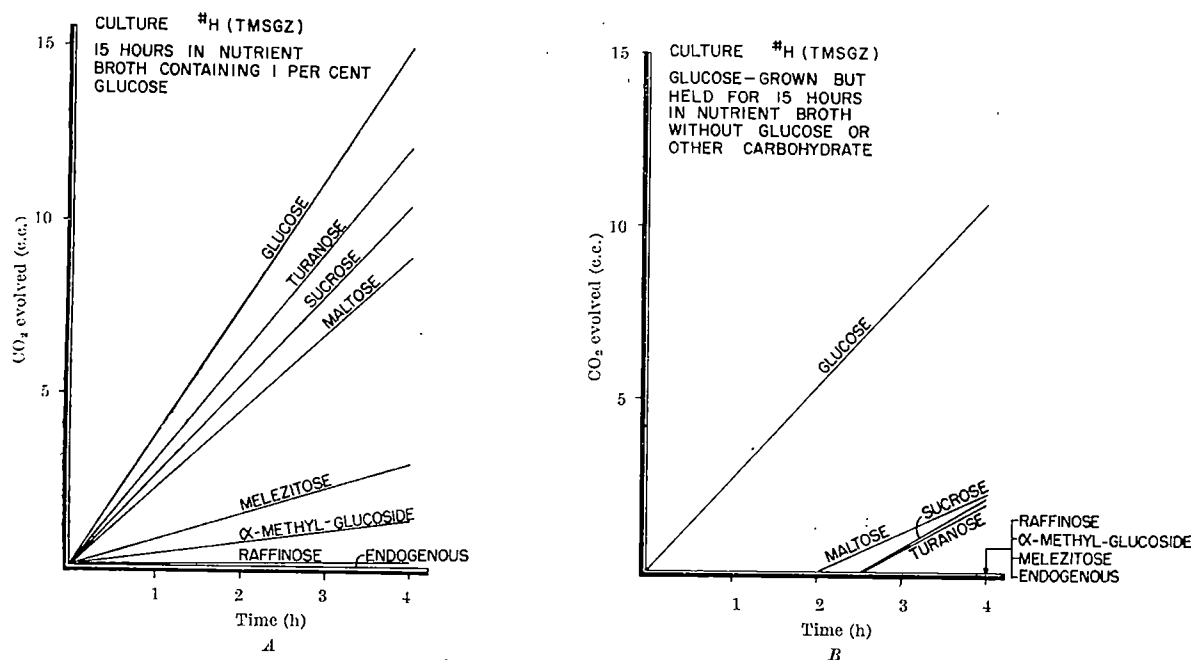


Fig. 3. Culture 'H' (TMSGZ) was grown in 2 per cent glucose nutrient broth for 24 h and then exposed to nutrient broth both with and without 1 per cent glucose for 15 h and tested for CO<sub>2</sub> production under the specified conditions (Figs. 1 and 2)

of several hours, was not due to mutation and selection but to adaptation of the total population of cells (presumably by the induction of melezitase). Cells of culture D1008-1 (which were unable to elicit melezitase in response either to alpha-methyl-glucoside or to melezitose) after adaptation to turanose, maltose or sucrose, were able to ferment alpha-methyl-glucoside and melezitose at zero time (Figs. 1A, B and C). The glucose-grown cells of this culture could ferment only turanose, maltose and sucrose, and the fermentation of these sugars occurred only after a lag period (Fig. 1D). The adapted cells underwent de-adaptation after 15 h exposure to a medium without the inductor. De-adapted cells could not ferment alpha-methyl-glucoside or melezitose, but were able to ferment turanose, maltose and sucrose after a lag period.

A totipotent culture 'H' (TMSGZ) was recently obtained by treating an ultra-violet-induced partial mutant, TMsgz, with melezitose which induced mutation from TMsgz to TMSGZ (ref. 6). Cells of this mutant have the characteristics of a totipotent MZ culture, because after they have been adapted either to turanose, maltose, sucrose, alpha-methyl-glucoside or melezitose, they can ferment all these alpha-glucosides without lag (Figs. 2A, B, C, D and E). However, culture 'H' is different from the normal MZ culture because it also ferments all five alpha-glucosides without lag after growth on glucose, without previous exposure to alpha-glucosides (Fig. 3A). One might infer that melezitase was a 'constitutive' enzyme in this culture. But investigation revealed that the activity of melezitase in the glucose-grown cells decreased when glucose was removed from the medium. The longer the cells were exposed to a medium with-

out glucose, the more the enzyme activity decreased. After the cells were held for 15 h in a broth containing all nutrients (described previously) but without glucose, they were unable to ferment either turanose, maltose or sucrose during the first 2 h in the fermentation test, and remained unable to ferment alpha-methyl-glucose and melezitose for 4 h (Fig. 3B). However, when the glucose-grown cells were held in phosphate buffer (pH 4.5) containing 2 per cent of glucose, for 15 h, the enzyme activity was maintained, revealing that melezitase was both induced and maintained by glucose in this particular mutant.

The induction of melezitase by glucose demonstrates the indeterminacy involved in specifying that an enzyme is 'constitutive'. If the inductor is a common and constantly present component of the cell, an inducible enzyme would be mistaken for a 'constitutive' enzyme<sup>7,8</sup>. The induction of an enzyme by one of the 'end-products' of its activity is contradictory to the concept of control by 'feed-back inhibition'.

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## AMINO-ACID SEQUENCE OF SPERM WHALE MYOGLOBIN

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THE circumvention of the problem of the insoluble 'core' by cleavage of the protein molecule with cyanogen bromide<sup>1</sup> made it possible to write an almost

complete amino-acid sequence of sperm whale myoglobin. However, three major tasks remained unfinished: (1) the determination of the sequences in two segments at the amino end of the protein; (2) the alignment of three blocks of sequences in the middle of the molecule; and (3) the

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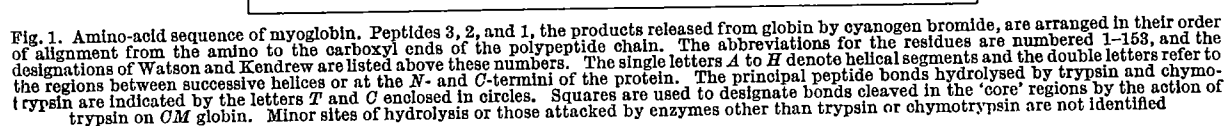




Table 1. AMINO-ACID COMPOSITION OF 'CORE' PEPTIDES FROM CM GLOBIN

The compositions of the 'core' peptides are expressed in terms of the molar ratios of the constituent amino-acids. Values for the principal constituents are shown in bold-faced type. With the exceptions of peptides 6 and 7, isolated from a chymotryptic hydrolysate, the peptides were released from carboxymethylated globin by the action of trypsin

Amino-acid	Peptide													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aspartic acid	0.14	0.05	—	—	—	—	—	—	—	0.03	—	0.10	0.04	—
Glutamic acid	3.07	2.01	2.03	1.90	1.06	—	—	—	0.04	—	0.93	1.06	0.04	—
Glycine	1.02	1.00	1.00	0.90	0.13	—	0.13	0.03	1.05	0.97	0.03	0.04	0.12	0.08
Alanine	0.10	0.08	—	—	0.07	—	—	0.98	0.03	2.06	—	1.02	0.78†	—
Valine	1.99	1.00	—	0.02	2.03	1.08	1.00	—	1.98	—	0.04	1.00	1.05	0.97
Leucine	2.92	0.99	0.99	0.02	1.88	—	—	—	1.00	2.03	1.08	0.98	1.05	1.06
Isoleucine	—	0.07	—	0.02	—	—	—	—	1.00	—	—	2.42(3)†	1.70(2)†	—
Serine	0.95	1.03	0.99	1.00	0.06	—	0.10	0.02	0.01	0.01	0.03	1.95	1.03	0.98
Threonine	0.04	—	—	—	—	—	0.04	—	1.00	0.91	—	—	0.01	—
Methionine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Proline	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Phenylalanine	—	—	—	—	—	—	—	—	—	—	0.99	—	—	—
Tyrosine	—	—	—	—	—	—	—	—	—	—	0.85	—	—	—
<i>di</i> CM Histidine	1.01	—	—	—	1.04	0.92	—	—	0.99	—	—	1.73*	1.89	1.01
Lysine	—	—	—	—	—	—	—	1.02	—	0.95	—	—	0.08	—
Arginine	—	—	—	—	—	—	—	—	—	—	—	0.88	0.98	0.99
Tryptophan	+	1.06	+	+	+	+	0.94	—	—	—	—	—	—	—
Integral No. of residues	12	7	7*	5	7	3	2	2	6	8	4	12	9	5

\* The *N*-terminal valine residue of this peptide is carboxymethylated, and therefore is not detected during the amino-acid analysis. For details see text.

† Isoleucine is generally not released quantitatively in 48 h of hydrolysis from either peptide 12 or 13, both of which contain the sequence Ala.Ile.Ile.His. In each of these examples the recovery of one residue immediately adjacent to the Ile residues was also low (compare value for *di* CM His in peptide 12 and the Ala value in peptide 13).

The compositions of the peptides in Table 1 indicate that the specificity of trypsin was not rigorously maintained. The aberrant cleavages are identical with those found after hydrolysis with chymotrypsin (compare Fig. 1) and are probably attributable to the manifestation of the chymotryptic activity associated with most samples of trypsin. The chymotryptic-like splits are more readily detected after extended periods of hydrolysis such as those (24 or 48 h) used in this investigation. Furthermore, relatively large quantities of trypsin were used, that is, 2–4 per cent of the weight of globin. In contrast to the results obtained after carboxymethylation, the core regions of the unreacted protein were not susceptible to chymotryptic-like hydrolysis during the exposure to trypsin<sup>9</sup>. The incidence of non-specific splits in the remaining regions of myoglobin was also lower than that for CM globin (Edmundson, A. B., unpublished results). Although the aberrant cleavages complicate the problems of fractionation and the establishment of overlaps, they do provide smaller fragments of what would otherwise be long tryptic peptides unsuitable for direct sequential analysis.

The detection of peptides collectively containing the 12 histidine residues in the form of their 1,3-dicarboxymethyl derivatives indicates that the reaction of bromoacetate with the imidazole groups was probably quantitative. Banaszak *et al.*<sup>2</sup> had previously observed the almost complete disappearance of unreacted histidine under similar conditions. In further agreement with their findings, the  $\epsilon$ -amino groups of lysine residues had not been attacked in any peptides examined (for example, peptides 8 and 10 in Table 1). A comparison of peptide 3 (compare Table 1) with the *N*-terminal peptide, No. 2, showed that the  $\alpha$ -amino group of *N*-terminal valine had been partially carboxymethylated. Peptide 3 was found to be identical in composition with peptide 2 except for the absence of unreacted valine, and to have approximately one more negative charge during ionophoresis at pH 6.4. Moreover, peptide 3 was ninhydrin-negative and did not yield an end group with Edman's phenyl-isothiocyanate method<sup>10–13</sup>. These results suggest that *N*-terminal valine was present in peptide 3, but in the form of its mono-carboxymethyl derivative.

### Chymotryptic Hydrolysis of Myoglobin

In an earlier article<sup>1</sup> the major sites at which myoglobin is hydrolysed with chymotrypsin were indicated in the structural formulae of the products of the cleavage with cyanogen bromide (compare Fig. 3 in ref. 1). The compositions of most of the peptides could be inferred from

this information, but the series had not been completed for the amino end of the protein. Its completion was accelerated by the use of the procedures found to be successful for the purification of similar or identical 'core' peptides from CM globin. The remaining chymotryptic peptides had previously been fractionated by the same general methods, although applied in a greater variety of combinations because of the large number (57) of peptides involved. Because of limitations of space, detailed compositions such as those in Table 1 will not be presented for these peptides, but the principal sites of cleavage are indicated in Fig. 1.

To obtain the long peptides desirable for the establishment of the overlaps, as well as to reduce the complexity of the mixtures to be fractionated, the duration of hydrolysis was at first limited to 3 h, and most of the peptides indicated in Fig. 1 were isolated from this type of chymotryptic hydrolysate. When it became apparent that the 'core' regions in general and the amino-terminal segment in particular were attacked more slowly than the remainder of the protein, the period was increased to 20 h. The latter hydrolysates were used principally for the preparation of larger quantities of peptides from the 'core' regions, and an exhaustive survey of the other components was not undertaken.

The peptide bonds hydrolysed were generally those predicted from the specificity of chymotrypsin—namely, those at the carbonyl ends of tryptophan, phenylalanine, tyrosine, methionine and, to a lesser extent, leucine and histidine (compare sequences given in Fig. 1). Among the unpredicted sites of hydrolysis were those involving such residues as aspartic acid (residue 20; compare Fig. 1), threonine (residues 67 and 70), alanine (residues 90, 94 and 125), glutamine (residues 91 and 128), and asparagine (residue 132). Two minor tryptic-like splits were detected after residues 63 and 77, which are members of highly basic centres with Lys.Lys.His and Lys.Lys.Lys sequences, respectively (compare Fig. 1).

If only unique sequences are considered, the chymotryptic peptides contain a total of 153 residues, identical in kind and number with the composition indicated by the original amino-acid analysis of myoglobin<sup>8,14</sup>. The values obtained<sup>1</sup> for the products of the cyanogen bromide cleavage fell short of this figure by approximately 0.6 of a valine and 0.7 of a glutamic acid residue. It was correctly assumed at that time that the composition of the amino-terminal segment (designated peptide 3 in Fig. 1), should have included an additional valine residue. Now the same conclusion can be applied to the number of glutamic acid residues.

### Identification of the Amides

The asparagine and glutamine residues were distinguished from their acidic counterparts by a combination of the following procedures: (1) paper ionophoresis of appropriate peptides at pH 6.4; (2) quantitative analysis of the amino-acids released from peptides by leucine aminopeptidase or carboxypeptidase A, and/or (3) direct identification of phenylthiohydantoins by the chromatographic techniques of Sjöquist and Edman<sup>11,12</sup>. The results of this investigation, incorporated into the sequence shown in Fig. 1, indicate that only 7 of the 27 possible residues are found in the amide form. This value coincides with that obtained by amino-acid analysis of the intact protein<sup>14</sup>. The ratios of amides to the total number of each type of residue was approximately the same for asparagine and glutamine: 2 (residues 122 and 132) out of 8 were asparagine and 5 (residues 8, 26, 91, 128 and 152) out of 19 were glutamine residues. Of special interest is the fact that 3 of the amides, residues 122, 128 and 132 are concentrated in one region, the last two occupying successive turns on  $\alpha$ -helix H (for nomenclature see ref. 17 and Fig. 1) and the first located in the corner segment between helices G and H. All three are constituents of the peptide Tryp 21 (refs. 8 and 14).

Before the final assignment of the amides in myoglobin it was necessary to consider the possibility of hydrolysis of these groups to their acidic forms. There are three examples of residues (8, 91 and 152) identified as amides in some peptides and as glutamic acid residues in others. This hydrolysis, which fortunately was far from quantitative, could have occurred during the fractionation of the peptides. An alternative explanation, not necessarily exclusive of the first, is based on the chromatographic heterogeneity of myoglobin. The glutamic acid forms of the labile amides were not detected in a 3-h chymotryptic hydrolysate of the principal chromatographic component. They were, however, found in chymotryptic and 'core' peptides from hydrolysates of the protein used without prior separation into five components on 'Amberlite IRC-50' (ref. 14). These results support the suggestion<sup>14</sup> that the chromatographic differences are largely attributable to differences in amide content, and therefore in charge. The three labile amides are in accessible positions, one (residue 91) close to the haem-linked histidine residue and the other two (residues 8 and 152) near the termini, where charge differences would probably be reflected in the chromatographic patterns.

### The Complete Sequence of Myoglobin

If the results in the preceding sections are included, there are now four principal lines of evidence on which the complete sequence of 153 residues can be written. (1) The chymotryptic peptides and (2) the three peptides released by cyanogen bromide contain all segments of the protein. (3) The soluble tryptic peptides together account for 70 per cent of the original molecule, and (4) the 'core' peptides from CM globin represent the remaining 30 per cent. This evidence is summarized in Fig. 1, which is divided into three sections corresponding to the peptides (designated peptides 3, 2 and 1 as in ref. 1) liberated by cyanogen bromide. The numbers of the constituent residues are given and the principal peptide bonds hydrolysed by trypsin and chymotrypsin are indicated by the letters *T* and *C* enclosed in circles. The squares refer to bonds cleaved in the 'core' regions by the action of trypsin on CM globin. The sequences were determined by a combination of the phenyl-iso-thiocyanate procedure and enzymatic end-group methods.

Fig. 1 is largely self-explanatory, and comments about most of the sequence have been made elsewhere<sup>1</sup>. However, the assignment of a few overlaps and the dipeptide sequence at the carboxyl end do require clarification. For example, the compositions given in Table 1 can be used

to determine the alignment of three blocks of sequences in the middle of peptide 2, for which both the *N*-terminal and *C*-terminal segments have already been identified<sup>1</sup>. Peptide 11 in Table 1, with its characteristic tyrosine residue, bridges the blocks marked I (residues 77–103) and II (residues 104–106) in Fig. 3 of ref. 1, and peptides 12–14 link group III (residues 107–115) to the *C*-terminal segment. By elimination, blocks I and II must provide the connexion between the terminal regions. Moreover, group I is the only one of the three to begin with lysine, and the composition of peptide 10 in Table 1 indicates that the *N*-terminal sequence is followed by lysine.

The results of the cyanogen bromide cleavage were used to postulate the approximate formulae of the long tryptic peptides from the 'core'<sup>1</sup>. From the structure of peptide 2, in combination with the compositions of peptides 1–7 in Table 1 and the sequences of chymotryptic peptides indicated in Fig. 1, it is now possible to give the following complete sequences of these fragments:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Val. Leu. Ser. Glu. Gly. Glu. Trp. Gln. Leu. Val. Leu. His. Val. Try. Ala. Lys															
(peptides 1–8 in Table 1)															
64	65	66	67	68	69	70	71	72	73	74	75	76	77		
His. Gly. Val. Thr. Val. Leu. Thr. Ala. Leu. Gly. Ala. Ile. Leu. Lys															
(peptides 9–10 in Table 1)															
103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118
Tyr. Leu. Glu. Phe. Ile. Ser. Glu. Ala. Ile. Ile. His. Val. Leu. His. Ser. Arg															
(peptides 11–14 in Table 1)															

The numbering of the residues is identical with that in Fig. 1, and the peptides accounting for the longer fragments are listed after the sequences.

The conflicting results about the dipeptide sequence at the carboxyl end of myoglobin<sup>14,15</sup> have recently been resolved by an investigation of a tripeptide (Tyr, Glu, Gly) released from this region by the action of pepsin. After treatment of the peptide with carboxypeptidase, glycine was the only amino-acid detected in the hydrolysate and has accordingly been assigned the *C*-terminal position. This observation confirms the tentative conclusion<sup>1</sup> that the chymotryptic peptide, Gln.Gly, was also derived from this segment.

The chemical investigation has been carried out in parallel with the determination of the structure by X-ray analysis, and it is of interest to comment on the correlation of the two approaches solely from the point of view of the amino-acid sequence. The three-dimensional structural features observed at a resolution of 1.4 Å and the concomitant interactions of amino-acid side-chains will be discussed in detail by Watson, Kendrew, and their colleagues at a later date. When the resolution achieved by the isomorphous replacement method had reached its limit of 2 Å, Kendrew *et al.*<sup>16,17</sup> were surprised to find that many amino-acid side-chains could be identified. The correlation with the present sequence was good in the segments of the molecule for which the compositions, and in a few cases the sequences, of the peptides were known from the chemical investigation. For the remainder, comprising the insoluble peptides of the tryptic series, only approximate overall compositions were available, and the 1:1 correspondence was understandably less precise. The comparison also indicates that the crystallographic identifications proved most difficult in the non-helical regions of the polypeptide chain.

Because of its high helical content (75–80 per cent), low molecular weight (17,816), and absence of disulphide bonds, myoglobin at present appears to be the most suitable protein for X-ray analysis. Even in this case, however, the experience of Kendrew *et al.* has indicated that the data must be extended to atomic resolution (less than 1.5 Å) before unequivocal sequences can be derived by X-ray analysis. Now that the positions of more than 90 per cent of the non-hydrogen atoms have been assigned, the agreement is complete in all these cases. Since the X-ray diffraction data from proteins seldom extend beyond 2 Å, it seems probable at present that the sequence information in most crystal investiga-



tions will have to be deduced by chemical methods and used by the crystallographer in the interpretation of his structural results.

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## LYMPHOCYTE FOOT APPENDAGE: ITS ROLE IN LYMPHOCYTE FUNCTION AND IN IMMUNOLOGICAL REACTIONS

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**B**LAST-CELL transformation and mitotic activity result when peripheral blood leucocytes from unrelated individuals are cultured together<sup>1</sup>. In order to observe the cellular interactions involved we used time-lapse photography to follow events in single donor and mixed cultures prepared without the addition of extraneous materials such as phytohaemagglutinin, heterologous serum or antibiotics. In the course of this investigation we observed a unique kind of attachment between lymphocytes and other cells. Lymphocytes appeared as anatomically polarized units with a foot appendage capable of firm attachment to the surface of cells or to cell debris. This method of sustained contact was different from 'peripolexis', the free movement of lymphocytes around the perimeter of a cell<sup>2</sup>, or the entry of lymphocytes into living cells and their wandering about, to which Pulvertaft has given the name 'emperipolexis'<sup>3</sup>. Although the attachment of lymphocytes to other cells was observed in cultures of leucocytes from a single donor, this activity was striking in mixed leucocyte cultures.

Venous blood containing 0.02 mg/ml. of heparin was allowed to settle for 1-2 h, and the cell-rich plasma was removed. The remainder of the specimen was centrifuged at 3,000 r.p.m. for 30 min to obtain additional plasma. The leucocyte-rich plasma was diluted with an equal volume of culture medium TC 109, and placed in large T-flasks for 20 min to allow polymorphonuclear leucocytes to attach to the glass. Cultures were placed in Carrel flasks and consisted of 3 ml. TC 109, 2 ml. plasma and approximately  $1.0 \times 10^6$  leucocytes per 5 ml. culture. Control cultures contained cells and plasma from a single donor, whereas in mixed cultures half the cells and plasma were furnished by each of two unrelated donors. Observations were made with an inverted phase microscope enclosed in a 37°C incubator, and fitted with a 16-mm time-lapse ciné camera. We observed cultures continuously for 6 days, and alternately photographed control and mixed cultures. At 12-h intervals a control and a mixed culture were centrifuged at 600 r.p.m. for 10 min and some of the sedimented cells were placed between 2 large No. 2 coverslips (43 × 50 mm and 22 × 40 mm). Thin cultures were sealed and observed continuously for 4-5 h. Remaining cells from each specimen were spread on coverslips as for routine blood films and stained with Wright and Giemsa stains.

During the first 24 h control and mixed cultures were similar in that polymorphonuclear leucocytes degenerated progressively and macrophages became more conspicuous

(Fig. 1A). A few lymphocytes firmly attached to other cells by means of the foot process, but such attachments were usually for short periods, rarely longer than an hour. This brief interaction between lymphocytes and other cells remained the only characteristic of control cultures during the entire 6 days of culture. Emperipolexis was not seen and no blast cells were present at the end of the culture period.

Mixed leucocyte cultures, however, contrasted sharply with controls after the first day (Fig. 1B and C). Lymphocytes tended to gather in the vicinity of macrophages and demonstrated peripolexis and emperipolexis. Time-lapse investigations showed rupture of occasional macrophages as a result of emperipolexis. On day 2 many lymphocytes were firmly attached to macrophages by means of their foot appendages, the attachment remaining fixed in spite of vigorous cell motions. Sometimes clumps of 10 or more lymphocytes were attached in a radial

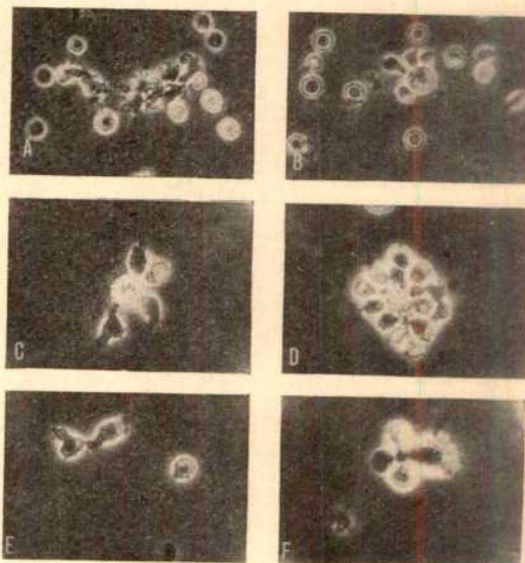
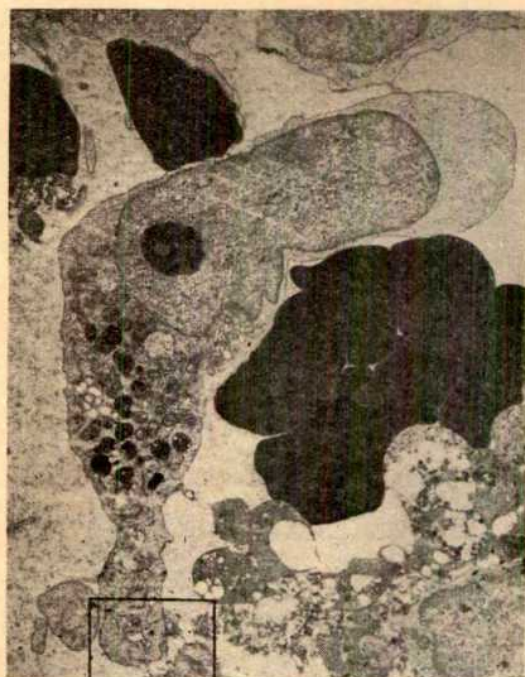
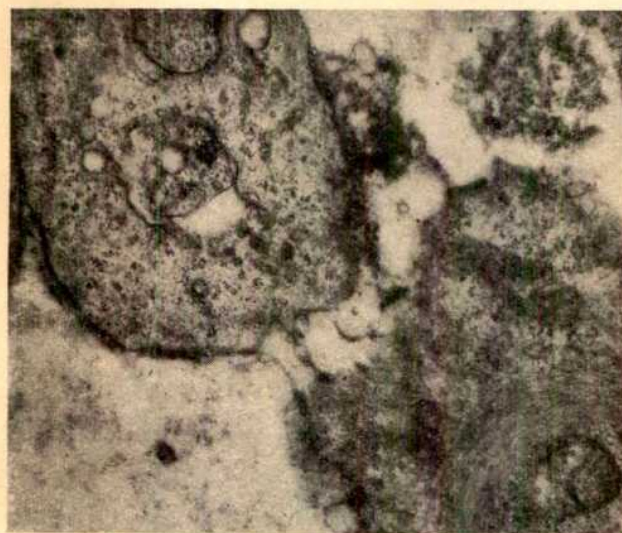


Fig. 1. Excerpts from 16-mm time-lapse phase microscopy study of cultured cells. A, Macrophages and 'inactive' lymphocytes in control culture at 24 h ( $\times 125$ ). B, Lymphocyte-macrophage interaction in mixed leucocyte culture at 24 h ( $\times 125$ ). C and D, Lymphocyte-macrophage interaction after the third day ( $\times 200$ ). E, Lymphocyte foot appendage and its firm attachment to macrophages is demonstrated. F, Lymphocyte-lymphocyte interaction via the foot appendages ( $\times 200$ ).





A



B

Fig. 2. A, Electron photomicrograph of a transformed lymphocyte from a phytohaemagglutinin-stimulated leucocyte culture to demonstrate the extended foot appendage ( $\times 7,520$ ). B, Higher magnification ( $\times 41,000$ ) of the distal end of the foot appendage showing the finger-like extensions between cells.

fashion to macrophages (Fig. 1D). On day 3 a few of the lymphocytes attached to macrophages began to transform and appeared several times larger than surrounding lymphocytes. An examination of fixed, stained cells from mixed cultures at 72 h showed that blast cells constituted 0.9 per cent of surviving lymphocytes. On day 4, transforming cells were recognizable by their increased size, and lymphocytes immediately surrounding them began to enlarge. Interaction between lymphocytes was observed frequently (Fig. 1E). On days 5 and 6 occasional transformed cells detached from macrophages, and as they migrated into the medium several small lymphocytes promptly attached themselves to the foot process of the blast cell (Fig. 1F). Mixed cultures stained on day 6 contained 0.2 per cent of cells in mitosis and 10.8 per cent transformed cells.

On high-power phase microscopy, the long, slender foot appendage appeared to end in a burr-like process that

contained a number of granules. Thread-like extensions projected from the end of the appendage forming a flexible connexion between the lymphocyte and other cells.

Efforts to obtain electron micrographs of the lymphocyte foot appendage in mixed leucocyte cultures were encumbered by the relatively low rate of blast transformation. Accordingly, for this phase of our investigation we examined sections of ordinary phytohaemagglutinin-stimulated leucocyte cultures in which the rate of blast transformation is high and lymphocyte foot appendages are a common phenomenon in our experience. Fig. 2A shows the foot appendage on a typical cell and Fig. 2B shows the delicate, finger-like projections.

Although the lymphocyte has long been recognized by its characteristic 'hand mirror' configuration, no functional significance has been attributed to the 'handle' of the 'hand mirror'. In this investigation it appeared as a specialized organelle by which lymphocytes attached to macrophages and other cells. It seemed to be part of a normal process which probably also occurs *in vivo*. Schoenberg *et al.*<sup>4</sup> have demonstrated a direct cytoplasmic connexion between lymphocytes and macrophages. Whether a cytoplasmic connexion exists through the foot process is not known; however, this specialized mode of attachment would seem to serve some purpose in lymphocyte function. The prolonged attachment to degenerated leucocytes suggested the possibility of absorption of products of autolysis, and another possible reason for the appearance of lymphocytes in an area of inflammation after the early emigration of leucocytes. Contact between lymphocytes could provide a method of transfer of RNA and other nucleic acids and materials without the necessity of cell division and death. Such an exchange would explain the prolonged retention of labelled nucleic acids in circulating lymphocytes<sup>5</sup> and obviate the necessity of postulating extreme longevity on the part of some lymphocytes<sup>6</sup>. Passage of instructive material on sustained contact between lymphocytes would provide a possible means of maintaining antibody-forming capacity for many years without the persistence of antigen in tissues.

The close association between macrophages and transforming lymphocytes was in agreement with the description of the immunological unit by Thiery<sup>7</sup> and Fishman's demonstration of the role of the macrophage in primary antibody reactions<sup>8</sup>. In our experiments only a small proportion of lymphocytes transformed initially. This evidence of heterogeneity was in accordance with the views of Burnet<sup>9</sup> and the *in vitro* results of Jerne and Nordin<sup>10</sup>. Events we recorded early in blast formation strongly suggested the transfer of stimulatory material from transformed cells to lymphocytes. This would provide an alternative method in addition to mitotic division for the development of islands<sup>11</sup> or 'clones'<sup>9</sup> of antibody-forming cells in the tissues of animals after primary antigenic stimulation. The extent of actual transfer must be determined experimentally, but the specialized mode of lymphocyte attachment seen in normal cultures and accentuated in 'stimulated' cultures points to the probability that this phenomenon plays a part in lymphocyte interactions in normal and pathologic states.

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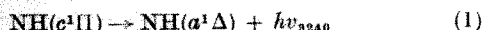


# LETTERS TO THE EDITOR

## ASTROPHYSICS

### Origin of $\text{NH}(A^3\Pi) \rightarrow \text{NH}(X^3\Sigma^-)$ Emission in Comets

THE presence of NH radicals in the coma of comets has been generally attributed to photodissociation of ammonia. However, vacuum ultra-violet photolysis<sup>1</sup> (1236 Å) of ammonia results in emission at 3240 Å:

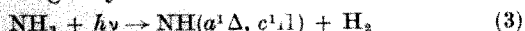


while the cometary emission at 3360 Å corresponds to:

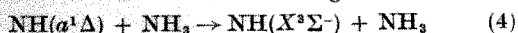


The energy-level diagram for NH showing the position of the states and the transitions under discussion is shown in Fig. 1. In the vacuum ultra-violet flash photolysis of ammonia<sup>2,3</sup>, the NH absorption band at 3360 Å ( $A^3\Pi \leftarrow X^3\Sigma^-$ ) was observed.

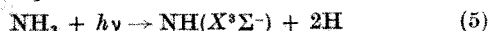
These results may be explained<sup>3</sup> by initial formation of NH in the singlet system:



with collision-induced transformation to ground state NH.



Ground state NH could also be formed directly in decomposition of  $\text{NH}_3$ :



Formation of  $\text{NH}(X^3\Sigma^-)$  and  $\text{H}_2$  is spin forbidden since the excited state of  $\text{NH}_3$  formed on absorption of light in the region 1220–1440 Å (state III) is a singlet<sup>4</sup>. Whatever the mechanism for production of ground state NH, it is evident that in photodecomposition of ammonia, NH is formed to some extent in the singlet system. Since the  $a^1\Delta \rightarrow X^3\Sigma^-$  is highly forbidden,  $\text{NH}(a^1\Delta)$  will have a long lifetime in absence of collision with other molecules. Evidence for the fact that radicals do not suffer collisions from the time of their formation near the nucleus until resonance fluorescence occurs further out in the coma comes from the observation that the  $\text{C}_2$  radical shows a vibrational and rotational distribution, which corresponds to a temperature of about 2500° K, while CN simulates

a very low-temperature distribution. A completely symmetrical molecule like  $\text{C}_2$  has no permanent dipole and is therefore unable to emit vibrational and rotational energy. Energy may therefore be transferred only by collisions. The fact that  $\text{C}_2$  retains excess vibrational and rotational energy suggests that it has suffered few, if any, collisions from the time of its formation to the time of its excitation by a fluorescence process. Thus in the coma of a comet, considerable concentration of  $\text{NH}(a^1\Delta)$  should accumulate if ammonia is the source of NH radicals. Emission from  $\text{NH}(c^1\Pi)$  at 3240 Å by a fluorescence process should be observed in cometary spectra along with the 3360 Å emission from the triplet system of NH. Since only the 3360 Å emission is observed, an alternative source of NH radicals which produces only the triplet system on photodecomposition is required.

Recent results obtained in our laboratory on emission spectra from hydrazine vapour during photolysis at the krypton resonance lines (1236 Å and 1165 Å) are pertinent to this problem. While the work was in progress, Becker and Welge reported<sup>5</sup> results on the emission spectra during photolysis of hydrazine which are in complete agreement with our observations. When hydrazine, carefully purified of ammonia, is photolysed at 50  $\mu$  mercury pressure, emission at 3360 Å is observed. This is undoubtedly the 0,0 band of the  $A^3\Pi \rightarrow X^3\Sigma^-$  NH transition. Emission is not observed at 3240 Å (0,0 band of the  $c^1\Pi \rightarrow a^1\Delta$  transition). Detection of emission at 3240 Å in presence of emission at 3360 Å is within the resolution of our grating, and emission at 3240 Å amounting to a few per cent of that at 3360 Å could have been detected. Thus photodecomposition of hydrazine appears to produce NH radicals in the triplet system only. On this basis we would suggest that the immediate precursor of NH radicals in comets is hydrazine or a hydrazine-type molecule and that the role of ammonia in the comet model needs reconsideration.

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## RADIOPHYSICS

### Dependence of the Critical Frequency of the Ionospheric E-layer on Solar Zenith Angle and the Annual Variation in E-layer Ionization

It is generally known that  $f_oE$ , the critical frequency of the ionospheric E layer, varies in a fairly regular manner with a law of the form:

$$f = f_o \cos^{\lambda} \chi \quad (1)$$

where  $\chi$  is the solar zenith angle. This form can be applied to both diurnal and annual variations, and generally both  $f_o$  and  $n$  are different for these two variations. To distin-

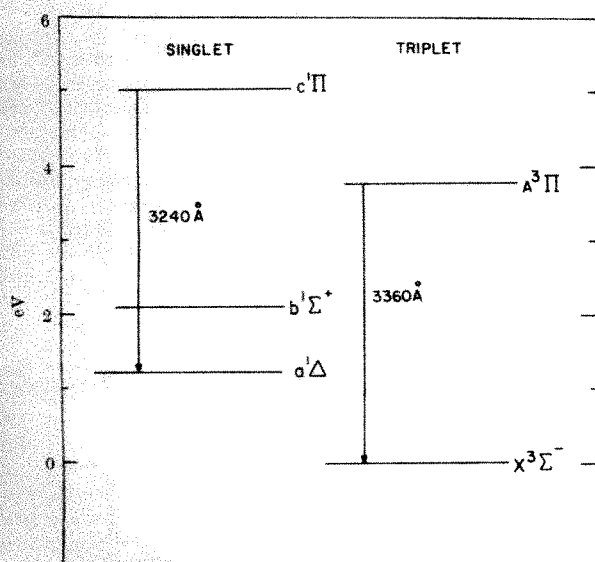


Fig. 1. Energy-level diagram of NH

guish these two cases we use  $f_a$  and  $m$  for diurnal variations, and  $f_a$  and  $n$  for annual variations. It is known that  $m$  is greater than  $n$  except at stations in equatorial regions and at very high latitudes (see Fig. 1), while  $f_a$  is greater than  $f_a$  everywhere<sup>1</sup>.

There is another variation in  $f_oE$  with  $\chi$ : for a fixed local time (for instance, noon) at a fixed season,  $\chi$  has a variation with latitude; therefore, for a variation in  $f_oE$  accompanying this latitudinal variation in  $\chi$  we can write:

$$f = f_l \cos \chi \quad (2)$$

Values of  $f_l$  and  $l$  can be determined by using data from stations distributed in latitude.

Minnis<sup>2</sup> has argued that both  $f_a$  and  $f_a$  are not, in a strict sense of the word, the critical frequencies at the 'sub-solar point'. Applying (1) to a single station, we have no observed value of  $f_oE$  at small solar zenith angles except at stations at latitudes less than  $23.5^\circ$ ; therefore, the extrapolation of a series of observed values to a hypothetical condition  $\chi = 0$  is quite unreliable. It must, however, be noted that one can obtain an accurate value of  $f_oE$  at the sub-solar point by applying (2) to a series of noon  $f_oE$  observed for various stations. In this case, we always have a station not far from the sub-solar point; therefore, this short extrapolation can give a reliable estimate of  $f_oE$  at the sub-solar point.

It is also argued by Minnis that the marked annual variation in  $f_oE$  could be explained by the relation:

$$f_a = f_a \sec^{(m-n)} \chi_{\text{noon}} \quad (3)$$

The winter value of  $f_a$  is greater than the summer value because of the greater value of  $\chi_{\text{noon}}$  in winter. In this argument Minnis seems to discuss the seasonal component of variations and to attribute its origin to the fact that  $m > n$ . This could be another aspect of the fact that the winter value of  $f_oE$  is greater than the summer value for the same value of  $\chi$ , which was discovered and called the 'seasonal anomaly' by Appleton<sup>3</sup>. Since  $m$  varies very little between summer and winter<sup>1</sup>, the diurnal curve of  $f_oE$  plotted against  $\chi$  has almost the same slope for summer and winter. For annual variations, the data for smaller (or greater)  $\chi$  are contributed from the summer (or winter) observations; therefore, they give smaller (or greater) values of  $f_oE$  than the corresponding value in winter (or summer). Thus, the annual curve has a lower slope than the diurnal curve and from this it results that  $m > n$ .

It must be noted, however, that at equatorial stations such as Huancayo ( $12.0^\circ$  S. in geographic latitude) and Trinidad ( $10.6^\circ$  N.)  $m$  is smaller than  $n$  as is seen in Fig. 1, which is plotted from Table 1 of the review article by Robinson<sup>1</sup> and also includes the data by Minnis. This result cannot be explained by the 'seasonal anomaly' and

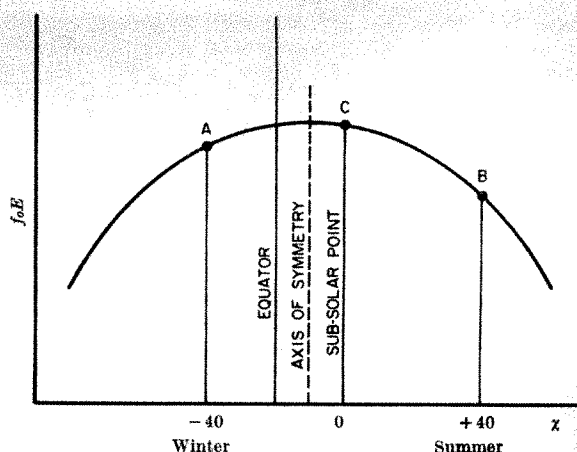


Fig. 2. Schematic illustration of the distribution of  $f_oE$  versus  $\chi$ , the solar zenith angle, for non-equinoctial months

it may suggest that the annual component of variations exceeds the seasonal component at low latitudes. It is reported<sup>2</sup> that at Lwiro ( $2.3^\circ$  S.) and Nha-Trang ( $12.0^\circ$  N.) the seasonal component of variations in  $f_a$  is less than one-tenth of that at Lindau ( $52.0^\circ$  N.) and Freiburg ( $48.0^\circ$  N.).

It is important to distinguish between the two facts that  $f_oE$  is greater in local winter than in summer for the same value of  $\chi$  at middle and high latitudes (about  $20^\circ$  to  $60^\circ$ ) and that  $f_oE$  at the sub-solar point is greater in December than in June. The former is a seasonal effect and may be explained by the fact that the maximum point (the axis of the symmetry) of  $f_oE$  versus  $\chi$  distributions is shifted from the sub-solar point ( $\chi = 0$ ) toward the equator. This was found by Appleton, Lyon and Turnbull<sup>4</sup> and myself<sup>5</sup> and is perhaps partly due to the vertical drift effect of the overhead  $S_q$  current system and the seasonal difference of its current intensity<sup>6,7</sup> and partly to a seasonal variation in the upper atmospheric parameters, such as temperature, temperature gradient, and composition. By comparing the point A with B in Fig. 2, which illustrates schematically the distribution of  $f_oE$  versus  $\chi$  in non-equinoctial months, we can easily see that the winter value is always greater than the summer for the same value of  $\chi$ , say  $40^\circ$ .

On the other hand, the latter fact is concerned with the world-wide component of variations in the E-layer ionization. The sub-solar value indicated by C in Fig. 2 may have some seasonal variations, but most of its variation is certainly due to the annual component of variations, because the sub-solar point is always in the summer hemisphere (or at the equator) and never in the winter hemisphere.

In previous papers<sup>5,8</sup> I tried to clarify the characteristics of the world-wide (non-seasonal) component of variations. For this purpose only sub-solar conditions were considered so that the seasonal component might become very small. The result that  $f_oE$  at the sub-solar point is greater in December than in June may be partly explained by the effect of the annual variation in Sun-Earth distance, but the observed variations are slightly larger than what can be attributed to this effect alone<sup>9</sup>. Thus, it is not possible to exclude entirely the possibility that there is a small annual component of variations in the E-layer ionization due to some unknown causes. Minnis stated that my previous result required an additional 'winter' ionizing agency. Actually, however, an additional electron density is needed in December solstice for the whole world.

In Fig. 1 it can also be seen that the indices  $m$  and  $n$  are very small and that  $m < n$  at stations at latitudes higher than about  $55^\circ$ . These are additional anomalies and may suggest the existence of some additional ionization at high latitudes, which has a slower variation with

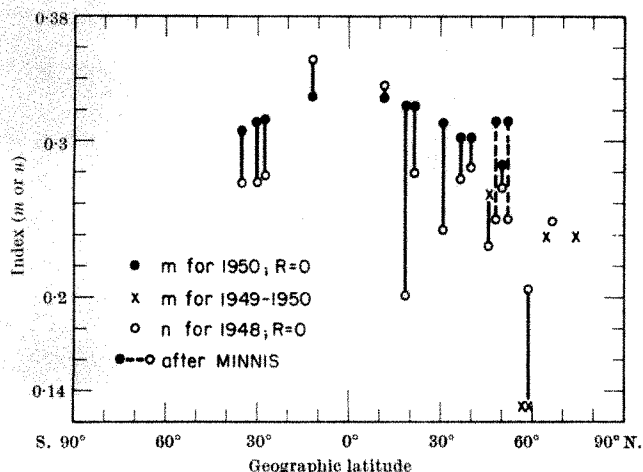


Fig. 1. Latitudinal dependence of the diurnal ( $m$ ) and seasonal ( $n$ ) indices in  $f_oE = f_a \cos^m \chi$  or  $f_oE = f_a \cos^n \chi$



than that given by (1), although attention must be directed to the fact that the determination of indices is seriously affected by possible errors in the observations of  $\epsilon_0 E$  at high latitudes because such data are restricted to large values of  $\chi$ .

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## GEOFYSICS

### Satellite Geoid and the Structure of the Earth

PROF. EGYED postulates<sup>1</sup> that harmonics of low degree of the geopotential arise from undulations of the core-mantle interface, and argues that the correspondence between highs and lows of the geoid surface and the isoporic foci of the vertical component of the geomagnetic field support this view. This argument is surely untenable. First, the correlation between magnetic and gravity fields is weak. This may be shown using either of Izsak's geoids<sup>2,3</sup> and the secular variation of the vertical (Z) or the eastward horizontal (Y) components of the geomagnetic secular variation field for the epoch 1942.5, given by Vestine *et al.*<sup>4</sup>. Values of the geoid heights and the magnetic field components were taken at corresponding 10° intervals. A weak correlation exists between the Y-components and the 'old' geoid but no correlation between any of the others:

Correlated variables	Correlation coefficient
Izsak's old and Z	+0.19749
Izsak's new and Z	-0.00536
Izsak's old and Y	+0.51797
Izsak's new and Y	-0.01935

Only the Z and the Y isopores were chosen because all other geomagnetic maps, whether isoporic or not, do not correlate as well.

Secondly, it does not seem possible to explain the 200 km high corrugation, which Egyed suggests in the core-mantle interface, by variation of chemical composition or temperature differences. As the density difference between the mantle and the core is 4 g/c.c., this undulation implies stress differences of  $10^{11}$  bars, greater than the breaking stress of iron or silicates at ordinary temperatures. If the phenomenon exists, the mechanism must be a hydro-magnetic one. The magnetic field at the Earth's surface may only be a weaker secondary field and the toroidal field threading and sustaining these undulations may be much greater. Equating the magnetic and hydrostatic pressures and assuming a density difference,  $\rho$ , of 4 g/c.c., a value for  $g$  of  $981 \text{ cm/sec}^2$  and a value of the undulations' height  $h$  to be  $2 \times 10^7 \text{ cm}$ , then:

$$H = \sqrt{\rho gh 4\pi} \approx 10^6 \text{ oersteds}$$

This value of the toroidal field at the core-mantle boundary is four orders of magnitude greater than those assumed by Bullard and Gellman<sup>5</sup> in their dynamo theory of the field. There is, however, a third and fatal objection to Prof. Egyed's idea.

The geomagnetic field drifts westwards at an average rate of  $1/5^\circ$  per year. Prof. Egyed's hypothesis must mean that the gravity anomaly is also rotating at the same rate of  $30^\circ$  every 150 years. The width of the gravity anomalies is of the order of  $30^\circ$ , so that the geoid surface must rise or fall by 30 m every 150 years. If the mantle is rigid over this short time scale, then this must mean that the ocean-levels change by about 50 m. As there is certainly no such recorded anomalous 'tidal' phenomenon, which, of course, would have flooded major cities, it must be concluded that the geoid does not drift.

In conclusion, the gravity anomalies of low degree arise from density differences in the mantle and it has been shown<sup>6</sup> that these are likely to arise from convection currents and, as a result, it does not seem possible to relate the geoid with the core-mantle boundary.

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THE aim of my article was to show that the theoretical result derived by Cook<sup>1</sup> (that the low-degree harmonics of the geopotential originate from the area of the core-mantle boundary) is in accordance with the results obtained by the reflexions of seismic waves from the core-mantle interface showing undulations of  $\pm 200 \text{ km}$  (ref. 2). It was shown also that the order of magnitude of geoid undulations corresponds to the gravity effect of the core undulation.

It was also mentioned that there is a correlation between the distribution of isoporic foci of vertical magnetic intensity and the geoid undulations, a positive centre being found always in the neighbourhood of a positive geoid height, and a negative centre in the vicinity of a geoid depression. This was valid except as to the geoid depression of Antarctica. Prof. Runcorn's remark, that the correlation became worse in the case of the new Izsak-geoid, is true. This can be interpreted, however, according to the results of Cook, in the sense that the

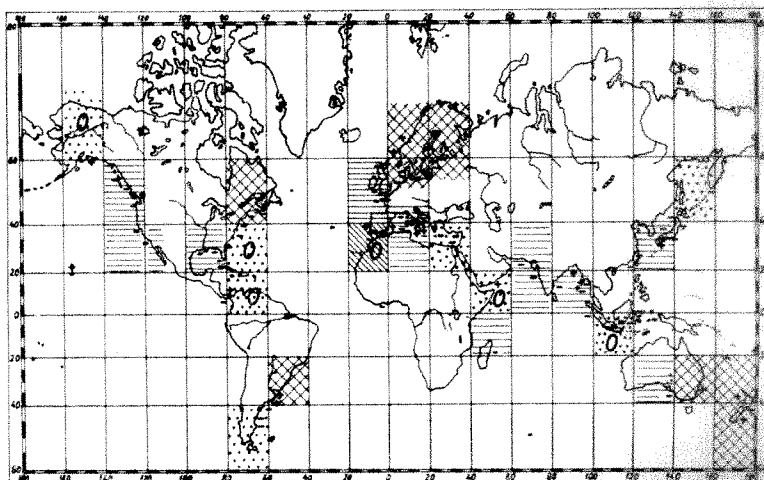


Fig. 1. Correlation between the vertical coastal movements and the satellite geoid, according to the data of Izsak and S. Polli. Cross-hatching, positive geoid undulation with uplifting coasts; horizontal lines, negative geoid undulation with sinking coasts; diagonal lines, no undulation and no change in the coast levels; dotted areas, contrasting undulations and coastal movements (zero undulations contrasting with positive or negative coastal movements are indicated by 0).

higher-order harmonics are affected first by the masses lying on higher level.

I agree with the second remark—and this is mentioned in my article—saying that the core-undulations cannot be explained by variations of chemical compositions or temperature effects. No difficulty arises, however, if the core is a high-pressure-phase transition of the mantle material only.

In my opinion the third objection is not serious. In the case of the tidal phenomenon also the Earth cannot be considered as rigid. It is still less rigid for a time interval 50,000 times longer.

It is remarkable that there is a correlation, not strong, but positive, between the vertical crustal movements<sup>2</sup> of the coasts and the satellite geoid. This contradicts an explanation by convection currents (Fig. 1).

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## PHYSICS

### Relativistic (Non-Linear) Oscillator

THE equation of motion of the relativistic oscillator (without damping):

$$\frac{d}{dt} \left( \frac{m_0 \dot{x}}{\sqrt{1 - \dot{x}^2/c^2}} \right) + kx = 0 \quad (1)$$

has been solved in terms of elliptic functions<sup>1</sup>. The result shows the frequency to decrease with the total energy, but does not make explicit how it is related to the amplitude of oscillation. A formula which is more amenable to experimental test is obtained when we approximate the original equation by:

$$\begin{aligned} \ddot{x} + \omega_0^2 x - \varepsilon x \dot{x}^2 &= 0 \\ \varepsilon &= \frac{3}{2} \frac{\omega_0^2}{c^2} \text{ and } \omega_0^2 = \frac{k}{m_0} \end{aligned} \quad (2.1)$$

It is found, then, that the frequency shows a red-shift:

$$\frac{\Delta\omega}{\omega} = -\frac{\varepsilon A^2}{8} \quad (3)$$

where  $A$  is the amplitude of oscillation. This result agrees with that derived by another method<sup>2</sup>.

The equation of motion of the relativistic oscillator (with damping) is, approximately:

$$\ddot{x} + \omega_0^2 x + b\dot{x} - \varepsilon x \dot{x}^2 = 0 \quad (4)$$

where  $b = \frac{\gamma}{m_0}$  and  $\gamma$  is the damping coefficient. The same red-shift occurs, in first approximation. However, the new frequency is quickly damped out so that this oscillator exhibits the linear frequency  $\omega_0$  (ref. 3).

Similarly, the equation of motion of a relativistic oscillator (without and with) damping which is driven by an external force may be written as:

$$\ddot{x} + \omega_0^2 x - \varepsilon x \dot{x}^2 = F \cos \omega_1 t \quad (5)$$

and

$$\ddot{x} + \omega_0^2 x + b\dot{x} - \varepsilon x \dot{x}^2 = F \cos \omega_1 t \quad (6)$$

where  $\omega_1$  is the driving frequency. The frequency-shift for the undamped, forced oscillator is:

$$\omega_1^2 - \omega_0^2 = -\left( \frac{\varepsilon A^2 \omega_1^2}{4} + \frac{F}{A} \right) \quad (7)$$

which, if  $\omega_1$  is assumed to be close to  $\omega_0$ , reduces to:

$$\frac{\Delta\omega}{\omega} = -\frac{\varepsilon A^2}{8} - \frac{F}{2\omega^2 A} \quad (8)$$

For the damped, forced oscillator the shift is:

$$\omega_1^2 - \omega_0^2 = -\frac{\varepsilon A^2 \omega_1^2}{4} - \sqrt{\left( \frac{F}{A} \right)^2 - b^2 \omega_1^2} \quad (9)$$

or, if  $\omega_1 \approx \omega_0$ :

$$\frac{\Delta\omega}{\omega} = -\frac{\varepsilon A^2}{8} - \sqrt{\left( \frac{F}{2\omega^2 A} \right)^2 - \frac{b^2}{4\omega^2}} \quad (10)$$

Thus, in both instances, the frequency-shift is essentially that of the free, undamped oscillator.

Using a light source of high intensity it may be possible to find the red-shift when the light is scattered in a suitable medium. For a ruby laser, having a wave-length  $\lambda$  6943 Å and an energy flux of  $2 \times 10^{12}$  photons/cm<sup>2</sup>/sec, a frequency shift  $\frac{\Delta\omega}{\omega} \approx 0.5 \times 10^{-4}$  is obtained, if the light is scattered in a plasma with frequency  $\omega_0 = 10^{12}$  and if the experimental value for the Rayleigh scattering cross-section of  $10^{-28}$  is used<sup>4</sup>.

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### Transmission of Laser Beams through Various Transparent Rods for Biomedical Applications

THE use of the laser as a surgical tool for the treatment of lesions in tissues other than those of the eye has been reported<sup>1</sup>. Most of these areas treated, however, are those that are readily accessible, making it possible to focus the laser beam directly on the lesion. To apply the laser as an optical knife for surgery, techniques must be developed to direct the laser beam from the laser head to inaccessible areas. This need to change the direction of the laser beam is required not only in the medical field but also in the field of communication.

If the laser beam is needed to impact internal neoplasms and lesions within the body during laser surgery, then it is desirable to use a medium that is safe, flexible, and able to transmit enough energy to be effective without deteriorating. Destruction of portions of pathological lesions has been accomplished by as little as 22 joules/cm<sup>2</sup> or less, but it may be necessary to transmit at least 110 joules/cm<sup>2</sup> or more to other areas, especially those tissues that are not pigmented.

Since the laser is light energy, optical instruments have been used to carry or to reflect this light. These include lenses, mirrors, prisms, and rods both of glass and of plastic. When the laser beam was transmitted through a glass lens of optical quality, approximately 10–12 per cent of its energy was lost. Similarly, when it was transmitted through a quartz prism about 8 per cent of its energy was lost with each reflexion and some of the surface was worn away with each shot. These readings were made with a TRG calorimeter and a Kiethley micro-voltmeter. This energy loss is due to absorption, reflexion and refraction of the light.

Mirrors cannot accept high-energy laser impacts without ruining their reflective coating. We have tried aluminium foil as a reflector with the ruby laser; the surface discoloured rapidly at very low-energy densities. Aluminium foil was used as shielding for the protection of tissues about the impact area. Investigations are under way with gold-plated mirrors for the neodymium and ruby laser.



We have also investigated fibre optics, special types of glass, and plastics. Fibre optics are glass rods with a core glass of one refractive index that is coated or jacketed with another glass of a lower refractive index. This allows light to be directed into one end, and by multiple total internal reflexions it is almost entirely transmitted out of the other end. Bundles of these fibres contain thousands of these rods that are, because of their small size, able to be bent into various angles and curves. Because of their flexibility, they would be an excellent tool for directing the light to internal components of the body. However, flexible bundles of fibre optics (each fibre being about 75 microns in diameter) are not able to take a very high energy without immediate destruction<sup>2</sup>. As little as 17 joules/cm<sup>2</sup> has been enough. Furthermore, these fibre bundles will accept very little energy before they begin to discolour and break down.

The use of non-flexible rods made of crown clad glass is also being considered. Crown clad glass is constructed similarly to that of a fibre optic rod. Thus, when light is directed through one end of a rod of crown clad glass it will be transmitted by internal reflexions to the other end. It has a glass core with a refractive index of 1.523 and a jacket of glass with a refractive index of 1.474. The diameter of the rods we used was 0.5 cm. A straight rod, 20 cm long, was able to transmit 70 per cent of the laser light that was focused on the input surface. This produced an output of 45 joules with an input of 63 joules. A rod 44.5 cm long, with a right-angle bend 30 cm from the end, was able to transmit about 35 per cent, but it shattered, like a small explosion, with an input of 52 joules (Fig. 1). A rod of the same length, bent in six places with gradual angles so that it resembled a semicircle, was able to transmit 50 per cent or as much as 20 joules with an input of 43 joules. Higher inputs were not attempted because of the damage to the right angle. However, experiments are under way to reinforce the areas around the bends with other glass by Dr. Thomas C. MacAvoy, of Corning Glass Co., and it is hoped that this type of rod will stand higher energies. While a rod of crown clad glass may be able to transmit from 50 per cent or even 70 per cent of the input energy, it is rigid and not desirable for lasing internal areas because it cannot be manipulated easily into various positions. However, significant tissue damage was obtained by laser transmission through crown clad glass.

After preliminary examinations on plates of clear epoxy plastics, transmission experiments were conducted on rods of this material. Some of these rods were rigid and others were very flexible. The diameters of the rods used were from 0.5 cm to 0.75 cm, and we used lengths from 15 cm to 70 cm. With the flexible rods we were able to get about 30 per cent transmission of light. These rods were able to accept up to 20 joules input energy, which is about 40 times what the flexible fibre optic bundles could

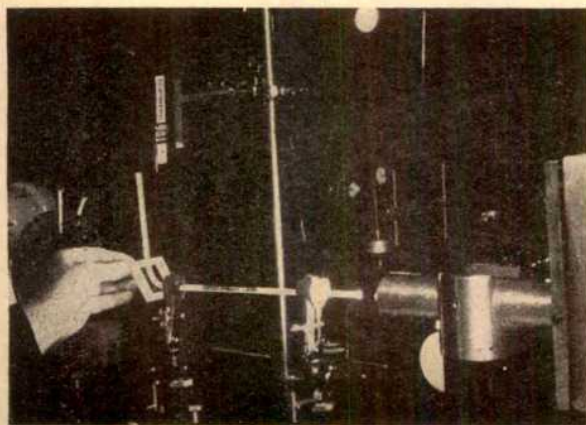


Fig. 1. Laser impact through right angle bend, crown clad glass

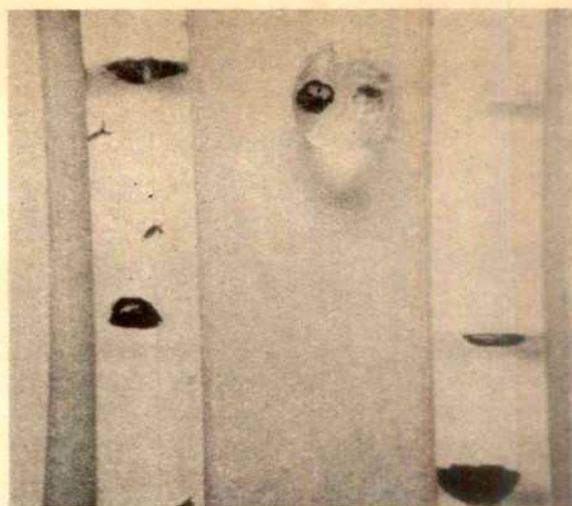


Fig. 2. The black areas of destruction are shown here on three different epoxy rods

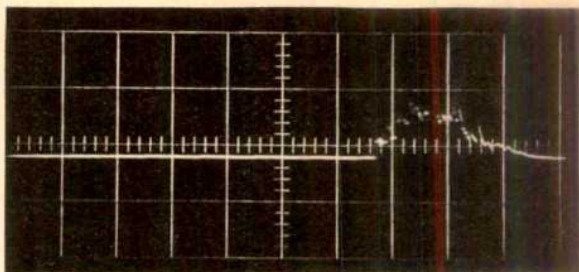


Fig. 3. Normal lasing as recorded by an SD-100 detector and a Tektronix 585 A oscilloscope

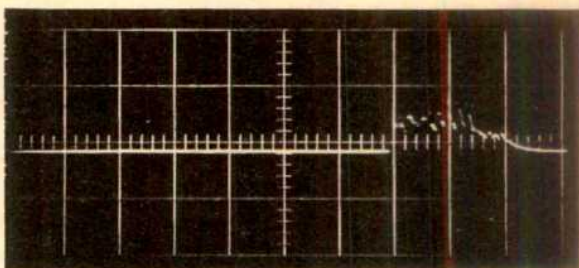


Fig. 4. Normal lasing as recorded by an SD-100 detector and a Tektronix 585 A oscilloscope after passing through an epoxy rod

withstand. However, at energies above 20 joules input, the epoxy became riddled with black areas of destruction (Fig. 2). When the epoxy was bent at the destroyed areas it broke, but when it was bent (even to 360°) over normal (non-damaged) areas it did not break. These rods did not have a jacketing of epoxy of a lower refractive index, and we have not yet tried coated rods. Thus, we have finally obtained a flexible material that can transmit a percentage of laser light and allow for free manipulation. The amount of energy density that can be accepted and transmitted is not very high, but the possibility of using better optical quality flexible epoxy that may be able to accept much higher energy densities is under investigation.

With the use of oscilloscopes we were also able to demonstrate that the laser light may still retain its coherency after being transmitted through the flexible epoxy bundle (Figs. 3 and 4). Rockwell (personal communication) has proposed the use of a beam-splitter, phototubes, and a dual trace oscilloscope to compare the beams from the same pulse to test coherency. Coherency of the laser beam is an important factor for laser use in



communications and is probably necessary for biomedical applications in order to achieve high-energy densities.

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### Oscillations in the Surface Resistance of Bismuth as a Function of Magnetic Field

KHAIKIN *et al.*<sup>1-3</sup> have observed several types of oscillations in the surface impedance of bismuth single crystals as a function of magnetic field directed parallel to the sample surface. These are cyclotron resonances (CR) of the Azbel-Kaner type<sup>4</sup>, quantum oscillations (QO) which arise because of the separation of allowed electron states into Landau levels, and a third type of oscillation (MO) attributed to the presence of magnetoplasma waves<sup>5,6</sup>. The different kinds of oscillations can readily be distinguished by the ranges of magnetic field over which they occur, and their relative amplitudes. At Khaikin's measurement frequency of 9.5 k Mc/s, C.R. due to electrons and holes occurred at fields below about 700 gauss for all directions of magnetic field with respect to crystal axes. The QO began to appear at fields above about 500 gauss. At fields above a few thousand gauss for certain field directions, large amplitude MO appeared and obliterated the QO.

Khaikin observed the oscillations using his very sensitive frequency-modulation method<sup>7</sup>, and made measurements on crystals of two principal orientations, one having the triad axis normal to the sample surface and the other having the triad and a diad in the plane of the surface. A detailed investigation of the cyclotron resonances was made, but the QO periods could be measured only for magnetic field directions near a diad (within 19° of a diad in the trigonal plane, or within 38° in the triad-diad plane).

We have made a preliminary investigation of a sample having the third principal crystal orientation (triad and a bisectrix in the plane of the sample surface), using a microwave bridge method with magnetic field modulation

and a superheterodyne detection scheme. The sample formed the end of a cylindrical  $H_{11}$  cavity resonating at 9.35 k Mc/s. The output of the bridge, proportional to either  $dR/dH$  or  $dX/dH$  (where  $R + jX$  is the surface impedance of the sample), was plotted against magnet current on an X-Y recorder. Two  $dR/dH$  traces for different magnetic field directions in the sample surface are shown. The scales are the same for both curves, and both were traced out at a rate of about one oscillation per sec. In (a) the CR can be seen at low fields and the QO appear at about 1.5 kgauss. Curve (b) was obtained for a direction of magnetic field nearer to the trigonal axis and is included in order to illustrate the relative magnitude of the MO.

We propose to carry out a detailed investigation of the orientation dependence of the various oscillation periods, and hope thereby to obtain information about the exact form of the energy bands in bismuth. A point of particular interest here is the extent of the departure of the bands from quadraticity<sup>8,9</sup>.

The measurements were made at a temperature of about 1.8° K on bismuth having a resistance ratio somewhat in excess of 100. Liquid helium facilities were made available through the provision of a grant by the Department of Scientific and Industrial Research.

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## ENGINEERING

### Stress Patterns in Steel Beams by Autoradiography

PREFERENTIAL dissolution of stressed metal crystals in a polycrystalline sample has been shown by Simnad<sup>1</sup> using <sup>60</sup>Co as a tracer.

In my investigation, preferential dissolution of tensile and compressive stressed areas in a simple cantilever beam experiment was observed.

The 8 × 0.5 × 0.5 in. beams were of mild (1018) steel having the following compositions: Mn 0.88 per cent, C 0.174 per cent, and S 0.036 per cent.

The yield strength was 55 kg/in.<sup>2</sup> and the ultimate tensile strength was 71 kg/in.<sup>2</sup>. The beams were electropolished to remove surface stresses in a mixture of 350 c.c. of 65 per cent perchloric acid and 640 c.c. of glacial acetic acid for 1 min with 20 amp at 25° C.

Two beams were inserted in a grooved steel block (Fig. 1) to a distance of 2 in. and clamped. The bending load was applied by bolting the free ends together so that the ends of both beams deflected toward each other.

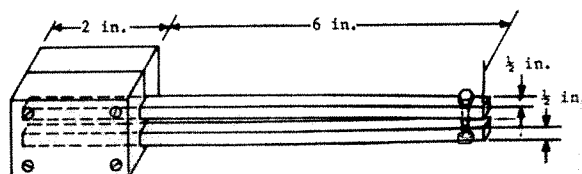


Fig. 1. Cantilever beam assembly

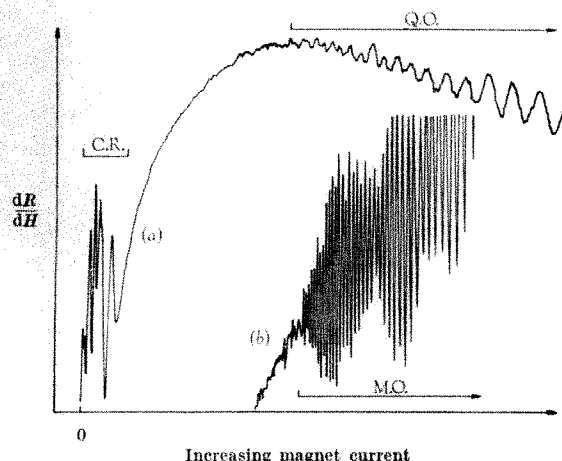


Fig. 1. (a) Trace of  $dR/dH$  versus magnet current showing cyclotron resonance oscillations at low fields and quantum oscillations at high fields. (b) Part of a similar trace for a different magnetic field direction showing magnetoplasma oscillations. The range of magnetic field covered was from zero to approximately 5 kgauss



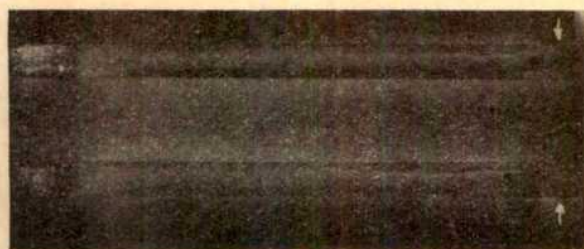


Fig. 2. Autoradiograph of 1018 steel beams previously stressed in a radiotracer solution. Arrows indicate where the beams were bolted together; lighter portions of beams indicate radioactivity

The load was well below the elastic limit of the steel. No permanent deflexion was observed.

The assembly was immersed, bolted end downward, in a solution of 1.05 N  $H_2SO_4$  containing 0.484 mg/ml.  $Fe^{++}$  and 0.0169 mc. of  $^{59}Fe$ . After an immersion time of 1 h at 25° C, the assembly was removed and rinsed until no tracer was found in the rinse water.

Autoradiographs were taken of the beams after releasing the stress load. The autoradiograph (Fig. 2) indicates that the dissolution of a polycrystalline metal beam occurs where either compressive or tensile stress is present. The zero axis (area under the lowest stress) was least attacked by the acid as shown by the light area (denoting radioactivity) on the photograph.

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## GEOLOGY

### Probable Life-forms from the Precambrian of the Witwatersrand System (South Africa)

DURING mineralographic work on Witwatersrand gold conglomerates from the Orange Free State goldfield, I encountered several peculiar micro-structures strongly resembling so-called 'mineralized bacteria'<sup>1-4</sup> and, in certain respects, some types of 'organized elements' recently under discussion as possible primitive life-forms in meteorites<sup>5-7</sup>. These structures are preserved in some of the minute detrital pyrite grains of the Elsberg A1 and A3 horizons occurring near the top of the Witwatersrand system, the deposition of which is believed to have been completed about 2,150 million years ago<sup>8</sup>. As the organized elements are indigenous to allogenic components of the rock, they are certainly considerably older.

Morphologically, two types of micro-structures may be discerned. Type 1 forms spherical to sub-spherical granules consisting of a central core-body enveloped in an outer ring suggesting the presence of an external sac (Fig. 1). The granules occur as isolated spots in the pyrite; only in a few cases are individuals seen to touch each other. They measure from 5 to 21  $\mu$  in diameter, the average values lying within the range of 11 to 16  $\mu$ . Internal structures of particular significance could not be distinguished. As a rule, core and outer ring seem to be mostly filled with quartz; occasionally cores displaying pyrite fillings have been observed. It is interesting to note that similar forms have been described by Ramdohr<sup>9</sup> from the Black Reef, that is, considerably higher in the South African stratigraphical column.

Type 2 is represented by spheroidal to elliptical globules surrounded by double-layered walls, each of the constituting layers appearing as a delicate stippled line (Fig. 2). These elements are somewhat smaller than those of type 1: they are between 4  $\mu$  and 16  $\mu$  across, their average ranging from 7 to 11  $\mu$ . In contrast to type 1, these forms very often tend to aggregate in clusters

resembling globose colonies. Neither of the two types described shows indications of spines, protuberances or other appendages.

Structural elements of this size and organization strongly suggest an origin as relics of primitive unicellular life-forms. There is no doubt that early life must have been largely microbial, a circumstance accounting for the fact that the fossil record in the dawn of geological history is deplorably scanty<sup>10</sup>. The pyrite now containing these relict-structures must, at the time of its formation, have provided extremely favourable conditions for the preservation of these bodies. Distinct morphological affinities can be established between the type 1 micro-structures (Fig. 1) and the common species of so-called fossil sulphur bacteria (with the exception of the characteristic 'framboidal' granulation usually present in the latter). Love and Zimmerman<sup>4</sup>, however, described Precambrian forms from the Mt. Isa shale (Australia) which also lacked this framboidal structure. On the other hand, the organized elements of type 2 (Fig. 2) with their apparently perforated walls seem to be somewhat exceptional as compared with forms hitherto known. Allowing for the fact that early micro-organisms must necessarily represent a morphological no man's land, it is preferred to refer to their relics simply as 'organized elements', a designation deliberately avoiding any unwarranted taxonomic specification. This seems the more necessary, as L. G. Love (personal communication), from his investigations of present-day sediments, found reasons to include into the morphological group formerly known as 'fossil bacteria' such taxonomically hetero-

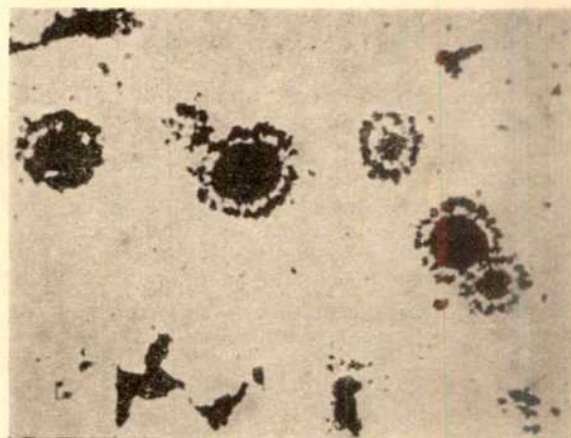


Fig. 1. Organized elements, type 1, from the upper Witwatersrand system (Elsberg A1 reef). (Polished section,  $\times 1,000$ )

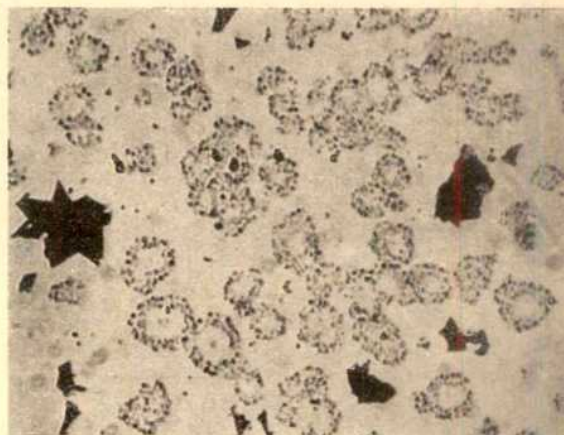


Fig. 2. Organized elements, type 2, from the upper Witwatersrand system (Elsberg A3 reef). (Polished section,  $\times 1,000$ )



geneous forms as pollen grains, spores of fungi and algal remains.

Unfortunately, the micro-elements described proved to be too scanty to enable large-scale maceration techniques to be applied, aiming at the isolation of organic material. All considerations as to a biogenic origin of these structures thus being based on purely morphological premises, it must justly be conceded that other possible modes of origin cannot be completely ruled out. Therefore, I would like to advance my conclusions with due reserve, stressing the observed facts rather than their possible (or even probable) explanation.

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## CRYSTALLOGRAPHY

### X-ray Line Broadening Patterns of Halogen Compounds

A SURVEY has recently been carried out into some features of the X-ray line broadening of various halogen compounds, in polycrystalline form, when they have been strained by mechanical working in a ball mill<sup>1</sup>.

Using the method described by Williamson and Hall<sup>2</sup>, strain and crystallite size can be estimated from the integral breadth ( $\beta$ ) by plotting  $\beta^*$  ( $= \frac{\beta \cos \theta}{\lambda}$ ) against

$d^*$  ( $= \frac{2 \sin \theta}{\lambda}$ ), when the slope gives a measure of the strain, and the intercept on the  $\beta^*$  axis gives a value for the reciprocal of the mean crystallite dimension. Integral breadths of the broadened profiles were determined by first separating the  $K\alpha_1$  peak from the  $K\alpha_1\alpha_2$  doublet by graphical means, and then measuring the area of the  $K\alpha_1$  peak above background level and dividing by the maximum peak amplitude. The broadening so obtained shows a general relationship with the order of the reflexion, and by plotting the integral breadth against  $h^2 + k^2 + l^2$  a smooth curve can be drawn to fit the points approximately. This curve would represent the relationship between integral breadth and  $h^2 + k^2 + l^2$  if the material were isotropic, and the deviation pattern of the points from the mean curve thus gives a qualitative measure of the anisotropy of the broadening. The magnitude of this deviation relative to the total broadening, for lithium fluoride and sodium bromide, is shown in Fig. 1.

Eight halogen compounds have been investigated and the deviation patterns of the broadening are shown in Fig. 2. The interesting feature of these patterns is that each is consistent, and characteristic of the particular material, though the relative amplitude of any pattern is dependent on the amount of broadening present.

The patterns show that the broadening of the (400) profile tends to be low relative to the mean, when compared

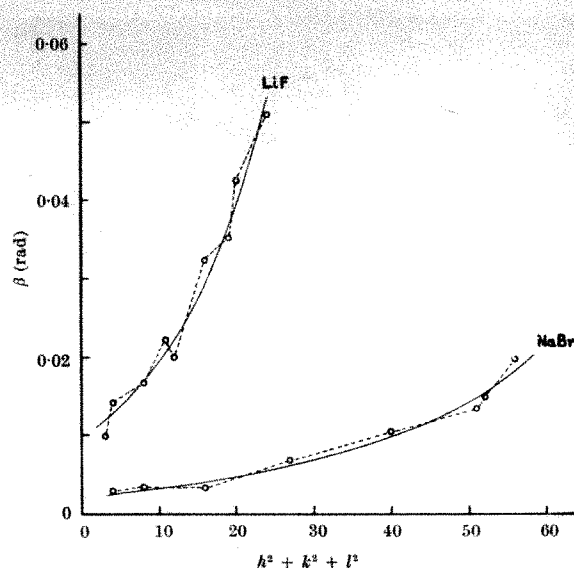


Fig. 1. Measured integral breadth of worked specimens of lithium fluoride and sodium bromide plotted against  $h^2 + k^2 + l^2$ . The 'scatter' of the points about the smooth curve constitutes the 'deviation pattern'.

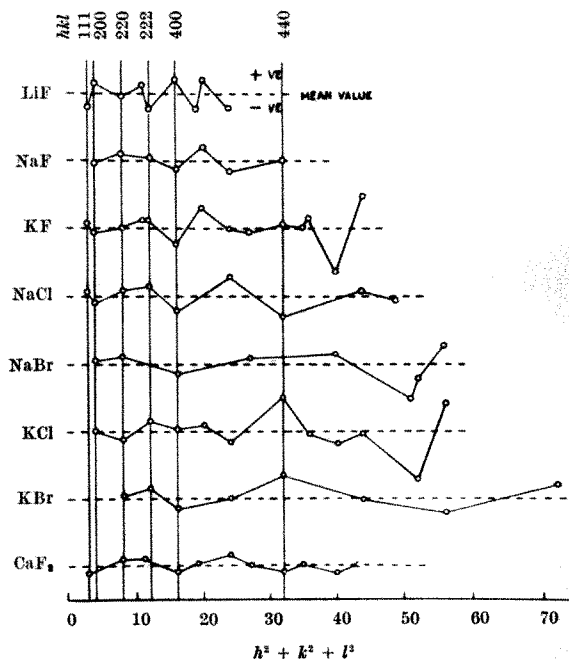


Fig. 2. Deviation patterns. Showing the relative deviation from a smooth curve of the measured integral breadth of certain halogen compounds, plotted against  $h^2 + k^2 + l^2$ .

with the (200) profile, where this is present, except in the case of lithium fluoride; the (222) profile, on the other hand, tends to be high relative to the mean when compared with the (111) profile. In terms of  $\beta^*$  versus  $d^*$  analysis this implies that (200)–(400) broadening tends to be associated with lower slope and higher intercept, that is, a less than average strain and smaller than average crystallite size in the [100] direction. The fact that the (222) profiles tend to show higher than average broadening, on the other hand, suggests greater than average strain and higher than average crystallite size.

These results are consistent with the known mechanical properties of these materials. That is, they tend to fracture easily on the (100) plane, hence one would expect a less than average particle size in the [100] direction and less than average value of strain. The converse applies



to the [111] direction, and these properties are confirmed by the deviation patterns already mentioned.

The anomalous pattern of lithium fluoride is outstanding, and this is also consistent with the mechanical properties as indicated by the elastic constants. In common with all cubic metals, except molybdenum, lithium fluoride has its maximum value of Young's modulus in the [111] direction and its minimum value in the [100] direction; whereas, in contrast, all other halides for which the elastic constants are available have their maximum and minimum values of Young's modulus in the [100] and [111] directions respectively. Deviation patterns of this type thus afford a means of estimating qualitatively the elastic constants of a substance when it is only available in a form which precludes direct measurement by other means.

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### Direct Observation of Surface Films on Gold

It has generally been assumed that naturally occurring surface films do not exist on gold since it has been classically observed that gold is stable in air. Shishakov<sup>1,2</sup> and Trillat *et al.*<sup>3</sup> have reported thin films of various gold oxides formed on gold surfaces on heating.

The purpose of this work was to investigate films thermally produced in air on gold surfaces using transmission electron microscopy and diffraction in conjunction with a recently developed technique to remove the films from the metal.

Surfaces of pure ( $\sim 99.97$  per cent) gold were produced by two methods. The first was to grow slowly a crystal horizontally from the melt in a graphite boat after the surface of the liquid had been scraped clean with a quartz scraper. When solidification was complete, the crystal was cooled to room temperature. The atmosphere was a mixture of argon and air. The crystals produced by this method were either single crystals or very large-grained polycrystals. The second method was to mechanically polish a coarse-grain polycrystal with alumina abrasive until as smooth a surface as possible was produced. The crystal was then heated in air to  $1,030^\circ\text{C}$  for 15 min and cooled. Both methods served to remove any previously existing surface films.

The technique used for isolating the surface films for electron microscopy has been described by Doherty and Davis<sup>4</sup>. On cooling, pits bounded by low-index crystallographic planes nucleated and grew under the film in the metal by the condensation of lattice vacancies. In all cases, it was observed optically that these pits had formed. At room temperature, a layer of cellulose nitrate was applied to the surface and stripped off, removing those areas of the surface film over vacancy pits. After shadowing with platinum at an angle of incidence of  $18^\circ$ , a thin film of carbon was deposited on to the cellulose nitrate. The cellulose nitrate was then dissolved away, and the carbon film containing the fragments of surface film was viewed in normal transmission in the electron microscope with a 50-kV beam. In all cases, observation was confined to a single grain.

Fig. 1 shows the appearance of typical fragments of surface film removed from a crystal solidified from the melt. The fragments of surface film from the mechanically polished crystals are similar. Their various geometrical shapes correspond to the morphologies expected of vacancy pits. Careful inspection of many electron micrographs showed that they do not cast a shadow, indicating that their thickness is not more than about

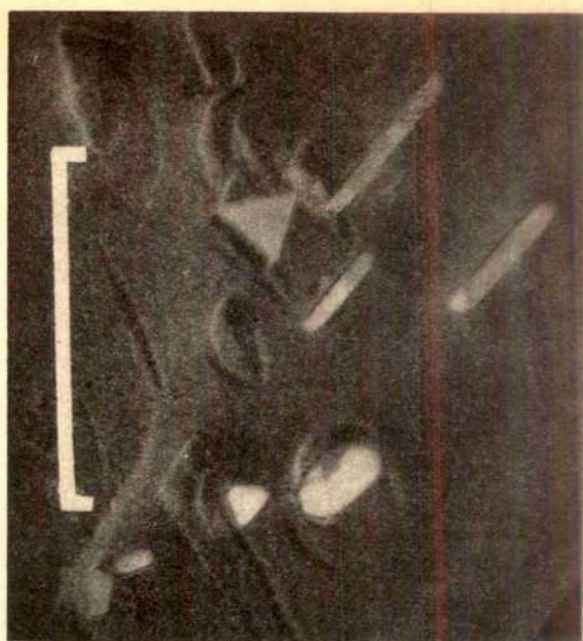


Fig. 1. Fragments of surface film (lighter than background due to photographic reversal) removed from a crystal solidified from the melt. Scale represents  $1\mu$ .

$25\text{ \AA}$ , the smallest height resolvable by this shadowing technique. Fig. 2 is a typical transmission electron diffraction pattern produced by an individual fragment of surface film from a mechanically polished crystal, and is similar to those produced in the case of the crystals solidified from the melt.

For the crystals solidified from the melt, the diffraction pattern produced by the fragments of surface film could be identified as that of a basal-oriented hexagonal lattice structure. On one orientation of the gold substrate which was close to the (221) plane, every fragment of surface film produced a diffraction pattern. The pattern showed that the fragments were not only basal-oriented, but were identically-oriented azimuthally, indicating that the



Fig. 2. Electron diffraction pattern produced by surface film removed from a mechanically polished crystal. The rings are produced by the platinum shadowing material.



surface film was a single crystal. An  $a_0$  lattice parameter of  $3.48 \text{ \AA} \pm 0.9$  per cent was calculated. This value was checked with the known hexagonal oxides, nitrides, carbides, cyanides and binary intermetallic compounds of those impurity metals detected by spectrographic analysis of the gold. It is in closest agreement with the  $a_0$  value of  $3.395 \text{ \AA}$  ( $c_0 = 5.080 \text{ \AA}$ ) obtained for bulk AuCN from X-ray measurements performed by the National Bureau of Standards<sup>5</sup>. On all other substrate orientations examined, only a small fraction of the fragments of surface film produced detectable diffraction spots. The patterns observed showed that these fragments were all basal-oriented but randomly oriented azimuthally, indicating that, with the exception of the one epitaxial case, all the surface films examined from crystals grown from the melt were polycrystalline. The  $a_0$  parameter was calculated to be  $3.48 \text{ \AA} \pm 1.1$  per cent, which is the same value as that obtained for the epitaxial film.

For all the mechanically polished crystals, only a small fraction of the fragments of surface film produced detectable diffraction spots. All the patterns could be identified as that of a basal-oriented hexagonal structure and were randomly oriented azimuthally. We thus conclude that these films were polycrystalline. From these patterns, an  $a_0$  lattice parameter of  $5.30 \text{ \AA} \pm 0.8$  per cent was calculated which is in closest agreement with the  $a_0$  value of  $5.28 \text{ \AA}$  obtained by Shishakov<sup>2</sup> using *in situ* electron diffraction, for thin films of  $\text{Au}_2\text{O}_3$  (hexagonal,  $c_0 = 6.75 \text{ \AA}$ ) formed on gold by heating to  $500^\circ \text{C}$  in oxygen. In addition, the pattern produced by one fragment could be interpreted as a hexagonal structure rotated  $70^\circ$  from the basal orientation about the  $a_1$  axis so that the beam lay in the  $[0221]$  direction. From this pattern, an  $a_0$  parameter of  $5.34 \pm 1.8$  per cent and a  $c_0$  parameter of  $6.56 \pm 1.6$  per cent were calculated.

Since, on all observed substrate orientations except (221), only a small fraction of the fragments of the surface films produced detectable diffraction spots, one cannot conclude whether or not there is any preferred orientation in these polycrystalline films. Presumably, due to the small mass of the fragments, only those fragments having low-index orientations produced spots of sufficient intensity to be detected.

For gold, the temperature range or ranges of vacancy pit nucleation during cooling are not known, and, since the range(s) could be quite broad, no quantitative conclusions can be drawn concerning the temperature or temperatures at which the surface films formed except that they formed somewhere between a temperature near the melting point of gold ( $1,063^\circ \text{C}$ ) and room temperature.

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## CHEMISTRY

### Rate Constant for the Reaction of $\text{O}^-$ with Oxygen

We have measured the rate constant for the reaction  $\text{O}^- + \text{O}_2 \rightarrow \text{O}_3^-$  by irradiating a solution of aerated  $10^{-1} \text{ N}$  potassium hydroxide with a short pulse of fast electrons and observing directly the growth of the transient species  $\text{O}_3^-$  which has an absorption maximum at  $4300 \text{ \AA}$  (refs. 1 and 2).

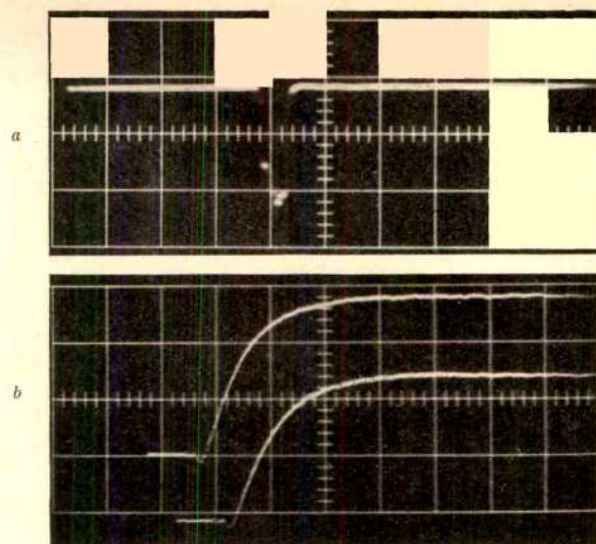


Fig. 1. *a*, Oscillogram showing the duration of the  $0.2\text{-}\mu\text{sec}$  electron pulse as indicated by the Čerenkov radiation. Abscissa scale,  $0.5 \mu\text{sec/cm}$ . *b*, Growth of absorption at  $4300 \text{ \AA}$  due to the species  $\text{O}_3^-$ . Ordinate scale,  $1.67$  per cent absorption/cm. Abscissa scale,  $2 \mu\text{sec/cm}$ . Solution:  $10^{-1} \text{ N}$  potassium hydroxide saturated with air and with nitrous oxide.

The apparatus and conditions of irradiation were generally similar to those already described<sup>1,3</sup>, but the electron pulse in this case had a duration of only  $0.2 \mu\text{sec}$  (see Fig. 1*a*) and delivered a dose of about  $1 \text{ krad}$  to the solution. To increase the  $\text{O}^-$  concentration the solution was saturated with nitrous oxide, which reacts with the electron to give  $\text{O}^-$ . To reduce the speed of the reaction of  $\text{O}^-$  with oxygen, we saturated the solution with air instead of pure oxygen.

Fig. 1 shows two successive growth curves for the species  $\text{O}_3^-$ , obtained by means of a monochromator, photomultiplier and oscillograph set to record at  $4300 \text{ \AA} \pm 150 \text{ \AA}$ . The light source used was a  $450\text{-W}$  xenon lamp. The input network of the oscilloscope had an integrating time constant of  $0.3 \mu\text{sec}$ .

The duration of the electron pulse is clearly indicated in the  $\text{O}_3^-$  growth curve by the initial negative peak due to Čerenkov emission. Each of these curves gives a linear first-order plot taking the origin at the point where the trace crosses the axis. The mean slope, based on several experiments, is  $0.76 \times 10^6 (\text{sec})^{-1}$ . The correction for the oscilloscope time constant leads in this case, paradoxically, to a slight reduction in the slope, and we estimate the first-order rate to be  $0.66 \times 10^6 (\text{sec})^{-1}$ . Since the oxygen concentration was  $250 \mu\text{M}$  the second-order rate constant,  $k$ , is  $2.6 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ .

We have not as yet found a complete absorption spectrum for  $\text{O}^-$  itself, but there is some evidence that it absorbs at wave-lengths near  $2600 \text{ \AA}$ . Fig. 2 shows the decay with time of the absorption at  $2600 \text{ \AA} \pm 100 \text{ \AA}$ .

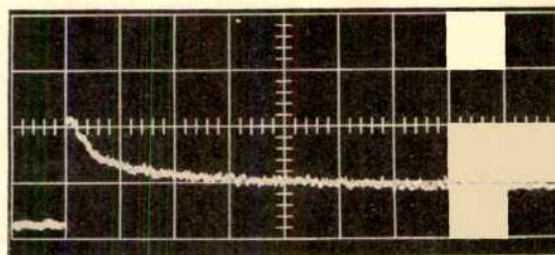


Fig. 2. Oscillogram showing the absorption at  $2600 \text{ \AA}$  following a  $2\text{-}\mu\text{sec}$  pulse to a  $10^{-1} \text{ N}$  solution of potassium hydroxide initially anoxic and containing  $8 \text{ mM}$  nitrous oxide. Ordinate scale,  $3.5$  per cent per cm. Abscissa scale,  $100 \mu\text{sec/cm}$ .



when an initially de-aerated solution of  $10^{-1}$  N potassium hydroxide containing about 8 mM nitrous oxide is irradiated by a single 2- $\mu$ sec pulse. The oscillogram shows two components, one of which has vanished in about 100  $\mu$ sec, while the other lasts for a relatively long time. The fast component is, we believe, due to  $O_2^-$ , and it lasts much longer than the  $O_3^-$  build-up in Fig. 1 because there is only a trace of oxygen in the solution, this having been formed by radiolysis during the pulse. The peak vanishes after two or three pulses have been given to an initially de-aerated solution. The long-lived component is due to  $O_2^-$ . It declines a little by reacting with the trace of  $O_3^-$  formed, and then remains practically constant for many msec.

We thank Mr. M. C. Crowley-Milling for the design of the fast-pulse circuitry, and Mr. Dieter Rebmann for help with its construction.

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### Polonium in Cigarettes—Spectroscopic Analysis

ALPHA-RADIOACTIVITY in cigarettes has recently been measured by two groups. Turner and Radley<sup>1</sup> suggested that  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  are not present in the raw tobacco at levels of activity of the same order as  $^{226}\text{Ra}$ , and concluded that the additional  $\alpha$ -activity taken into the lungs from the radium content in cigarettes is less than 1 per cent of the atmospheric radon inhaled by both smokers and non-smokers. Radford and Hunt<sup>2</sup>, however, maintained that polonium is more dangerous because of its volatility above 500° C. They identified the  $^{210}\text{Po}$  content of cigarettes by observing  $\alpha$ -activity of 138 days half-life, and estimated the radiation dose to bronchial epithelium from  $^{210}\text{Po}$  inhaled in cigarette smoke to be at least seven times that from background sources, and in localized areas possibly up to 200 times.

The investigation recorded here was undertaken in order to: (1) identify spectroscopically and measure the amount of  $^{210}\text{Po}$  as well as other polonium isotopes in cigarettes; (2) present a fast and reliable detection system capable of determining accurately minute quantities of  $^{210}\text{Po}$  (of the order of  $10^{-2}$   $\mu\text{c}$ .) as well as of mixtures of  $\alpha$ -active isotopes.

Polonium was extracted from whole cigarettes using a wet ashing procedure<sup>3</sup>. The polonium samples deposited on silver foils were then placed at a distance of a few mm from a 2-cm<sup>2</sup> solid-state detector, which was coupled into a low-noise, charge-sensitive preamplifier and a multi-channel analyser<sup>3</sup>. The effective solid angle was  $3.8 \pm 10$  per cent steradians. The overall energy spread of a monoenergetic  $\alpha$ -group was about 50 keV. Alphas from known sources of  $^{241}\text{Am}$ ,  $^{238}\text{U}$ ,  $^{212}\text{Po}$ , and  $^{212}\text{Bi}$  were used for energy calibration. This procedure can determine energies of unknown  $\alpha$ -groups to  $\pm 15$  keV. Two samples, each containing polonium from two cigarettes, were measured. The two samples were prepared from cigarettes of the same brand but from different cartons and were both found to contain  $0.45 \pm 0.10$   $\mu\text{c}$ . per cigarette, in agreement with Radford and Hunt<sup>2</sup>. The quoted uncertainty is mainly due to the uncertainty in solid angle and in plating efficiency. Background was 0.1–0.2 counts/h and was less than 1 per cent of the sample activity. A polonium  $\alpha$ -spectrum accumulated for 6 h

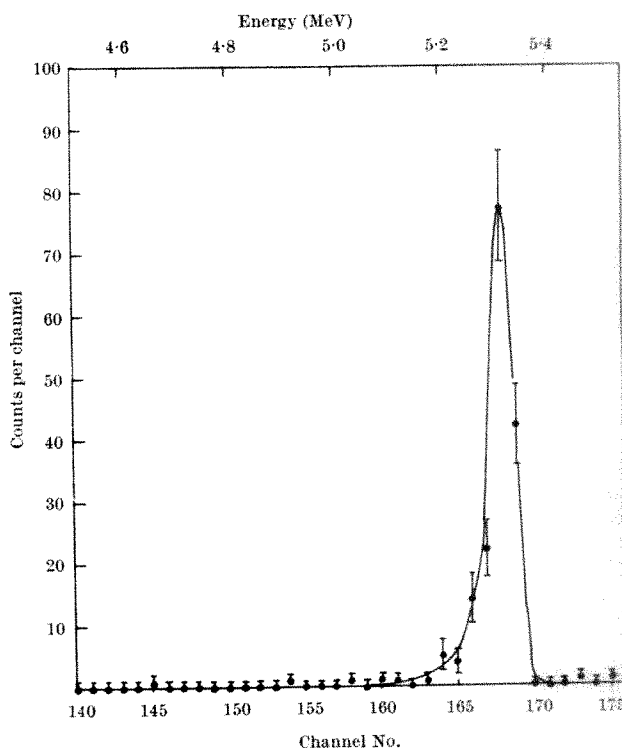


Fig. 1. Polonium  $\alpha$ -spectrum accumulated for 6 h from a two-cigarette sample

from a two-cigarette sample is shown in Fig. 1. The location of the peak corresponds to an energy of  $5.310 \pm 0.015$  MeV, in excellent agreement with the value of 5.305 MeV for  $^{210}\text{Po}$ .

Although all polonium isotopes are  $\alpha$ -active, no  $\alpha$ -activity due to any other polonium isotopes was observed, with an upper limit of 1 per cent. This result is not surprising since all polonium isotopes which belong to the radioactive series have too short half-lives to be observed in our experiment, and there is no apparent process which can yield other polonium isotopes in large amount. There is an agreement to within 15 per cent between the activity of our two samples and between our samples and those of Radford and Hunt<sup>2</sup>. Though this agreement may be fortuitous, it at least suggests that the amount of polonium per regular size American cigarette (about  $2 \times 10^5$  atoms) does not fluctuate extensively, and may reflect the homogenizing procedure used in the production of cigarettes.

We have also performed a preliminary measurement of the polonium collected on a glass-wool filter when whole cigarettes were smoked in a stream of oxygen inside a heated flask. This preliminary measurement indicates that most of the polonium follows the smoke, in agreement with the findings of Radford and Hunt<sup>2</sup>. If we assume that about 10 per cent of the polonium content of the cigarette eventually decays in the lung, the amount of  $^{210}\text{Po}$  activity at equilibrium for a person smoking 50 cigarettes a day can reach a level of 400  $\mu\text{c}$ . Unfortunately, biological data for the efficiency of the lung for the uptake and retention of polonium in cigarette smoke are not available to test this assumption<sup>4</sup>. Such a value, although not alarming, can be a cause for concern if concentrated in small areas of the respiratory system<sup>2</sup>. We believe that the questions of polonium retention and localization are very important and should be fully investigated. We also have not yet investigated the question of equilibrium in the cigarette between  $^{210}\text{Po}$  with a half-life of 138 days and one of its parents  $^{210}\text{Pb}$  with a half-life of 22 years. This question is significant if ageing is to be considered as a possible remedy. The dependence of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$  concentrations on factors such as rain<sup>5</sup>,



soil, irrigation, fertilizers, etc., should also be investigated. It is also conceivable that proper glass filters might retain a significant part of the inhaled polonium, as indicated by Radford and Hunt<sup>2</sup> and by our preliminary measurement.

Other polonium-absorbing materials such as silver or nickel should also be considered. In any event, we strongly believe that  $\alpha$ -spectroscopy, which utilizes apparatus similar to that described here, can be a powerful tool in the investigation of all these questions. It could also help in the investigation of radioactivity of polluted air from factory smoke and automobile fumes. Such an experimental system is practically free from background. It permits determination and identification of minute quantities of  $\alpha$ -active isotopes. Counting efficiency of up to 100 per cent, and with no appreciable loss in energy resolution, can be achieved with the use of 2 solid-state detectors connected in parallel in a 4 $\pi$  arrangement, and sources plated on very thin foils. In short, a spectroscopic system is unambiguous and fast, and is therefore preferable to systems which utilize analysis by half-life.

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### Synthesis of Substituted Phenyl- $\beta$ -D-xylopyranosides

INVESTIGATIONS into the specificity of a  $\beta$ -xylosidase required the synthesis of a number of substituted phenyl- $\beta$ -D-xylosides. A very important method is the Helferich reaction in which the appropriate phenol and the fully acetylated monosaccharide are heated in the presence of an acidic catalyst. To favour the formation of xyloside-acetates with the  $\beta$ -anomeric configuration, *p*-toluenesulphonic acid and  $\beta$ -tetra-*O*-acetyl-xylose were used<sup>1</sup>.

The corresponding xylosides were prepared by catalytic deacetylation of the acetates.

Tetra-*O*-acetyl- $\beta$ -D-xylose was prepared according to Vogel<sup>2</sup> and twice crystallized from ethanol: m.p. 127°–128°.

The xylosetetraacetate (31.8 g; 0.1 mol.) and the appropriate phenol (0.4 mol.) were fused together and the molten mixture treated with a solution of *p*-toluenesulphonic acid (0.6 g) in a mixture of acetic acid and acetic anhydride (95:5; 50 ml.). The mixture was heated under reduced pressure at 100° for 1 h. After cooling, the resulting syrup was dissolved in chloroform (200 ml.), thoroughly washed with ice-cold N sodium hydroxide and water, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated *in vacuo*. The resulting syrup was then crystallized from the appropriate solvent to constant m.p. and optical rotation.

The *ortho*-substituted phenol, *o*-chlorophenol, failed to react in the presence of *p*-toluenesulphonic acid even at higher temperature. Jermyn<sup>3</sup> likewise reports inability to obtain *o*-chlorophenyl-tri-*O*-acetyl- $\beta$ -D-glucoside by this method. However, we succeeded in obtaining the *o*-chloroderivative by a modified Michael synthesis<sup>4</sup>. To a solution of *o*-chlorophenol (8.95 g; 0.07 mol.) in acetone (192 ml.), potassium hydroxide (3.36 g) in methanol (50 ml.) and acetobromoxylose (20 g; 0.059 mol.) were added. After 5 h at room temperature the mixture was

Table 1. ACETYLATED  $\beta$ -D-XYLOPYRANOSIDES

	M.p. cryst. solvent	( $\alpha$ ) <sub>D</sub> <sup>20</sup>	Yield	C	Analytical value	
					H	Cl
<i>p</i> -cresyl $\text{C}_{15}\text{H}_{19}\text{O}_5$	114° Methanol	-45.1° c, 4	48	<i>f</i> * 59.0 <i>c</i> 59.0	6.1 6.0	—
<i>m</i> -cresyl	112° Methanol	-48.2° c, 4	37	<i>f</i> 58.8	6.1	—
<i>o</i> -cresyl	112°–113° Methanol	-53.6° c, 2	32	<i>f</i> 58.7	6.1	—
<i>p</i> -chloro- <i>m</i> -cresyl $\text{C}_{15}\text{H}_{17}\text{O}_5\text{Cl}$	134°–135° Ethanol	-45.0° c, 2	32	<i>f</i> — <i>c</i> —	—	5.8 5.9
<i>p</i> -chlorophenyl $\text{C}_{17}\text{H}_{15}\text{O}_5\text{Cl}$	129°–130° Ethanol	-48.6° c, 2	54	<i>f</i> — <i>c</i> —	—	9.2 9.2
<i>m</i> -chlorophenyl	108°–109° Ethanol	-52.2° c, 2	47	<i>f</i> —	—	9.2
<i>o</i> -chlorophenyl	130°–132° Ethanol	-75.5° c, 2	30	<i>f</i> —	—	9.2
<i>f</i> , found.	<i>c</i> , calculated					

Table 2.  $\beta$ -XYLOPYRANOSIDES

	M.p. cryst. solvent	( $\alpha$ ) <sub>D</sub> <sup>20</sup>	Yield	C	Analytical value	
					H	Cl
<i>p</i> -cresyl $\text{C}_{15}\text{H}_{19}\text{O}_5$	162°–163° Methanol	-42.0° c, 4	89	<i>f</i> 60.0 <i>c</i> 60.0	6.7 6.7	—
<i>m</i> -cresyl	145°–146° Methylethyl- ceton	-45.0° c, 2	93	<i>f</i> 59.8	6.7	—
<i>o</i> -cresyl	163° Methylethyl- ceton	-48.0° c, 2	95	<i>f</i> 59.5	6.7	—
<i>p</i> -chloro- <i>m</i> -cresyl $\text{C}_{15}\text{H}_{17}\text{O}_5\text{Cl}$	178° Ethylacetate	-39.5° c, 2	79	<i>f</i> — <i>c</i> —	—	18.0 12.9
<i>p</i> -chlorophenyl $\text{C}_{17}\text{H}_{15}\text{O}_5\text{Cl}$	156°–157° Ethylacetate	-41.6° c, 2	72	<i>f</i> — <i>c</i> —	—	13.6 13.6
<i>m</i> -chlorophenyl	149°–150° Water	-47.8° c, 2	81	<i>f</i> —	—	13.7
<i>o</i> -chlorophenyl	175°–176° Water	-57.0° c, 2	80	<i>f</i> —	—	13.6

filtered and evaporated *in vacuo*. The residue was dissolved in chloroform (100 ml.), washed with ice-cold 5 per cent sodium carbonate and water, dried ( $\text{Na}_2\text{SO}_4$ ), evaporated *in vacuo* and crystallized from ethanol.

The tri-*O*-acetyl- $\beta$ -D-xylopyranosides shown in Table 1 were prepared in this way (all rotations were determined for chloroform solutions).

*Preparation of substituted phenyl- $\beta$ -D-xylopyranosides*<sup>5</sup>. 1 g of the corresponding acetate, dissolved or suspended in 10-ml. dry methanol, was treated with 3 ml. of a freshly prepared solution of sodium methoxide (0.5 g of sodium in 100 ml. of methanol). After standing a few hours at room temperature the sodium was removed with a slight excess of 'Dowex 50 W' ( $\text{H}^+$ ). The solution was filtered, evaporated *in vacuo* and crystallized from a suitable solvent. Table 2 shows the  $\beta$ -D-xylosides prepared (all rotations were determined for methanol solutions).

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### Analysis of the Absorption Spectrum of Solutions of Uranyl Compounds

THE absorption spectra of solutions of uranyl compounds between 20,000 and 28,000  $\text{cm}^{-1}$  show some features which have, at least to our knowledge, not been stressed in the literature.

Indeed, if  $\Delta\nu$  is plotted against decreasing values of  $\nu$  the results shown in Fig. 1 are obtained;  $\Delta\nu$  being the

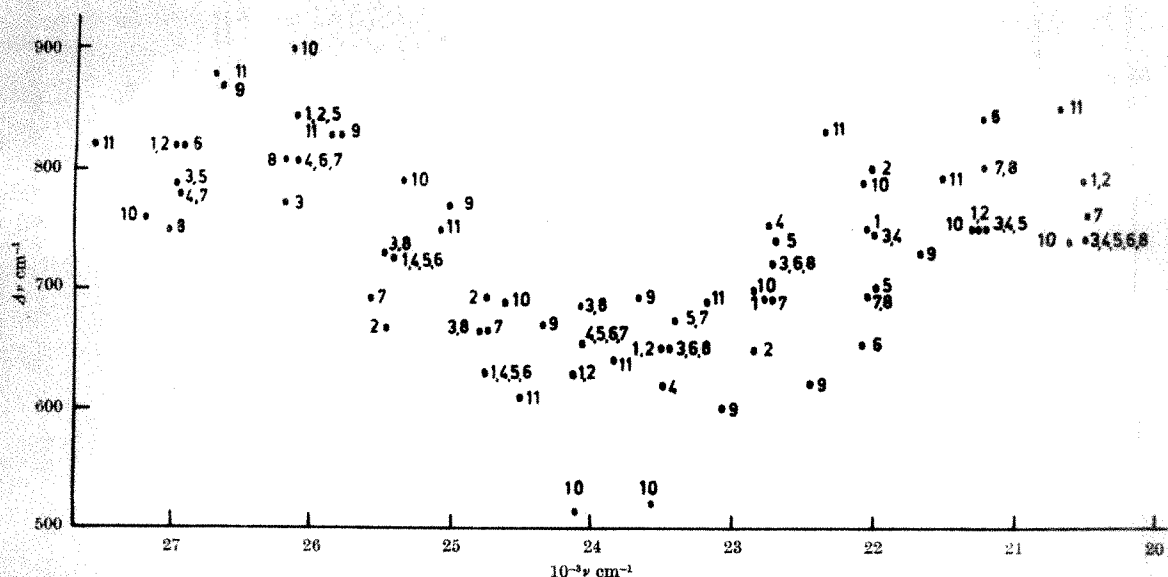


Fig. 1. Vibronic intervals as a function of the wave-numbers of the vibronic maxima in the absorption spectra of solutions of uranyl compounds. The figures next to the points refer to different spectra

difference in wave-number values for two successive absorption maxima expressed in  $\text{cm}^{-1}$  and  $\nu$  being the wave-number at which these maxima appear, also in  $\text{cm}^{-1}$ . The  $\Delta\nu$  values are plotted against the lower of the two wave-number values  $\nu$ , from which they are obtained.

Fig. 1 was established from data of eleven different spectra for uranyl perchlorate, nitrate and phosphate dissolved at various concentrations in water, alcohol and acetone. Vibronic analysis data from Jezowska-Trzebiatowska and others<sup>1,9</sup> are not reported on the figure, although they actually do fit rather well on it.

At some wave-numbers in the absorption spectra of uranyl the vibronic maxima are rather poorly defined. This is certainly so, along with the co-excitation of others than the uranyl symmetric vibration with electronic excitation, at the origin of the scatter of the points on Fig. 1. However, Fig. 1 does show an important feature.

One can distinguish a regularity in the values of  $\Delta\nu$  for different values of  $\nu$ . Roughly one can say that  $\Delta\nu$  equals approximately  $800 \text{ cm}^{-1}$  between  $27,500$  and  $25,500 \text{ cm}^{-1}$ ,  $650 \text{ cm}^{-1}$  between  $25,500$  and  $22,500 \text{ cm}^{-1}$ , and some  $750 \text{ cm}^{-1}$  between  $22,000$  and  $20,000 \text{ cm}^{-1}$ . Around  $25,500$  and  $22,500 \text{ cm}^{-1}$  the  $\Delta\nu$  values are intermediate between  $650$  and  $800 \text{ cm}^{-1}$ .

These three spectral regions, which one can distinguish according to  $\Delta\nu$  values, coincide strikingly with the regions which have been proposed by several authors for three electronic transitions in the  $20,000$ – $28,000 \text{ cm}^{-1}$  absorption spectrum of uranyl<sup>2-5</sup>. Therefore, any decrease in  $\Delta\nu$  for increasing  $\nu$  values, which could be considered as significant of a convergence of bands within each one of the three regions, could be used to calculate potential energy curves for the three electronically excited states  $^3\Pi_{2u}$ ,  $^3\Pi_{1u}$  and  $^3\Pi_{0u}$  (ref. 4) of the uranyl ion.

In fact, the  $\Delta\nu$  values within each region are, for the reasons just mentioned, too irregular and there are too few of them to consider them as belonging to a convergent series of bands. Nevertheless, we did so and calculated in a classical way<sup>6</sup> potential energy curves for the excited states already mentioned here. We used the Badger relation as given by Jones<sup>7</sup> for uranyl in its ground-state to calculate equilibrium internuclear distances U–O for each of the three excited states. The energy of the  $0'-0''$  bands for each of the three excited states are estimated by extrapolation from the positions of the  $1'-0''$  and  $2'-0''$  bands in the three electronic transitions suggested in an earlier communication<sup>5</sup>.

The potential energy curves given on Fig. 2 were calculated from the spectrum of uranyl perchlorate in water (the most favourable case: points 1 on Fig. 1). The potential energy values of Fig. 2 were calculated in  $\text{cm}^{-1}$  for the ground-state energy equal to  $0 \text{ cm}^{-1}$ .

Although from a theoretical point of view these curves may be far too inaccurate, their comparison with experimental results does give them some significance. Indeed, the calculated value of  $28,690 \text{ cm}^{-1}$  for the asymptote of the potential energy at infinite atom separation in the  $^3\Pi_{0u}$  state roughly corresponds to the appearance of a continuum at about  $28,200 \text{ cm}^{-1}$  in most uranyl absorption spectra. The corresponding calculated value of  $32,250 \text{ cm}^{-1}$  for the  $^3\Pi_{1u}$  state strongly recalls a feature of the uranyl absorption spectrum reported by Pringsheim<sup>8</sup>. He distinguishes band structure superposed on a continuum up to about  $32,573 \text{ cm}^{-1}$  in the absorption spectrum of  $1/20 \text{ M}$  uranyl sulphate in water.

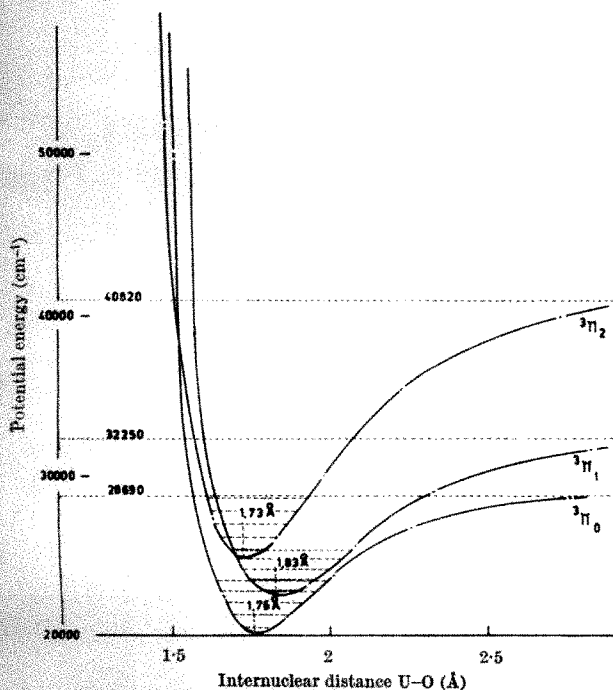


Fig. 2. Potential energy curves for the lowest excited states of the uranyl ion in solution

Application of the Franck-Condon principle to the proposed relative positions of the potential energy curves appears, at first sight, to agree with the observed difference in intensity distribution between the absorption and phosphorescence spectra of uranyl compounds. (Phosphorescence of uranyl is used here instead of fluorescence, as suggested by McGlynn and Smith<sup>4</sup>). In absorption the  $2'-0''$  band of the  ${}^3\Pi_{1u} \leftarrow {}^1\Sigma_g^+$  (ref. 5) transition appears to be the most intense one, while in emission it is the  $1'-0''$  band of the transition  ${}^3\Pi_{0u} \rightarrow {}^1\Sigma_g^+$  which appears to be the most intense band.

The irregularities in the band structure of the uranyl absorption spectrum have been considered as indicative of "distinct negative vibrational anharmonicity"<sup>9</sup> of uranyl in its lowest excited state. The present analysis of this absorption spectrum yields an explanation for these irregularities which is in good agreement with other evidence<sup>4,5</sup> for the triplet character of the lowest excited state of uranyl.

This analysis is now being applied to the absorption spectra of a great number of uranyl compounds in order to check its general validity.

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### Some New Co-ordination Compounds of Copper(II) Chloride

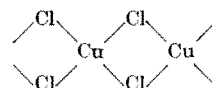
WE wish to report the preparation of a series of 1:1  $\text{CuCl}_2$  ligand compounds which appear to have polymer type structures. The ligands used in this investigation were 1,2-diazine (pyridazine); 1,3-diazine (pyrimidine); 1,4-diazine (pyrazine); 1,2,4-triazole; 1,10-phenanthroline and 2,2'-bipyridine.

Interest in this area of investigation arose from our belief that a comparison of the copper(II) complexes formed with bidentate ligands in which the two nitrogen donors have different relative locations in the ligand molecule might provide insights into the structures of these complexes. Moreover, the crystal structures of the 1,2,4-triazole- $\text{CuCl}_2$  complex reported by Jarvis<sup>1</sup> led us to expect that the preparation of a variety of  $\text{CuCl}_2$ -ligand polymers should be possible.

All compounds herein reported were prepared by the addition of excess solid  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  to the ligand dissolved in water. Chemical analysis (for example, the  $\text{CuCl}_2$ -1,2-diazine complex yielded the following analysis: calc.: Cu, 29.61; Cl, 33.05; found: Cu, 29.65; Cl, 33.14.  $\text{CuCl}_2$ -1,4-diazine. Calc.: Cu, 29.61; Cl, 33.05; found: Cu, 29.21; Cl, 32.74) indicated a chlorine to copper ratio of 2:1 and a  $\text{CuCl}_2$  to ligand ratio of 1:1. All the compounds were green, and the mull spectra of these compounds showed maxima near  $1.4 \times 10^4 \text{ cm}^{-1}$ , and all

exhibited strong absorption in the ultra-violet. The minimum in absorption between the two observed bands in every case was near  $1.8 \times 10^4 \text{ cm}^{-1}$ .

Comparison of the observed mull spectra with mull and solution spectra of numerous other copper(II) compounds leads to the conclusion that the bands centring near  $1.4 \times 10^4 \text{ cm}^{-1}$  are due to  $d-d$  transitions (ligand field bands) and that the intense ultra-violet bands arise from ligand absorption and/or charge transfer processes. Since the ligand field at the cupric ion is provided almost entirely by the nearest neighbour environment, the marked similarity in band position and shape suggests that the copper(II) ion has nearly the same local environment in all these complexes.



The model which appears to be most consistent with these facts is one which places cupric ions in chains and in which the ligands are utilized as cupric ion-cupric ion links. The low water solubility of the compounds supports the chain-type polymer structure. In terms of this model, a ligand with adjacent nitrogens as in 1,2,4-triazole and 1,2-diazine connects copper(II) ions within a chain as shown schematically in Fig. 1. Ligands with non-adjacent nitrogens within a single ring such as 1,3-diazine and 1,4-diazine connect different chains as shown schematically in Fig. 2. (The stereochemistry of the nitrogens in the phenanthroline molecule suggests that this copper chloride complex would be of the type shown in Fig. 1. However, the possibility of rotation about the carbon-carbon bond in 2,2'-bipyridine allows that it could act as an intra- or inter-chain link.) A model of this type has also been proposed for  $\text{NiCl}_2$ -1,4-diazine complex by Lever, Lewis and Nyholm<sup>2</sup>. In each of the proposed structures, then, the copper(II) ion has a co-ordination number of six, lies at or near the centre of four in-plane chloride ions, and has one ligand nitrogen above and another below the plane.

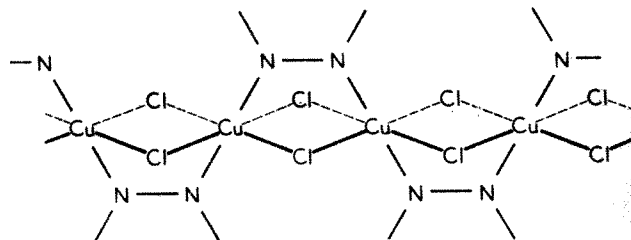


Fig. 1. Schematic representation of intra-chain bonding in copper chloride-ligand polymers

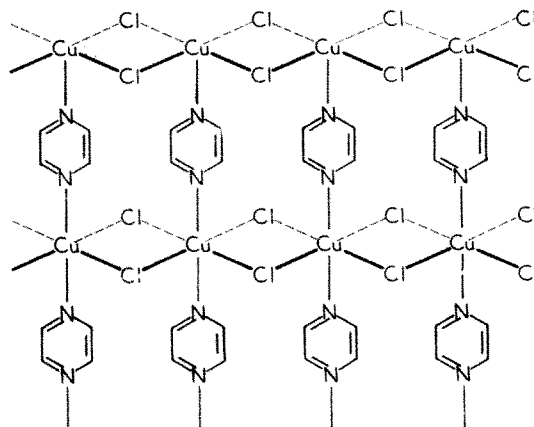


Fig. 2. Schematic representation of inter-chain bonding in copper chloride-ligand polymers



Other possible structures have also been considered. For example, a model involving copper in tetrahedral co-ordination was considered unlikely on the grounds that a  $1.4 \times 10^4 \text{ cm}^{-1}$  band (with no evidence for additional  $d-d$  bands between  $1.4 \times 10^4 \text{ cm}^{-1}$  and  $4.5 \times 10^3 \text{ cm}^{-1}$ ) is not at all consistent with  $d-d$  bands of copper compounds known to be tetrahedral<sup>3</sup>. A four co-ordinate model ('square planar') was also considered unlikely because of the different steric requirements of the ligands which would be expected to produce differences in the optical spectra. No such differences have been observed.

We believe that the information at present available supports the six-fold co-ordination of the copper(II) ions; however, only a single-crystal X-ray examination can elucidate the exact details of these structures. Attempts are now in progress to grow single crystals of these compounds and the results of magnetic and optical measurements will be reported later.

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## BIOCHEMISTRY

### Glucose Metabolism and $\alpha$ -Keto-acids in Rat Brain and Liver *in vivo*

GLUCOSE is the major substrate for the brain<sup>1,2</sup>, which utilizes a very high proportion of the bodily glucose requirement<sup>3</sup>. Whittam<sup>4</sup> has calculated that about 40 per cent of the energy produced from glucose metabolism in the brain is required for ion transport mechanisms important in maintaining the physiological function of the tissue. Over the past few years a number of workers<sup>5-8</sup> have demonstrated the high rate of incorporation of carbon-14 from glucose into brain amino-acids, particularly glutamic acid. It has been suggested<sup>6</sup> that the amino-acids have a special role as intermediates in the brain; that their formation and oxidation constitute a major pathway of glucose metabolism in the brain. However, the characteristically high concentration of free glutamate in the brain, in contrast to other tissues, renders methods of investigations which are based on the labelling of the amino-acid pools, rather than specific activity measurements on individual amino-acids isolated from the tissues, rather inconclusive. As has been suggested<sup>9</sup>, such results could be due to quantitative differences in glycolysis and transamination reactions between the brain and the liver.

This communication reports the main results of an investigation into the incorporation of carbon-14 from glucose into the  $\alpha$ -keto-acids in rat brain and liver. The previous emphasis has been on glutamate which has been shown to be involved in potassium transport<sup>2,10</sup>, so this work has been carried out *in vivo* since potassium transport, and possibly therefore glutamic acid metabolism, is suboptimal in cerebral cortex slices<sup>11</sup>. These experiments form part of a more extensive investigation which will be published in detail shortly.

Glucose-U-<sup>14</sup>C (15  $\mu\text{C}$  in 0.5 ml. saline, obtained from the Radiochemical Centre, Amersham) was injected subcutaneously into 100 g male rats of the Wistar albino Glaxo strain (obtained through the courtesy of Dr. C. M. Mauritzen, Department of Biochemistry, University of Melbourne). At various times (2-40 min) after injection, the rats were killed by decapitation, the blood was

immediately collected in heparinized beakers and deproteinized by pipetting a measured volume (usually 1 ml. into cold 6 per cent perchloric acid. Also immediately after death, the tissues were rapidly excised and frozen in liquid nitrogen. The time from decapitation to complete freezing of the tissues was usually effected within 1 min and never exceeded 1.5 min. The tissues were weighed, dropped while still frozen into cold 6 per cent perchloric acid (6 ml./g) and dispersed in a 'Teflon'-pestle homogenizer (A. H. Thomas Co., Philadelphia). After centrifugation the protein residues were washed by resuspension in cold perchloric acid.

Two-thirds of the combined supernate and washings was brought to room temperature (20-25°) and reacted with one-tenth the volume of 2 : 4-dinitrophenylhydrazine (0.2 per cent in 2 M HCl) for 30 min. (The remainder of the acid extract was neutralized with KOH at 0° and the precipitated KClO<sub>4</sub> was removed by centrifugation. This neutral extract was stored frozen for subsequent isolation of amino-acids and sugar intermediates.) The keto-acid dinitrophenylhydrazones were extracted into ethyl acetate (10 + 5 ml.) at 0° in the absence of light by buzzing on a Vortex shaker. All extractions were performed at 0° in the dark with a Vortex shaker since it was found that a brown contaminant, similar to that reported by Neish<sup>12</sup>, appeared on subsequent chromatograms if these precautions were not observed, or if mixing was by aeration. The ethyl acetate extract was washed once with water (5 ml.) and the dinitrophenylhydrazones were purified by extraction into 10 per cent Na<sub>2</sub>CO<sub>3</sub> (2 ml.) and washed with ethyl acetate (5 ml.). After dropwise acidification with chilled concentrated HCl, the dinitrophenylhydrazones were re-extracted into ethyl acetate and washed with water according to the usual procedure<sup>13</sup>. The extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed to *in vacuo* at < 10°. The residue was dissolved in the minimum volume of ethyl acetate, transferred to a micro-test-tube and concentrated to about 20  $\mu\text{l}$ . in a gentle stream of dry nitrogen. The total extract from each tissue sample was applied in 2  $\mu\text{l}$ . aliquots to thin-layer plates of cellulose and chromatographed at 3° in the dark in butan-1-ol : ethanol : 0.5 M ammonia (7 : 1 : 2 by vol.) as previously described<sup>14</sup>. The plates were dried in an air-stream, after which the discrete spots were clearly visible. Only rarely was a spot of oxaloacetic acid dinitrophenylhydrazone discernible, and then in too small amounts (about 0.2-0.5  $\mu\text{g}$ ) to allow for accurate quantitation.

Each spot was scraped off the plate into a micro-test-tube and the derivative was eluted into cold 10 per cent Na<sub>2</sub>CO<sub>3</sub> (0.5 ml.) by 'buzzing' and rapid centrifugation in a Servall SST. The extracts could then be estimated directly at 380 m $\mu$  (ref. 15) in 0.4 ml. micro-cuvettes of the 'Spectrochem' (Hilger and Watts). However, since radioactivity measurements were to be performed, it seemed advantageous to estimate the keto acids in the same sample as that to be counted. The Na<sub>2</sub>CO<sub>3</sub> extracts were acidified (HCl) at 0° by buzzing in the presence of ethyl acetate (0.6 ml.); the ethyl acetate extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and estimated directly at 360 m $\mu$ . The molar extinctions of the dinitrophenylhydrazones (pyruvate : 19,800; oxaloacetate : 25,600;  $\alpha$ -oxoglutarate : 23,500) were very similar to those obtained<sup>15</sup> in Na<sub>2</sub>CO<sub>3</sub> at 380 m $\mu$ , so no sensitivity was lost. The recovery of synthetic keto-acid derivatives through the whole process ranged from 96 to 102 per cent. In all the tissue extracts, in which storage-time under acid conditions was kept to a minimum (the extractions and estimations were all performed within the same day under controlled conditions of light and temperature), no separation of *syn-anti* isomers<sup>14</sup> was observed.

Radioactivity measurements were made using a Packard 'Tricarb' model 314 EX liquid scintillation counter at -10° in the scintillator described by Patrick<sup>16</sup> except that it was found necessary to include dioxane (10

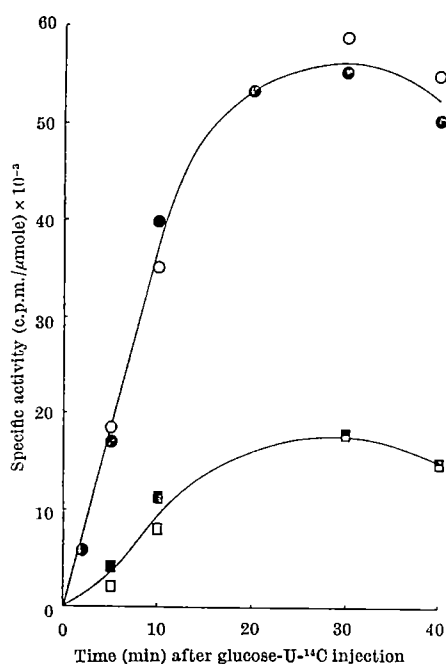


Fig. 1. Uptake of carbon-14 from circulating blood glucose-U-<sup>14</sup>C into keto-acids isolated from rat tissues. Each point is the average of 4-6 experiments. Brain keto-acids: pyruvate (●), α-oxoglutarate (○); liver keto-acids: pyruvate (■), α-oxoglutarate (□)

per cent by volume). The absolute efficiency was 70 per cent and no quench correction was found to be necessary for dinitrophenylhydrazones up to 15 μg in 10 ml. scintillator. Glucose, after isolation by paper chromatography<sup>17</sup>, was estimated with glucose oxidase<sup>18</sup> and counted in the Packard using the scintillator described by Bruno and Christian<sup>19</sup>. In contrast to their report, water did quench—the efficiencies observed for standard <sup>14</sup>C-toluene were: in 10 ml. scintillator, 65 per cent; with 0.5 ml. H<sub>2</sub>O, 62.5 per cent; with 1.0 ml. H<sub>2</sub>O, 60 per cent. Samples of 0.5 ml. were counted and the 62.5 per cent efficiency was extrapolated to 70 per cent for comparison with the keto-acids.

The incorporation of the carbon-14 from glucose into the keto-acids is shown in Fig. 1, where the specific activities (c.p.m./μmole) are plotted against the time (min) after injection of <sup>14</sup>C-glucose. It can be seen that whereas there is no difference in the carbon-14 content of the individual keto-acids from the same tissue, the brain keto-acids are labelled more rapidly and attain a higher proportion of the carbon-14 from the circulating glucose than do the keto-acids in the liver—in a pattern similar to that reported for the amino-acids<sup>5,6</sup>. Whereas some variation was observed in the specific activities of intermediates isolated from identically-treated individual animals, this variation was observed also in the labelling of the circulating glucose, and the relative specific activities (c.p.m./μmole keto-acid versus c.p.m./μmole blood glucose) were quite consistent, especially in the longer time experiments as shown in Table 1. At 30 min

Table 1. INCORPORATION OF CARBON-14 INTO KETO-ACIDS ISOLATED FROM THE BRAIN AND LIVER OF RATS AFTER INJECTION WITH GLUCOSE-U-<sup>14</sup>C

Time (min) after injection	R.S.A. Brain keto-acids		R.S.A. Liver keto-acids	
	Pyruvate	α-Oxoglutarate	Pyruvate	α-Oxoglutarate
2	4(4) ± 0.4			
5	10(6) ± 0.3	12(4) ± 1.2	3(4) ± 0.4	2(4) ± 0.2
10	16(6) ± 1.1	13(4) ± 1.4	4.5(4) ± 0.9	3(4) ± 0.1
20	13(2)			
30	14(6) ± 0.5	14(6) ± 0.4	4(6) ± 0.03	3.5(6) ± 0.02
			(P < 0.001)	
40	12(2)		4(2)	

Male rats were injected subcutaneously with 15 μC glucose-U-<sup>14</sup>C, and the keto-acids extracted and isolated as described in the text. The results are expressed as relative specific activities (R.S.A.).

R.S.A. =  $\frac{\text{counts/min/μmole free keto-acid}}{\text{counts/min/μmole blood glucose}} \times 100$  (number of animals) ± S.E.

after injection, the relative specific activity of the brain pyruvate or α-oxoglutarate is 3-4 times higher than that of the liver acids, the difference being highly significant ( $P < 0.001$ ).

These results suggest that the rapid labelling of the amino-acid pool in brain is a reflexion of a rapid general utilization of glucose, since the brain keto-acids are also more highly labelled. They support the view<sup>3</sup> that more active glycolysis and rapid isotopic exchange by transamination between α-keto-acids and amino-acids can adequately account for the rapid incorporation of carbon-14 from glucose into glutamate.

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## Growth Hormone Action on Rat Liver RNA Polymerase

HYPOPHYSECTOMY of the adult rat results in a loss of weight and protein content of the liver and other tissues<sup>1</sup>. These effects can be reversed by treatment of the rat with growth hormone. This control by growth hormone of protein biosynthesis is reflected in alterations in the ability of ribosomal preparations of liver and other tissues to incorporate amino-acids into protein *in vitro*<sup>2</sup>. The hormonal control of protein synthesis can be at least partially explained in terms of hormonal control of the rate of synthesis of messenger and other RNA<sup>3,4</sup>.

One possible point at which growth hormone could exert its effect on RNA synthesis is through the activity of the RNA polymerase enzyme<sup>5</sup> (*E.C.2.7.7.6*). The effect of growth hormone on the activity of this enzyme was, therefore, investigated.

Rats were killed by decapitation and the liver rapidly removed into ice-cold 0.5 M sucrose containing 3 mM magnesium chloride, cut up with scissors, and washed free of blood. The liver was homogenized in the same medium, filtered through gauze and a crude nuclear pellet prepared by centrifugation at 600g for 5 min. The crude pellet was suspended once in the sucrose-magnesium chloride medium and recentrifuged at 600g. The pellet was suspended in 2.2 M sucrose<sup>6</sup> and centrifuged at 22,000 r.p.m. for 1 h in the SW 25 rotor of the Spinco model L centrifuge. The pellet produced by this procedure was free of red blood cells and contained very few whole liver cells on observation by light microscopy. These purified

nuclei were suspended in 0.05 M *tris* buffer, pH 8. The protein/DNA ratio of the nuclei was about 4.5/1 and there were no significant differences in this ratio between the nuclei prepared from differently treated rats. DNA was determined by the method of Burton<sup>7</sup>, and protein by the method of Lowry *et al.*<sup>8</sup>, using thymus DNA and bovine serum albumin as standards.

The RNA polymerase activity of the nuclei was assayed by a slight modification of the method of Goldberg<sup>9</sup>. The reaction was started by adding nuclei to 0.5 ml. of a mixture containing 100 mM *tris* buffer pH 8.2, 5.0 mM mercaptoethanol, 20 mM sodium fluoride, 3 mM manganese chloride, 60 mM potassium chloride and 0.5 mM each of ATP, GTP, CTP and UTP. One of these nucleoside triphosphates was labelled with <sup>14</sup>C or <sup>3</sup>H. After addition of the nuclei the tubes were incubated at 37° for varying periods and the reaction stopped by the addition of 1 ml. of saturated sodium pyrophosphate solution. 1 mg of bovine serum albumin carrier was added followed by 5 ml. of cold 0.5 M perchloric acid to precipitate the protein and nucleic acids. The precipitate was washed twice with cold perchloric acid, twice with ethanol and twice with ethanol: ether (1:1). The dried precipitate was either dissolved in formic acid and plated on to weighed metal planchets and the radioactivity assayed in a gas-flow counter, or the dried material was dissolved in hyamine and counted in a scintillation counter. The results, expressed as  $\mu$ M of labelled nucleoside triphosphate incorporated per mg of DNA added have been corrected for zero time values. The latter were less than ten c.p.m.

Table 1. CHARACTERISTICS OF RNA POLYMERASE ASSAYED IN RAT LIVER NUCLEI

	$\mu$ M <sup>14</sup> C-ATP incorporated/mg DNA 20 min incubation	60 min incubation
Complete medium	488	450
Omit GTP	40	—
Omit UTP	45	—
Omit GTP	100	—
Add 100% DNAase	55	—
Add 50% actinomycin	33	—
Add 0.05 ml. satd. ammonium sulphate	2,680	6,400

The results, depicted in Table 1, show that incorporation is dependent on the addition of all four of the nucleoside triphosphates and on the presence of native DNA. Incorporation is greatly stimulated by a medium of high ionic strength as was previously noted<sup>9</sup> for the system from HeLa cells. Maximal stimulation on raising the ionic strength of the medium was obtained by the addition of 0.05 ml. of saturated ammonium sulphate (saturated at 4° C). It was noted that incorporation is complete in 15 min if ammonium sulphate is not added, but in the presence of high concentration of ammonium sulphate incorporation continued for at least 90 min.

The effect of growth hormone on the activity of the RNA polymerase measured in this system is shown in Table 2. It can be seen that hypophysectomy of the rat results in decreased activity of the RNA polymerase and that the injection of 1 mg of human growth hormone into the rat 12 h before death increased the enzyme activity both in hypophysectomized rats and in normal rats. However, the stimulating effect of growth hormone was only seen in the absence of high concentrations of ammon-

ium sulphate; raising the ionic strength of the assay medium abolished the growth hormone effect. It should be mentioned that addition of growth hormone *in vitro* in the presence or absence of ammonium sulphate has had no significant effect on the labelling of the RNA by the nuclear preparations.

Gorski<sup>10</sup> found that oestrogen treatment of rats resulted in an increase of RNA polymerase activity of uterus but that the presence of high concentrations of ammonium sulphate abolished the oestrogen effect.

Widnell and Tata<sup>11</sup> have shown that thyroid hormone treatment of rats resulted in an increase in liver RNA polymerase activity after about 16 h. They also showed<sup>12</sup> that growth hormone stimulated RNA polymerase activity and that the effects of the two hormones were additive. Their investigations<sup>13</sup> led them to conclude that the action of ammonium sulphate could be explained by unmasking of a second DNA-dependent RNA polymerase activity. An alternative explanation<sup>9</sup>, which our own results favour, is that raising the ionic strength increases the incorporation of nucleoside triphosphates into RNA because of some activation of the DNA-RNA polymerase enzyme complex. If this were the case it would be possible to argue that the action of growth hormone *in vivo* is similar to that of high concentrations of ammonium sulphate *in vitro*; that is to say, that growth hormone acts by facilitating the formation of the DNA-enzyme complex, or perhaps the movement of the enzyme relative to the DNA template. This possible mechanism of growth hormone action and other possibilities are being investigated.

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### Blood Content of Rat Liver Homogenates

EVEN though the presence of blood in tissue homogenates is quite evident, its implication in the interpretation of results is generally ignored. In work on rat liver tissue, this attitude results from an under-estimation of the blood content by earlier investigators<sup>1-4</sup>. In order to gauge to what extent blood 'contamination' must be taken into account, a very accurate determination of blood content of homogenates is essential. A simple and accurate method, free from the errors inherent in procedures used so far, is described here. It consists in (1) complete extraction of haemoglobin from liver with sodium deoxycholate (DOC) followed by a specific spectrophotometric determination of haemoglobin; (2) evaluation of blood content in homogenates by comparing it with the blood from the same animal.

Table 2. GROWTH HORMONE TREATMENT OF RATS ON LIVER NUCLEAR RNA POLYMERASE ACTIVITY

	$\mu$ M <sup>14</sup> C-GTP incorporated/mg DNA No A.S.*	+0.05 ml. satd. A.S.
Normal rats	326	1,920
Normal rats given 1 mg human growth hormones 12 h previously	700	1,910
Hypophysectomized rats	218	1,880
Hypophysectomized rats treated with growth hormone	392	1,800

\* A.S., ammonium sulphate.



Determinations were made on 2 groups of rats (Wistar sub-strain *WAG*, fasted, adult males). In group *A*, livers from rats anaesthetized with ether were used; in group *B*, livers were removed after decapitating and bleeding the rat. After ligaturing the median lobe, the portal vein was severed and the liver was quickly perfused with one of several isotonic solutions through the inferior vena cava. The liver was removed and a 2.5 per cent homogenate was prepared by homogenizing separately the perfused and non-perfused (median) lobes, in physiological salt solution containing 0.5 per cent (w/v) DOC. The median lobe represents the whole tissue (*W*) the blood volume of which is to be evaluated, and the perfused lobes (*P*) are used as the control tissue. Whole blood samples were obtained, in *A*, by cardiac puncture after the median lobe had been ligatured and, in *B*, by collecting after decapitation. The homogenates were centrifuged at  $150,000g_{av}$  during 30 min. The film formed on the surface was discarded and the clear supernatant was collected. Using the extract from *P* as blank, the optical density of the extract from *W* was measured at 542 m $\mu$ . Simultaneously the optical density determinations of the blood samples, after 200-fold dilution with 0.5 per cent DOC, were also made.

The values obtained for blood content are given in Table 1: an average value of  $21.09 \pm 2.38$  (*S.D.*) as g blood/100 g tissue for non-exsanguinated and one of  $8.60 \pm 1.67$  (*S.D.*) for exsanguinated rats were obtained. These results call for three remarks: (1) results achieved by Gibson *et al.*<sup>5</sup> permit us to correlate the haemoglobin concentration of blood in the hepatic vessels with that of the total circulating blood, since, according to these authors, both haematocrit values are the same, which, however, is not the case with all organs; (2) variations in the values in *B* are greater than in *A*; this could evidently be attributed to the unequal extent of bleeding in the different animals after decapitation; (3) these values are much greater than the different values reported: 3.7–5.2 per cent<sup>4</sup> for non-exsanguinated and 2.3–6 per cent<sup>1</sup>, 1.5–5.2 per cent<sup>2</sup>, 1.86 per cent<sup>3</sup> for exsanguinated rats.

Table 1. BLOOD CONTENT IN RAT LIVER HOMOGENATES

	Exp. No.	Optical density at 542 m $\mu$		Blood content of homogenate	
		0.5% blood in 0.5% DOC	2.5% homogenate in 0.5% DOC	In ml./100 g liver *	In g/100 g liver †
Nonexsanguinated animals	1	0.522	0.459	17.52	18.48
	2	0.589	0.511	17.36	18.30
	3	0.486	0.526	21.64	22.81
	4	0.535	0.506	22.38	23.49
	5	0.576	0.632	21.92	23.11
	6	0.469	0.452	19.27	20.30
Exsanguinated animals	11	0.594	0.227	7.65	8.07
	12	0.580	0.216	7.45	7.86
	13	0.590	0.217	7.36	7.76
	14	0.559	0.287	10.26	11.83
	15	0.550	0.264	9.60	10.12
	16	0.558	0.157	5.62	5.94
	17	0.485	0.182	7.51	7.91
	18	0.538	0.264	9.82	10.35

\* Values calculated by the following formula:

$$\frac{\text{optical density of 2.5\% homogenate} \times 100}{\text{optical density of 0.5\% blood} \times 5}$$

† Specific gravity of rat blood, 1.054.

The main sources of possible error have been totally eliminated in our experiments. That there is no adsorption and that the extraction of haemoglobin from DOC-treated tissue is quantitative is shown, for each animal, by the experiments where known and increasing amounts of blood were added to homogenates from *P*. In Fig. 1, the optical densities at 542 m $\mu$  of the DOC extracts were plotted against the concentration of blood in the homogenates. The resulting straight line intersects the origin, thereby indicating a total absence of haemoglobin adsorption on the cell components. Further, the average optical density of the extract from *P* containing 0.5 per cent blood has been found, for 14 individual experiments, to be

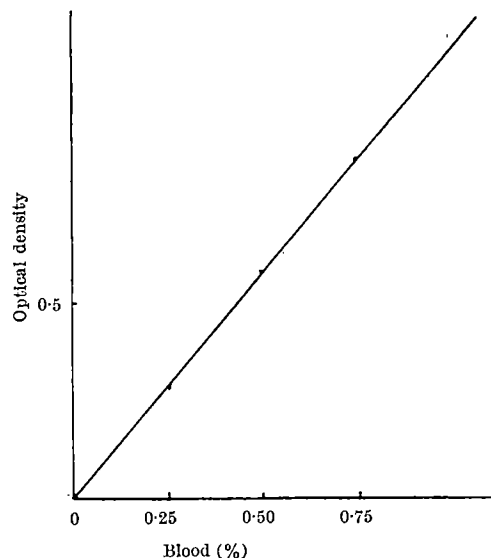


Fig. 1. Relative concentration of added blood recovered from the perfused tissue homogenates. Optical density of supernatants obtained from tissue treated with 0.5 per cent DOC, measured using similar supernatants from perfused tissue as blanks

identical with that of DOC extract of 0.5 per cent blood alone ( $0.545 \pm 0.042$ ). (Adsorption could explain low values obtained by Holzer<sup>1</sup> and Pragay's<sup>2</sup> techniques.) Error due to turbidity is avoided by preparing homogenates in saline solution, thereby permitting sedimentation of residual cell components. Interference caused by the coloured substances of the liver (bile, pigments, cytochromes, flavins, etc. . . .) is overcome by using supernatants from perfused liver as the blank; the absorption spectra 2 and 3 in Fig. 2 of the DOC-treated blood and the homogenate are, indeed, identical.

Regarding the method of estimation of haemoglobin, convincing evidence could be obtained to suggest that it accounts for the total haemoglobin content of the blood: (1) Although the quantity of reduced haemoglobin is not appreciable in our blood preparations, as could be expected, all determinations were made at 542 m $\mu$ , a wave-length at which the extinction coefficients of haemoglobin and oxyhaemoglobin are equal<sup>6</sup> and, therefore, effects due to any possible variation in the oxyhaemoglobin/haemoglobin ratios are abolished. This should also make it possible to have a rough estimate by directly comparing the absorption of the supernatants from tissue homogenates to the average extinction coefficient of rat blood:

$$\left( E_{1 \text{ cm}, 542}^{0.5\%} = 0.545 \right)$$

(2) The absolute similarity in the spectrum of blood treated and not treated with DOC shows the absence of denaturation of oxyhaemoglobin by DOC.

This method of haemoglobin determination could be applied to all tissues that could be perfused. From the results obtained in these investigations it could be established that the amount of blood present in liver homogenates is not negligible, in whatever manner the animal may be killed. It follows that allowances have to be made for blood contents as a rule or, better still, that investigations have to be carried out on perfused livers. In the results expressed per unit weight of tissue or per unit weight of nitrogen, errors are only appreciable in the case of substances the activity or concentration of which is different in blood and in tissue. This permits us to assume that the values are inaccurate for the determination of concentrations of glucose-6-phosphate, 3-phosphoglycerate, adenosine di- and tri-phosphate malate, etc.<sup>7</sup>,

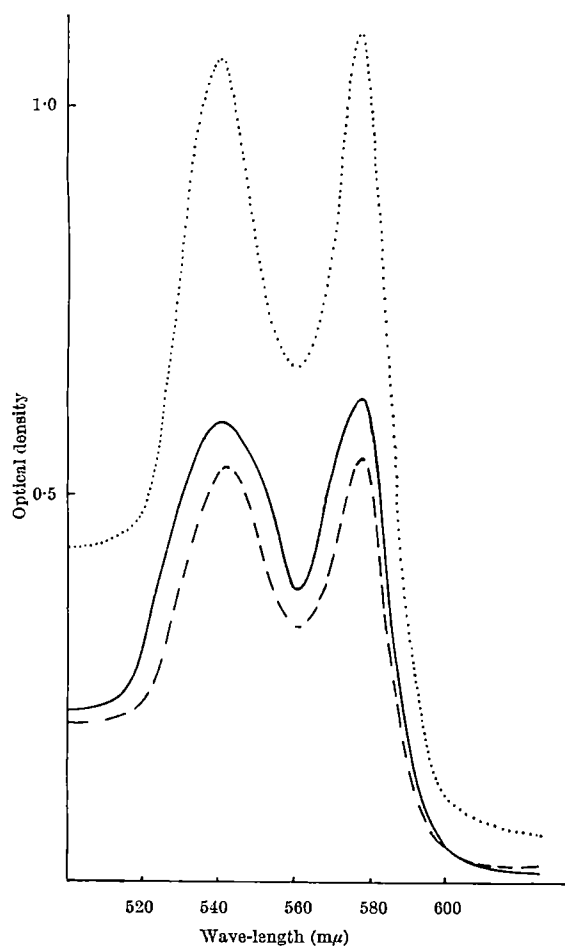


Fig. 2. Absorption curves for blood and rat liver supernatants. ··· 0.1 per cent blood; --- 0.5 per cent blood in 0.5 per cent DOC; — 2.5 per cent tissue homogenate treated with 0.5 per cent DOC

or the enzyme content in adenosine deaminase, ribonuclease, cholinesterase, acid phosphatase, aldolase, etc.<sup>8</sup>, in liver tissues. In so far as values are expressed in terms of DNA, blood content in DNA not being appreciable, the error is greater and this holds for all substances which are to be found in blood.

Finally, it should be emphasized that contamination by blood particularly affects analyses of both nuclei and cell sap isolated by differential centrifugation. Corpuscles, indeed, only contaminate the former, whereas plasma and what haemoglobin has been freed contaminate the latter. This introduces errors to the extent of 20 per cent in the nitrogen determination, for example, on the liver cell sap obtained from exsanguinated rats, and more than 40 per cent on the cell sap obtained from non-exsanguinated rats.

These artefacts occur in all organs, such as kidney, lung, spleen, where there is an intense blood supply.

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### Effect of Curare on the Incorporation of <sup>32</sup>P-Orthophosphate in Rat Gastrocnemius Muscle

SOME biochemical changes in mitochondria isolated from pigeon breast muscle after section of motor nerve have been reported<sup>1</sup>, and the turnover of high-energy phosphate was markedly slowed after nerve section (Cessi *et al.*<sup>2</sup>). These reports suggest that some regressive biochemical changes of the main energy-producing cell structures are brought about by denervation.

Since the discovery of the effect of curare in inducing muscle paralysis, much work has been done on its structure and pharmacological actions<sup>3</sup>. It is known to antagonize acetylcholine, which is believed to transmit nerve impulses from a motor nerve to skeletal muscle. A protein which binds curare, acetylcholine and related compounds has been isolated from electric organs<sup>4</sup> and was identified as the receptor protein. Curare is said to combine with this receptor protein, on which acetylcholine normally acts to initiate the propagation of nerve impulse; it therefore blocks the motor terminals, causing the failure of contraction after stimulation. In this sense, curarization might be regarded as a sort of chemical denervation. The aim of these experiments was to observe whether some metabolic changes, especially in the turnover of high-energy phosphates, could be induced by blocking the neuromuscular transmission with *d*-tubocurarine.

Albino rats (weighing approximately 300 g) were injected with *d*-tubocurarine (400 μg/kg) after inserting a metal cannula into the trachea for artificial respiration by a respirator under pentobarbital sodium anaesthesia (80 mg/kg). To check the degree of curarization, an electrode was connected with the sciatic nerve and electrical stimulus was given by a stimulator. Muscle reactivity was recorded on a kymograph from the time when curare was injected. When the kymograph showed the reappearance of muscle contraction, another dose of curare was injected, maintaining the fully curarized state continuously.

Two hours after the curare injection, <sup>32</sup>P-orthophosphate (1 mc./kg) was injected intraperitoneally. Two hours after the radioactive phosphorus injection, gastrocnemius muscle was rapidly removed from the animal and an extract was made with 0.6 M perchloric acid according to the method described by Buffa *et al.*<sup>5</sup>. The extract was neutralized with potassium hydroxide and, after separation of potassium perchlorate, introduced into a column of 'Amberlite IRA-400', formate form, 400 mesh, 1 × 18 cm. The elution was carried out with the formic acid-formate system described by Hurlbert *et al.*<sup>6</sup>, with slight modifications. The radioactivity and the extinction at 260 mμ of the effluent were measured continuously and recorded. 3-ml. fractions were collected and the phosphorylated compounds identified by the ultra-violet spectrum were measured on a Beckman DU spectrophotometer. The specific activities of labile phosphates were determined by measuring the concentration of phosphorus after hydrolysis (18 min, 2 N sulphuric acid, 100° C) with the method of Martin and Doty<sup>7</sup> and the radioactivity of the same butanolic extract was measured with a well-type Geiger-Müller counter. The control group was treated in the same way, except for the curare injection.

The incorporation of <sup>32</sup>P-orthophosphate into the organophosphate was considerably inhibited after 4 h of continuous curarization (Table 1). The inhibitory effect of curare might be due to an inhibition of phosphorylation mechanism, or to an interference with phosphate entry, or transport to the muscle cell. In physically denervated muscle, Cessi *et al.*<sup>2</sup> reported lower specific activity of the phosphate involved in energy transformation and suggested that this may simply reflect a decreased demand for ATP in the paralysed muscle. It is also quite possible to suppose that the metabolism of muscle in completely relaxed state by curare could be diminished and the

Table 1. EFFECT OF CURARE ON THE RATE OF  $^{32}\text{P}$ -ORTHOPHOSPHATE INCORPORATION. VALUES ARE PRESENTED AS THE SPECIFIC ACTIVITY OF TISSUE INORGANIC PHOSPHORUS BEING TAKEN AS 100, AND AS THE MEAN OF THREE EXPERIMENTS

	Specific activities relative to inorganic P of tissue	
	Control	Curarized
ATP/IP	55.3 $\pm$ 3.6	39.2 $\pm$ 3.8
ADP/IP	44.1 $\pm$ 2.9	30.4 $\pm$ 1.2
PCP/IP	73.5 $\pm$ 9.2	51.3 $\pm$ 10.1
BP/IP	814.2 $\pm$ 17.1	1,641.9 $\pm$ 52.9

ATP, ADP, PCP, BP, IP: Specific activities of adenosine triphosphate, adenosine diphosphate, phosphocreatine phosphate, blood inorganic phosphorus, tissue inorganic phosphorus, respectively.

Table 2. CONCENTRATION OF ADENINE NUCLEOTIDES IN CONTROL AND CURARIZED GROUP

	Micro moles of adenine nucleotides per g of fresh tissue	
	Control	Curarized
AMP	0.54 $\pm$ 0.14	0.58 $\pm$ 0.14
ADP	1.02 $\pm$ 0.33	0.83 $\pm$ 0.34
ATP	4.42 $\pm$ 0.72	3.44 $\pm$ 0.84

requirement of energy would also be decreased as an example of self-regulation or feedback in a biological system. The little differences in the concentration of adenine phosphates seem to support this view. However, the marked difference in the ratio of specific activity of blood phosphate to inorganic phosphorus of tissue in curarized muscle suggests that another factor is responsible for the phosphate entry or transport. As the detailed mechanism of the efficient cellular organization for fast resynthesis and recycling of the nucleotide is still obscure, it remains to be established whether some possible changes in the membrane permeability by curare-receptor complexes have some effect on this slowing down of high energy phosphates.

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## Two-dimensional Separation of Nucleic Acid Bases on Cellulose Layers

THIN-LAYER chromatography on cellulose layers has been shown to be an effective method for separation and quantitative determination of nucleic acid bases, nucleosides and nucleotides<sup>1-5</sup> which has considerable advantages over paper chromatography. Keck and Hagen<sup>5</sup> recently reported a one-dimensional thin-layer chromatographic separation of the four major bases of RNA and DNA. The present communication describes a two-dimensional procedure which in this laboratory has proved useful for separating more complex mixtures of nucleic acid bases.

**Preparation of cellulose layers.** 20 g cellulose powder MN 300 (Macherey and Nagel, Düren, Germany) are suspended in 120–130 ml. distilled water, homogenized in a Waring blender for 15–20 sec, and coated on degreased glass plates in the usual way, using a Stahl-type applicator<sup>6</sup> adjusted to a slit width of 0.5 mm (Desaga, Heidelberg, Germany). The plates are allowed to dry at room temperature for 10–15 h.

**Chromatographic procedure.** Chromatography is carried out in closed rectangular jars filled with solvent to a height of about 0.8 cm. It is not necessary to saturate the tank atmosphere with solvent vapours. Solvents used are: (1) methanol/concentrated hydrochloric acid/water (70:20:10, v/v), for the first dimension; (2) *n*-butanol/

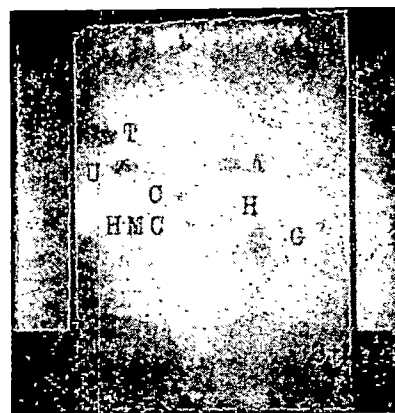


Fig. 1. Two-dimensional separation of nucleic acid bases on a 0.5 mm-cellulose layer. A solution of 0.5–1.5  $\mu\text{g}$  of each compound was applied in ten 1  $\mu\text{l}$ . portions to the start spot. Solvents: see text. First dimension from right to left, second dimension from bottom to top. Development distances: 10 cm in either direction. The black areas of the photograph are parts of the layer which were removed before the second development. Total development time: about 150 min. T=thymine; U=uracil; C=cytosine; HMC=5-hydroxy-methyl cytosine; A=adenine; H=hypoxanthine; G=guanine. (Photography in short-wave ultra-violet light)

methanol/water/concentrated ammonium hydroxide (60:20:20, v/v), for the second dimension. The plate is dried between the two developments for 3–5 min in a current of cold air, then for 5 min in a current of hot air (60°–70° C) in order to remove hydrochloric acid as completely as possible.

Fig. 1 shows a separation of a mixture of bases. The main advantages of this method are its speed, sensitivity and sharpness of resolution.

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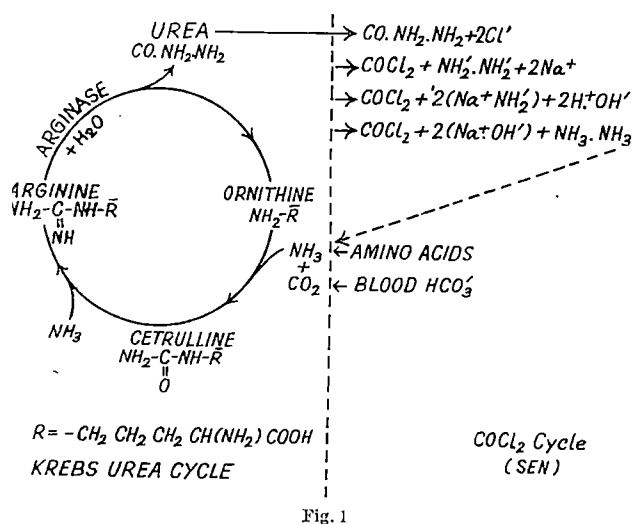
## Carbonyl Chloride Cycle in Uraemia

THE role of carbonyl chloride in the production of uraemic coma has been emphasized in previous publications<sup>1,2</sup>. It has been further demonstrated that urea reacting with ammonium chloride *in vivo* produces carbonyl chloride with the liberation of ammonia. In view of the enormous number of cases of uraemia already handled by the author it appears that a plausible suggestion with regard to the biochemical steps involved in the production of carbonyl chloride and associated compounds is called for.

Urea is formed in the liver in the cycle described by Krebs<sup>3</sup>, involving the mediation of arginase. In the production of uraemic coma the conversion of urea to carbonyl chloride is involved<sup>1</sup>. The intermediate steps may be outlined as follows:

In the first stage of the reaction one molecule of urea reacts with two molecules of free chlorine ( $\text{Cl}^{\cdot}$ ) to produce carbonyl chloride and  $2\text{NH}_2$ . Further reaction with two molecules of free sodium ( $\text{Na}^+$ ) results in two molecules of sodium amide in addition to the carbonyl chloride already existing. Finally, reaction with two molecules of water yields free ammonia and two molecules of sodium





hydroxide. The free  $\text{NH}_3$  thus formed enters again into Krebs urea cycle.

The entire reaction of the  $\text{COCl}_2$  cycle as suggested above is diagrammatically represented in Fig. 1.

The mode of reaction suggested seems to be consistent with the known facts, and it probably represents the true state of affairs in the production of  $\text{COCl}_2$ .

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### Photoreceptive Pigments for Anthocyanin Synthesis in Apple Skin

LIGHT is required for anthocyanin synthesis in many plant tissues. Appreciable synthesis of idaein (cyanidin 3-galactoside) in the apple skin,  $1.0 \times 10^{-5}$  moles/cm<sup>2</sup> of skin, requires 7 joules/cm<sup>2</sup> incident energy at the action maximum near 650 m $\mu$  and about three times as much energy in the region of the subsidiary maximum between 430 and 480 m $\mu$  (ref. 1). This high-energy photoreaction, which will later be referred to as the HER, usually must be followed by a photoreaction establishing the photomorphogenic pigment, phytochrome, in the far-red absorbing form ( $P_{fr}$ ) which requires about  $3 \times 10^{-3}$  J/cm<sup>2</sup> for half saturation at the action maximum near 655 m $\mu$  (ref. 2). A number of photomorphogenic responses of plants including control of flowering and etiolation suppression, but not seed germination, involve both a high-energy reaction and the action of  $P_{fr}$  (ref. 3). The photoreceptive pigment has not previously been identified for the HER in apple skin or other objects and  $P_{fr}$  has not been shown to be necessary for idaein synthesis.

Our findings indicate that the photoreceptor for the HER in apple skin is the photosynthetic system. The maximum near 650 m $\mu$  in the action spectrum for the anthocyanin formation<sup>1</sup> corresponds to the red absorption maximum of chlorophyll *b*. A broad subsidiary maximum between 420 and 470 m $\mu$  is in the region of a broad maximum effective for excitation of system 2 for photosynthesis. The apple skin contains chlorophyll in the hypodermal cells in which anthocyanin synthesis takes place. Low-temperature ( $-196^\circ\text{C}$ ) spectroscopy of the apple skin revealed the presence of chlorophyll-*b*, chlorophyll-*a* (abs. max. 670 m $\mu$ ), chlorophyll-*a* (abs. max. 680 m $\mu$ ), and  $P_{700}$  in ratios approximately those of green plants. Photosynthesis is weakly displayed. Apple skin

scraped free of underlying tissue, under an irradiation of 3.5 mW/cm<sup>2</sup> (the order of  $1 \times 10^{-6}$  Einsteins/cm<sup>2</sup> min) in the region of 400–680 m $\mu$  (illumination of 1,000 ft. candles with a white fluorescent source), gave an oxygen evolution of  $4 \times 10^{-8}$  moles/cm<sup>2</sup> min, which was about twice the oxygen consumption in darkness. The rate of photosynthesis relative to respiration, however, varied between samples of skin and some did not reach compensation under such conditions.

Chlorophyll fluorescence from scraped apple skin was measured to determine if both photosynthetic pigment systems 1 and 2 (ref. 4) were functional. One manifestation of the dual pigment system relating directly to the photosynthetic enhancement phenomenon in green plants is the change in fluorescent yield of chlorophyll-*a* which accompanies supplementary radiation in the red and far-red regions<sup>4,5</sup>. Measurements on apple skin showed a 30 per cent greater fluorescent yield following supplementary red radiation as compared with far-red radiation. These measurements indicated that the dual pigments of photosynthesis and the associated electron transport chain were functioning. The light-induced changes in fluorescence were inhibited by more than 75 per cent by  $2 \times 10^{-6}$  M DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea). This reagent is known to abolish the fluorescent yield changes by blocking the photosynthetic electron transport chain<sup>4,5</sup>.

The rate of anthocyanin synthesis in Arkansas apple skin floating on 10 per cent sucrose solution, after an induction period, varies linearly with intensity above 0.3 mW/cm<sup>2</sup> irradiation in the region of 600–680 m $\mu$ . A concentration of  $1.0 \times 10^{-6}$  M DCMU inhibited the rate of synthesis in the linear region by 50 per cent, which was also the degree of inhibition of oxygen evolution. The proportional change in photosynthesis and anthocyanin formation was closely similar for several DCMU concentrations leading to inhibitions between 0 and 100 per cent (Fig. 1).

The rate of anthocyanin synthesis in the skin of mature apples held for three months in storage was enhanced about two-fold by sucrose. It is evident that sucrose, either stored or supplied, and other substrates which were partially endogenous, rather than direct photosynthetically-derived compounds, supply the carbon substrate for the HER. The impression previously held was that anthocyanin synthesis in many green plants is inhibited rather than enhanced by chlorophyll<sup>6</sup>. The observed enhancement of anthocyanin synthesis as chlorophyll

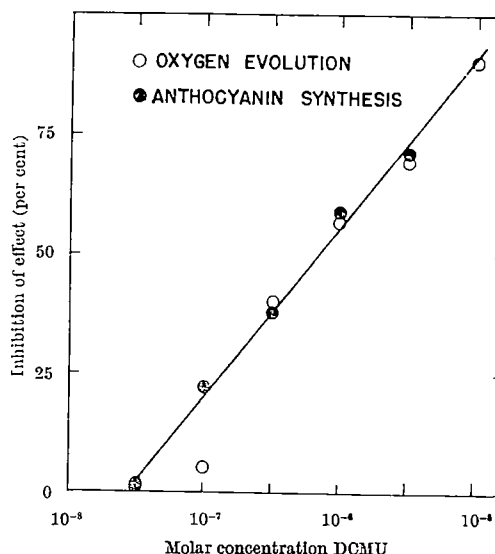


Fig. 1. Inhibition of photosynthesis (oxygen evolution) and anthocyanin synthesis in apple skin by 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU).

decreases probably arises, however, from increased sugar supply. This is strikingly evident in the red colorations of many leaves in autumn and of young growth in the spring when the days are cool.

Apple skin produces ethanol and acetaldehyde in darkness<sup>7</sup>. This production is immediately terminated by adequate radiation with alternative production of idaein. The metabolic reaction in which the HER enters is accordingly considered to be in the region of pyruvate utilization. The chlorophyll system is apparently contributing directly to this reaction on irradiation. The manner of the contribution, however, is unknown and might consist in the supply of an oxidant, a reductant, an energy or phosphate source (adenosine triphosphate) or, less likely, in direct energy transfer. The control might also be effected by a side reaction of the electron transport chain. In the apple skin the action spectrum for anthocyanin synthesis resembles pigment system 2 in having a pronounced contribution by chlorophyll-b, but in other tissues the HER action maxima agree more closely with pigment system 1. Such variability might be expected, but at this juncture it remains to be examined.

Presence of phytochrome in the P<sub>fr</sub> form, which is produced with maximum effectiveness in the region of 600–680 mμ, is necessary for display of the HER in several carefully examined responses<sup>8</sup>. Failure of its detection in others apparently arises from nuances of the P<sub>fr</sub> action. In anthocyanin synthesis in sorghum cultivar 'Wheatland', P<sub>fr</sub> action follows the HER by an average interval of about 4 h, which is the time required for substrates produced by the HER to undergo dark reactions before undergoing enzymatic change by P<sub>fr</sub> (ref. 8). The low effectiveness of anthocyanin synthesis in apple skin dictates long times of irradiation at high intensities for appreciable synthesis. In the apple skin the radiation most effective for HER is also most effective for establishing P<sub>fr</sub>, and periods of irradiation are long compared with 4 h. Both facts tend to obscure P<sub>fr</sub> action. The action, however, was successfully demonstrated. Arkansas apple skins floating on 10 per cent sucrose irradiated for 16 h (3.5 mW/cm<sup>2</sup> in the 400–680 mμ region, 1,000 ft. candles white fluorescent) to overcome an induction period, were placed in darkness for 24 h before an additional 24-h irradiation period (3.5 mW/cm<sup>2</sup>). The skins were then returned to darkness for 24 h at 20° C for completion of idaein synthesis. They contained  $1.70 \times 10^{-8}$  idaein/cm<sup>2</sup>. In other lots of skin, subjected to brief far-red irradiation at the start of the first dark period to convert the phytochrome to the red-absorbing form, the idaein content was reduced to  $1.42 \times 10^{-8}$  M/cm<sup>2</sup>. A second short irradiation of another lot with red light to re-establish P<sub>fr</sub> gave  $1.75 \times 10^{-8}$  M idaein/cm<sup>2</sup>. Maintenance of P<sub>fr</sub> throughout the 24-h period by a continuous red irradiance of 2 μW/cm<sup>2</sup> enhanced the idaein content to  $2.65 \times 10^{-8}$  M/cm<sup>2</sup>. These enhancements are evidence of P<sub>fr</sub> action on the substrate supplied by the HER.

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## PHYSIOLOGY

### Adrenal Medullary Hormones and Sensitization of Mice to Histamine by the Histamine-sensitizing Factor of *Bordetella pertussis*

*Bordetella pertussis* cells, or extracts from them, increase the sensitivity of mice to histamine, serotonin, toxic materials, various stresses and anaphylaxis. This profound effect, observed to different degrees in various strains of mice, is almost entirely duplicated by adrenalectomy<sup>1,2</sup>. For this reason, it has long been suspected that the mode of action of *B. pertussis* involves the adrenal glands either directly or indirectly<sup>1,3</sup>. *B. pertussis*-treated mice can be protected from histamine death by injection of cortisone or hydrocortisone, but treatment with epinephrine 30 min before histamine challenge was not effective. Consequently, it has been assumed that the steroid hormones were the significant factor in the histamine sensitization phenomenon<sup>1,2</sup>. In adrenalectomized mice, it has been demonstrated that both epinephrine and steroids protect mice from histamine death<sup>3</sup>. It should be stressed that no evidence has been uncovered to show that the histamine-sensitizing factor (HSF) extracted from *B. pertussis* cells directly affects the adrenal glands. No histological abnormalities or changes in the amount of steroids produced have been observed<sup>2</sup>. To account for this lack of direct effect on the adrenal gland, it was proposed that the *B. pertussis* effect might be due to an indirect effect at a site removed from the adrenal glands, such as blocking the site of action of an important adrenal hormone.

Fishel *et al.*<sup>4,5</sup> have recently postulated a mechanism involving mainly the adrenal medullary hormones. They have found that substances such as dichloroisoproterenol (DCI) or nethalide, which block the 'beta-adrenergic' receptors, produce effects which mimic the increased susceptibility of *B. pertussis*-treated mice to histamine and serotonin. Their hypothesis is that *B. pertussis* interferes with the beta-adrenergic receptors, thus producing an imbalance between the reactivity of beta and alpha receptors to epinephrine, and they assume that this increased reactivity of the alpha receptors to epinephrine accounts for the greater sensitivity to histamine and serotonin. This hypothesis then assigns an important part to the medullary hormones. However, this view does not explain why adrenalectomized mice show an increased sensitivity to histamine, and suggests that exogenous epinephrine should not protect HSF-treated mice from histamine shock.

This preliminary note substantiates previous work on adrenalectomized mice<sup>6</sup> and rats<sup>6</sup> concerning the involvement of the medullary hormones in the sensitization to histamine and indicates that HSF may block the effect or the site of action of epinephrine and possibly other catechol amines.

Adrenalectomized mice react in many ways like HSF-treated mice. It was recently found that removal of the adrenal medulla alone produced increased histamine sensitivity. A representative experiment is given in Table 1. Demedullated animals became highly sensitive to the injection of histamine. This effect was not due to a degeneration of the whole adrenal since healthy cortex tissue was found on histological examination at 33 days post-operation. The medulla, however, at the time the sections were taken, showed little, if any chromaffin tissue in the mice that died of histamine shock. Medullary tissue was present in the 'demedullated' mice that survived the shock. It was found that as little as 1.25 to 2.50 μg of *l*-epinephrine given intravenously protected demedullated or adrenalectomized mice from an intraperitoneal challenge with 0.5 mg of histamine base (Table 2).

The prime requirement for protecting mice from histamine challenge by epinephrine is that it must be given

Table 1. HISTAMINE SENSITIVITY OF ADRENAL DEMEDULLATED MICE\*

Group	Results D/T†
Demedullated	13/20
Sham operated	1/20

\* CFIV female mice were used. The sensitivity of mice was similar 2 or 33 days after demedullation. The challenge dose of 0.5 mg histamine base (as the diphosphate salt) was given intraperitoneally.

† D/T: deaths/total number of animals.

Table 2. PROTECTION OF ADRENALECTOMIZED AND ADRENAL DEMEDULLATED MICE FROM HISTAMINE SHOCK BY *l*-EPINEPHRINE

Group	Amount of <i>l</i> -epinephrine $\mu$ g/mouse	Results D/T*
Adrenal demedullated	0.0†	7/10
	2.5	0/10
	5.0	0/9
	0.0†	14/15
Adrenalectomized	0.02	9/15
	1.25	3/15
	2.50	2/15
	0.0†	14/15

\* D/T: deaths/total number of animals.

† Received 0.2 ml. of saline instead of epinephrine.

Table 3. PROTECTION OF HSF-TREATED MICE FROM FATAL HISTAMINE TOXICITY WITH *l*-EPINEPHRINE\*

Treatment $\mu$ g <i>l</i> -epinephrine/mouse	Results D/T†
0.0	10/15
2.5	7/15
5.0	9/15
7.5	4/15

\* CFIV 6-week-old mice were given either 5 or 10  $\mu$ g HSF i.v. 24 h before challenge with 0.5 mg histamine base given i.p. Treatment with *l*-epinephrine was given i.v. one-half min after challenge. Control mice received saline instead of *l*-epinephrine.

† D/T: deaths/total number of animals.

shortly after the histamine challenge. Although it is known that large doses of relatively insoluble steroids (4 mg of cortisone or hydrocortisone) given intraperitoneally 24 h before histamine shock protect mice, smaller and more nearly physiological doses apparently do not protect them. In the present work, 50  $\mu$ g/mouse of corticosterone failed to protect adrenalectomized mice from histamine shock, when the steroid was given from 24 h to 30 min before challenge. Moreover, no demonstrable synergistic or additive effect between the steroid and *l*-epinephrine was shown.

These results indicate that, if adrenal hormones are indeed involved in the activity of HSF, the process might mainly be through interference with the action of medullary hormones, as suggested by Fishel *et al.*<sup>4,5</sup>. If the mode of action of *B. pertussis* is that of blocking adrenergic receptors, it should be more difficult to protect *B. pertussis* mice with catechol amines such as *l*-epinephrine. Preliminary results indicate that this is actually the case (Table 3). If the mechanism of HSF can be assumed to be a blocking of receptor sites in the effector organs, this blockage must be quite irreversible since it lasts for a long period of time (3 weeks or more) and cannot easily be overcome by an excess of catechol amines.

As indicated earlier, the hypothesis of Fishel *et al.*<sup>4,5</sup> proposes that *B. pertussis* interferes with the beta-adrenergic receptors, thus producing an imbalance between the alpha- and beta-adrenergic receptors to the action of epinephrine. This imbalance is responsible for the increase of sensitivity to histamine and serotonin. In other words, the assumption is made that the alpha receptors become hyper-reactive to the action of epinephrine. Since, in our hands, epinephrine protected mice from histamine death, one wonders whether the interpretation given by Fishel and co-workers is entirely correct. Their hypothesis, however, has merit and should be thoroughly tested. The production of histamine sensitization by beta-adrenergic blocking agents, such as DCI and nethalide, has been confirmed in our laboratories. These agents, however, act only for a few minutes while the *pertussis* effect is long-lasting (3 weeks or longer). This investigation of the part played by medullary hormones indicates that catechol amines might be the most important hormones involved in protecting mice against histamine and that *B. pertussis* or the HSF

extracted from these cells may act by interfering with the action of these hormones. If the active substance of *B. pertussis* actually blocks some adrenergic receptors, it may prove to be an extremely powerful drug with possible practical applications. A more detailed report of these findings will be published later.

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### Effect of Trypsin on Resting Potential of Frog Muscle

PAST work on the exposure of cells to protease has indicated either that the proteins on the outer surface of the cell do not contribute to the physiological properties of the membrane or that they are not available to enzymatic action. The external application of proteases to squid, lobster, or frog axons does not seem to alter the resting potential or the excitability of these cells<sup>1-4</sup>. Erythrocytes exposed to trypsin for up to 24 h show no change in permeability compared with normal cells<sup>5</sup>. However, external application of protease has been shown to produce injury potentials in muscle<sup>2</sup>.

In the work recorded here the effect of trypsin on the resting potential of single fibres of the sartorius muscle of the frog, *Rana pipiens*, was investigated. This system was chosen in order to examine the effects of protease on the electrical properties of single cells which are not surrounded by a protective cell layer, such as exists around nerve fibres. The resting potentials were measured with a standard electrophysiological recording set-up and an intracellular micropipette, the resistance of which varied from 10 to 15 M $\Omega$ . Recordings were made from fibres on the underside of the fibre bundle.

The experimental muscles were exposed to a trypsin solution (1.0 mg/ml. in frog Ringer's solution; pH 7.0-7.2) for 25 min and then washed for 5 min in normal Ringer's solution. Control muscles were left in Ringer's solution without trypsin for the same period of time. The resting potentials were recorded from 25 fibres in each muscle. Only one muscle was used from each frog in order to allow all measurements to be completed within 50 min after the frog was killed. Measurements of the resting potentials before and after treatment with trypsin were not made on the same fibres, due to the frequent occurrence of spontaneous contractions while in the trypsin solution. The trypsin solutions were made up just before their use. All experiments were carried out in August and September. The ambient temperature was 23°  $\pm$  1° C for both the treated and non-treated groups.

The results are shown in Fig. 1. The resting potentials of 100 untreated fibres ranged from 20 to 90 mV. The mean potential was 78 mV, the median 80 mV, and the standard deviation was  $\pm$  9.7. The 100 trypsin-treated fibres had resting potentials ranging from 10 to 80 mV, with a mean of 50 mV and a median of 58 mV. These resting potentials showed a bimodal distribution with the modes between 30-40 mV and 60-70 mV. Twenty fibres remaining in trypsin solution for 50 min showed an almost complete loss of resting potential, with a range of 4-20 mV. Twenty-six fibres were treated with trypsin solution which had



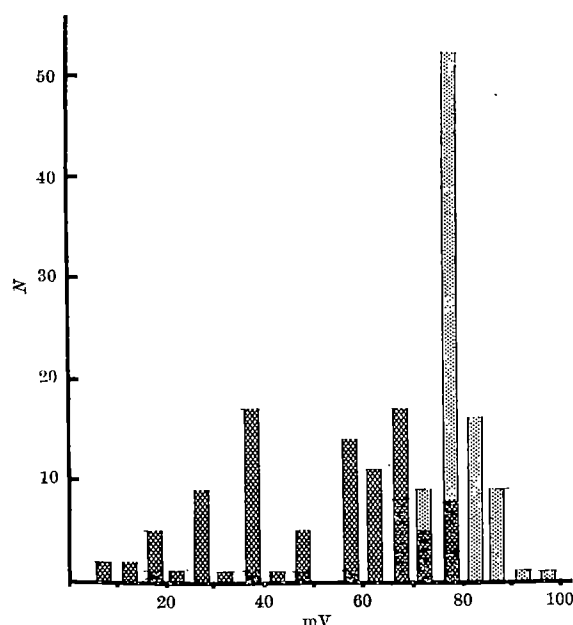


Fig. 1. Distribution of resting potentials. Cross-hatched bars represent the trypsin-treated fibres; dotted bars represent the non-treated fibres

been heated in Ringer's solution brought to pH 12 by sodium hydroxide. Cooling and returning the solution to normal pH by hydrochloric acid, before treating the muscle fibres, partially reactivated the trypsin<sup>6</sup>. The results of this control for enzymatic activity were a median resting potential of 70 mV and a mean of 69 mV. Because of the results of this control, complete hydrolysis of the trypsin, by refluxing in concentrated sulphuric acid, was not considered necessary.

The results of this investigation indicate that trypsin in the external medium affects the electrical properties of the muscle cell. It should be noted that this effect need not have been brought about by the hydrolysis of peptide bonds, since trypsin, like many proteases, has amino-acid amidase and esterase activity<sup>7</sup>. The bimodal distribution of the resting potentials in the trypsin-treated fibres probably represents measurements from fibres on the surface of the bundle and from fibres just below the surface layer. The latter would be exposed for less time to the trypsin, which would have to reach it by diffusion.

These results differ markedly from those on other excitable cells where the protease, applied to the outside of the cell, apparently had no effect on the resting potential. This difference could be due either to a difference in the chemical or physiological properties of the nerve membranes or the ability of the surrounding Schwann cell to act as a barrier to diffusion of protease to the axolemma. In the muscle cell, where protease has more direct access to the cell membrane, the intact state of the outer surface of the membrane does appear to play a part in maintaining membrane properties.

Lehmkuhl and Sperelakis<sup>8</sup> reported that when isolated chick ventricular tissue, which is normally quiescent, has been separated into individual cells by trypsin treatment, these cells show spontaneous electrical activity and contractions. Those authors conclude that all ventricular cells are capable of intrinsic pacemaker activity. They also reported that the isolated cells have resting potentials about 13 mV below those of cells in the intact tissue, a depolarization probably sufficient to produce the repetitive activity. As already mentioned here, the sartorius muscles often showed spontaneous contractions in trypsin solution. This depolarization and, therefore, the repetitive activity may possibly be attributed to the action of trypsin rather than to an intrinsic property of the ventricular cell which is somehow masked in the intact tissue.

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## An Effect of Cyanide and Nitriles on Connective Tissue

IN the course of an investigation on a reduction in tensile strength of the skin of the rat's tail, produced by incubation in physiological saline<sup>1</sup> and thought to be due to production of acid metabolic products, the effects of various metabolic inhibitors were examined, including cyanide. This produced an increase in tensile strength which was so large and unexpected that it seemed worth further investigation.

The material used was the whole thickness of the tail cut into rings (belts) 2.5 mm wide and broken by loading stepwise at constant rate to the point of rupture. Time to rupture was 2-5 min in most cases and the same rates of loading were used for control and test samples. These rings were placed in a saline solution (modified Krebs-Ringer phosphate<sup>2</sup>) containing potassium 6 mM, magnesium 1 mM, phosphate 18 mM, sulphate 1 mM, sodium 154 mM and chloride 126 mM, the last two varying slightly with pH, to which test substances had been added. In this solution they were either incubated at 37° C for 2-3 h or more commonly left overnight at 4° C in a refrigerator and then broken in Locke's solution at 37° C.

The following is a summary of the main features of this cyanide effect.

Potassium cyanide (A.R., British Drug Houses) in concentration  $10^{-3}$  M increased the tensile strength of the whole skin at pH 7.5 by 50-100 per cent above the value for untreated rings tested immediately after removal of the tail. The minimum concentration to produce an effect was between  $10^{-5}$  M and  $10^{-4}$  M at this pH. This is the range for inhibition of many enzymes<sup>3</sup>, though for some it extends lower (for example, cytochrome oxidase,  $10^{-8}$  M).

At pH 6 with  $10^{-3}$  M potassium cyanide the effect is relatively larger, a 3-5-fold increase in tensile strength over that in saline alone at the same pH. (Without cyanide at this pH tensile strength is reduced to about one-third the untreated value<sup>4</sup>.)

The effect is on the dermis, not the epidermis, because it is found after the latter has been removed.

The effect is complete at 37° C in 1-2 h and is reversible by washing in physiological saline at approximately the same speed as it comes on in the presence of cyanide.

Methyl cyanide has a similar effect, but at concentrations about  $10^3$  higher. Ethyl cyanide has not been investigated in detail, but is less active still. The activity of methyl cyanide, together with the activity of potassium cyanide at pH 6, indicates that the effect is not due to the cyanide ion.

$\beta$ -Aminopropionitrile (fumarate,  $10^{-2}$ - $10^{-1}$  M), a substance producing lathyrism *in vivo* associated with reduction of tensile strength of tissues including skin<sup>5,6</sup>, had relatively little effect by itself but reduced the effect of cyanide. Though this antagonistic effect to cyanide is not entirely specific to lathrogenic agents it nevertheless

suggests the possibility that these might compete for sites on collagen or interfibrillary material with naturally occurring substance resembling cyanide in properties. This, however, is pure speculation.

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### Linearization of Evoked Responses to Sine Wave-modulated Light by Noise

THE use of sine wave-modulated light in psychophysics<sup>1,2</sup> has directed attention to problems of linearity in the signal transmission in the visual system.

The first electrophysiological experiments with modulated light have revealed strong non-amplitude dependent distortions<sup>3,4</sup>. One of the most striking phenomena is that in most subjects with the modulation at 5 c/s a nearly pure 10 c/s response is found in the occipital leads of the EEG.

A tentative explanation was presented<sup>4</sup> in which the signal was supposed to be distorted in an early stage of the system. This distorted response, or a combination of it and a rather undistorted response, would then be processed in the cortical areas in a way comparable with selective filtering with the filter frequency set at 10 c/s. It was shown that there are subjects in which indeed resonance effects occurred at approximately 10 c/s, this being at, or near, their  $\alpha$ -rhythm frequency. It was suggested that at low percentages of modulation the quantal and neural noise may exceed the variations occasioned by the modulation.

In an earlier paper<sup>5</sup>, an estimate of the quantal noise near subjective threshold at 10 c/s was given. The conclusion was that with certain suppositions about quantum efficiency, etc., the variations in the light due to quantal noise became of the same magnitude as the amplitude of the modulation. Since this was calculated for a field of 22', it can be expected that for one single receptor the modulation-noise ratio will be considerably smaller. Although such estimates are open to criticism, it was suggested that in a case where the noise exceeds the actual signal, in some non-linear systems the distortion would disappear. This leaves the question open whether and in what way the signal-to-noise ratio in the actual case will have to be improved before the stage of distortion is reached, regarding the large distortions found at modulation depths as low as 3 per cent.

For a number of non-linear systems of the rectifying type the influence of noise on the response functions was calculated and it was found that a sine wave with noise or a non-correlated periodical signal, such as a triangle, sine wave, etc., added, will be less distorted by rectification procedures than a pure sine wave.

This suggested the following experiment: A well-trained subject was stimulated with white light from a television projection tube which could be modulated electronically<sup>6</sup>. The stimulation was performed with a large field at moderate intensity, in the range of 50–200 lux equivalent. The level of illumination had little influence on our results. The responses of the subject for 10 per cent modulation were averaged by a CAT computer and are presented for

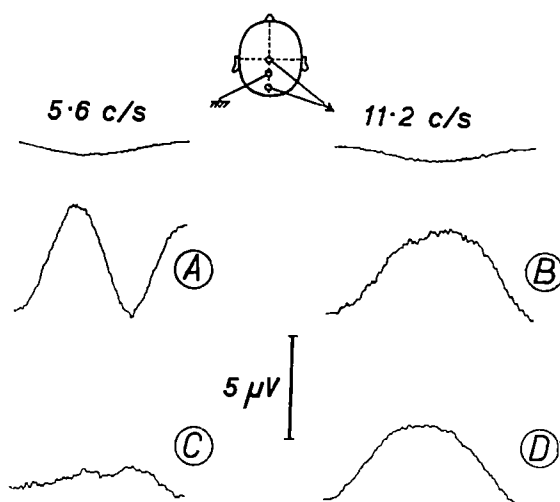


Fig. 1. Occipital responses to 5.6 and 11.2 c/s. Diffuse illumination. 10 per cent modulation, natural pupil, approximately 200 lux equiv. Upper trace: modulated light. Medium trace: responses to sinusoidal stimulation (A and B). Lower trace: responses to sinusoidal stimulation and noise (C and D).

two-eye stimulation in Figs. 1A and B for 5.6 and 11.2 c/s. It is seen that the 5.6 c/s gives a pure second harmonic response, whereas the response at 11.2 c/s displays mainly the fundamental frequency. If now gaussian noise resulting in a signal-noise ratio  $A^2/2\sigma^2 = 0.04$  ( $A$  being the amplitude of the sine wave,  $\sigma$  being the standard deviation of the noise) and a band-width 15–25 c/s is added to the modulating signal (1C and 1D) the second harmonic at 5.6 c/s stimulation nearly disappears, whereas the fundamental response at 11.2 c/s stimulation is much less affected, if at all. This may depend on the influence of saturation phenomena.

If one eye is stimulated with the sine wave modulation and the other eye separately at the same time with only noise, not much change in the responses to 5.6 and 11.2 c/s is found, compared with responses to stimulation of one eye with sine wave modulation and the other eye with steady light. This, and other arguments, suggest that most probably the distortion is not caused at a late stage in the system. If these results are valid also for the spontaneous noise inherent in the retinal processes, a kind of integration has to be assumed to increase the signal-noise ratio before the distorting stage is reached.

The possibilities for further work with this method are evident, and one of these is the adding of high-frequency sine waves to get an impression of the high-frequency attenuation up to the stage of distortion. By comparing responses at 5.6-c/s stimulation with the addition of non-related high-frequency sine waves we could deduce that the high-frequency attenuation from 25 to 60 c/s is less than would be expected from the so-called De Lange curves, a problem discussed earlier by Van der Tweel<sup>7</sup> and Levinson<sup>2</sup>.

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## PHARMACOLOGY

## Antitussive Activity of Medazonamide

Most of the known antitussive agents also possess, in varying degree, other pharmacological properties, such as the analgesic, antihistaminic, local anaesthetic and spasmolytic ones (Silvestrini and Maffii<sup>1</sup>). These activities are often responsible for, or directly connected with, specific clinical side effects that may limit the effect. In particular, the antitussives that show analgesic effect produce depression of respiration and possibly tolerance, while antihistaminic antitussives often lead to drowsiness, and the local anaesthetic ones usually have a high toxicity.

For these reasons the search for new agents, possessing almost exclusively antitussive properties, is still pursued in many pharmacological laboratories. In the course of a systematic investigation on a new series of pyridazine compounds which have been prepared by Teotino and Cignarella<sup>2</sup>, it appeared that 2-methyl-4,5-dihydro-3-pyridazinone-6-carboxamide ('medazonamide') possesses antitussive properties in doses that do not produce any other pharmacological effect. Moreover, medazonamide was found to be practically non-toxic both in acute and chronic tests.

Medazonamide depresses and inhibits the cough induced in guinea-pigs by inhalation of acrolein vapours according to a method previously described<sup>1</sup>. Median effective doses and acute toxicity of medazonamide and some other known antitussive agents are given in Table 1.

Table 1. ANTITUSSIVE ACTIVITY (GUINEA-PIGS) AND ACUTE TOXICITY (MICE) OF MEDAZONAMIDE AND OTHER ANTITUSSIVE AGENTS

Compounds	Cough inhibition $ED_{50}$ (mg/kg s.c.)	$LD_{50}$ (mg/kg) s.c.	$LD_{50}$ (mg/kg) i.v.
Codeine HCl	4	370 (ref. 3)	80 (ref. 3)
Morphine sulphate	5	311 (ref. 4)	255
Narcotine	3-5	390 (ref. 5)	—
Dextromethorphan	6	275 (ref. 3)	37 (ref. 3)
Benzononatine	3	230 (ref. 6)	9 (ref. 6)
Medazonamide	5	>1,000	>1,000

Medazonamide, at doses of 5 mg/kg subcutaneously, or 20 mg/kg orally, exerts inhibitory activity on coughing induced in dogs by aerosol of  $H_2SO_4$  (0.5 N), according to the method described by Winter and Flataker<sup>7</sup>.

Appreciable analgesic activity free from sedation is shown only at doses of 50 and 100 mg/kg subcutaneously and intraperitoneally in rats (method described by Randall and Selitto<sup>8</sup>) and mice (Eddy and Leimbach<sup>9</sup>). An intraperitoneal dose of 500 mg/kg of medazonamide produces significant inhibition of formalin-induced oedema of the rat hind-paw.

Frequency and amplitude of respiration are not modified after intravenous injection of 10 mg/kg of medazonamide in rabbits (anaesthetized with chloralose, 50 mg/kg intraperitoneally, and ethyl urethane, 1 g/kg subcutaneously) or after subcutaneous administration of 20 mg/kg in rats (anaesthetized with phenobarbitone, 120 mg/kg intraperitoneally). Arterial blood pressure of dogs (anaesthetized with pentobarbital sodium) is not changed by intravenous doses of 5 and 10 mg/kg.

At intravenous doses of 5 and 10 mg/kg medazonamide does not produce bronchial spasm in guinea-pigs. Codeine elicits bronchial spasm at doses of 7 mg/kg subcutaneously and 3-5 and 10 mg/kg intravenously.

At concentrations up to 10  $\mu$ g/ml. medazonamide is without any spasmolytic effect on contractions of isolated small intestine, induced either by barium chloride or acetylcholine (rat) or by histamine (guinea-pig).

Acute toxicity of medazonamide is extremely low (see Table 1). In fact, it causes no deaths in mice even after administration of 1 g/kg by various routes, and it differs, therefore, from all other known antitussive drugs, including the most recently discovered ones<sup>10,11</sup>.

In subacute and chronic toxicity tests, carried out in dogs at daily oral doses of 200 and 100 mg/kg for 4 weeks and 6 months respectively, medazonamide has been shown

to be well tolerated and free from side-effects even at high dose-levels and over prolonged treatment periods.

The results of the afore-mentioned pharmacological investigation demonstrate that medazonamide is an effective antitussive agent practically devoid of any pharmacological side-effects.

An extensive clinical study has shown that medazonamide significantly reduces the cough caused by chronic bronchitis in man (Nicolis and Pasquariello<sup>12</sup>). In 'double blind' conditions a dose of 800 mg of the substance appeared to be as effective as 60 mg codeine. The lack of any side-effect in man has been confirmed also for doses much higher than the effective ones.

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## Chloroquine in Dextran Oedema

EXPERIMENTS in our laboratory have shown that chloroquine blocks the actions of endogenous as well as exogenous histamine in guinea-pigs<sup>1</sup>. Chloroquine in these experiments was also found to block the action of exogenous 5-hydroxytryptamine (5HT). Further experiments were undertaken to study the action of chloroquine on the endogenous 5HT, which has been shown by several workers to be released by dextran in rats and mainly accounts for the oedema<sup>2,3</sup>. The cholesterol content of the adrenal glands of these rats was also estimated to determine whether the adrenals were involved in the action of chloroquine on dextran oedema.

A group of 10 male albino rats (100-150 g) were injected daily with chloroquine sulphate (10 mg/kg) intramuscularly. An equivalent amount of normal saline was injected into a similar group of 10 control rats. On the 31st and 38th day each animal was injected intraperitoneally with 1 ml. of 6 per cent dextran solution per 100 g body-weight. The dorsoplantar thickness was measured by means of a calliper rule as described by Setnikar *et al.*<sup>4</sup> to assess the degree of oedema. The animals were killed on the 45th day and the adrenal glands were removed. Total cholesterol was estimated by means of the Lieberman-Burchard reaction<sup>5</sup>. Results are summarized in Table 1.

It is seen from the table that chloroquine causes a reduction in the dextran oedema. That the oedema is not completely prevented shows a partial antagonism to the endogenous 5HT. These results are in conformity with *in vitro* results<sup>1</sup>. Chloroquine was found to cause a slight

Table 1. EFFECT OF CHLOROQUINE ON DEXTRAN OEDEMA AND ADRENAL CHOLESTEROL IN RATS

	Mean increase in dorso-plantar thickness (mm) $\pm$ (S.D.)	Mean cholesterol content of adrenals (g/100 g) $\pm$ (S.D.)
Control	2.33 $\pm$ 0.33 (2.0-3.0)*	2.66 $\pm$ 0.55 (1.87-3.02)
After chloroquine	0.87 $\pm$ 0.57 (0.0-1.5)	3.87 $\pm$ 1.03 (1.95-7.43)

\* Figures in the bracket indicate range.



increase in adrenal cholesterol. Thus it appears that the prevention of dextran oedema by chloroquine is not dependent on the adrenal hormonal secretions.

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## Phenazine Methosulphate in Cyanide Toxicity

THE blocking action of cyanide ion on cellular respiratory enzyme cytochrome oxidase is of considerable biological importance. *In vitro* action of phenazine methosulphate<sup>1</sup> (PMS) appears somewhat similar to that of the terminal respiratory enzymes in the cell. The following experiments would suggest that PMS might be effective, *in vivo*, as a temporary substitute of the cyanide-blocked respiratory enzymes.

Adult albino rats and mice of either sex were used. All substances were dissolved in water and used within 3 h. PMS solution was protected from light by thick black paper. Respiratory arrest in animals was taken as the end-point.

The dose of PMS (15 mg/kg) used in these experiments was apparently well tolerated by rats and mice. Sodium cyanide (10 mg/kg) alone invariably killed all animals. In mice, 15 mg/kg of PMS proved more effective than 7.5 mg/kg. Increasing the interval between PMS and cyanide injections from 0.25 min to 4 min decreased the protecting action of PMS. Those animals which PMS or nitrite failed to protect against cyanide lived longer than the ones receiving only cyanide. PMS solution lost considerable activity after 3 h exposure to daylight. Animals protected by PMS or nitrite against cyanide often suffered brief unconsciousness followed by sedation. Animals surviving for 2 h usually recovered completely. In rats, PMS given by the intraperitoneal route 3 min after subcutaneously injected cyanide could protect 69 per cent of animals.

Table 1. SURVIVAL OF MICE AND RATS AFTER CYANIDE TREATMENT, WITH AND WITHOUT PROTECTIVE PRE-TREATMENT

First drug (mg/kg)	Interval between first and second drug (min)	Second drug (mg/kg)	No. animals died and average survival time (min)	No. and % of animals protected against cyanide
Mice				
NaCN, 10	Nil	Nil	24 (3)	Nil (0%)
PMS, 15	0.25	NaCN, 10	8 (35)	22 (73%)
PMS, 15	4	NaCN, 10	5 (19)	1 (17%)
PMS, 7.5	0.25	NaCN, 10	6 (24)	4 (40%)
PMS, 15*	0.25	NaCN, 10	7 (35)	Nil (0%)
NaNO <sub>2</sub> , 80	4	NaCN, 10	4	11 (73%)
Rats				
NaCN, 10	Nil	Nil	20 (15)	Nil (0%)
NaCN, 10	3	PMS, 15	8 (29)	18 (69%)

\* PMS solution in glass container exposed to daylight for 3 h. NaCN injected into rats by the subcutaneous route. All other injections by the intraperitoneal route. Room temperature 16°–34° C.

Sodium nitrite, the well-known antagonist of cyanide *in vivo*, was used for comparison. It acts by converting haemoglobin into methaemoglobin; the latter forming the comparatively stable complex cyanmethaemoglobin. Unlike PMS, nitrite was effective when injected 4 min before cyanide. This suggests that its mode of action is different. Survival of 73 per cent of mice and 69 per cent of rats suggests what is otherwise an invariably fatal dose of cyanide is considered significant.

## Nitrogen Oxides in Tobacco Smoke

BECAUSE of their considerable pharmacological significance, the presence of nitrogen oxides in tobacco smoke has been the subject of a number of reports in the past few years<sup>1–4</sup>. In general, the choice of experimental techniques has been such that the relative amounts of the principal components, nitric oxide (NO) and nitrogen dioxide (NO<sub>2</sub>), in cigarette smoke have not been clearly established.

Although NO and NO<sub>2</sub> are both readily absorbed in the human lung, their physiological action is reported to be quite different. NO is found to be approximately five times less toxic than NO<sub>2</sub> (ref. 5), and recently it has been found that NO is approximately six times less active as a mammalian ciliastatic agent<sup>6</sup>. These considerations made it desirable to develop an analytical technique which would provide quantitative estimates of the amount of each oxide in tobacco smoke. With such a technique it is found that almost all of what had previously been considered as a mixture of oxides is, in fact, the less active nitric oxide.

Although NO undergoes oxidation to NO<sub>2</sub> by the well-known termolecular reaction  $2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2$ , the rate of conversion is found to be relatively slow at the concentrations existing in cigarette smoke. It is calculated that approximately 500 sec would be required in undiluted smoke, and more than 5,000 sec would be required in the more dilute system within the human lung, for the oxidation of half the NO to NO<sub>2</sub>. Since these times are very much greater than those available during the inspiration, absorption and expiration of a puff of smoke, NO can be considered as an independent agent in its pharmacological action.

Because of the considerable difference in volatility of NO (B.P. –151° C) and NO<sub>2</sub> (B.P. 21° C), a cold adsorption trap can effectively be used to separate these gases. Following such a separation the NO is allowed to oxidize to NO<sub>2</sub>, and the concentrations of both are individually estimated by the Saltzman procedure<sup>7</sup>. Our smoking and smoke collection system consisted of a train containing an efficient particulate smoke filter, a flow-limiting orifice, a small U trap, and an evacuated terminal gas collection flask. The functions of the filter, orifice and terminal flask have been described elsewhere<sup>8</sup>, and it is necessary to note here that they provide a means of withdrawing a single 40-ml. puff of smoke of normal duration from a lighted cigarette, and of separating the gaseous mixture from the entrained smoke particles. The 55-ml. terminal collection flask both provides the suction necessary for puffing and also serves as a quantitative container for the gas mixture and its component NO during the reaction with 10 ml. of the Griess-Ilosvay reagent contained therein. The 7-ml. U trap, which is packed with glass helices and 1.0–1.5 grams of silica gel deactivated with 20 per cent H<sub>2</sub>O, is cooled to dry-ice temperatures. This trap largely retained such NO<sub>2</sub> as is present, which subsequently can be estimated by elution and reaction with Griess-Ilosvay reagent.

Experiments with pure gases and mixtures of NO and NO<sub>2</sub> each diluted with nitrogen to the approximate level of nitrogen oxides in cigarette smoke were performed. It

Table 1. NITROGEN OXIDE CONTENT OF CIGARETTE SMOKE

Cigarette	Gas in U trap ( $\mu$ l./puff)	Gas in flask ( $\mu$ l./puff)	NO		NO <sub>2</sub>		Total	
			( $\mu$ g/puff)	(p.p.m.)	( $\mu$ g/puff)	(p.p.m.)	( $\mu$ g/puff)	(p.p.m.)
Non-filter (fourth puff)	4.9	22.7	38.2	715	0	0	38.2	715
Filter, A (fourth puff)	3.1	14.2	24.0	442	0	0	24.0	442
Filter, B (lighting puff)	5.0	22.2	37.4	698	0	0	37.4	698
(fourth puff)	5.7	27.6	46.6	871	0	0	46.6	871
(last puff)	5.8	31.8	54.0	1,008	0	0	54.0	1,008
(fourth puff, whole smoke)	5.6	24.4	41.2	768	0	0	41.2	768
Filter, C (fourth puff)	7.4	24.3	40.5	755	1.2	25	41.7	780

was found that the U trap captured 79 per cent of the NO<sub>2</sub> introduced, while 77 per cent of the NO passed into the terminal flask. Overall recoveries of 90–99 per cent of the two gases were achieved, the slight losses probably occurring through irreversible absorption of some of the gases on silica gel and during the manipulations required in the analytical procedure. Utilizing the pure gas data, simultaneous equations were formulated which were used to resolve the NO and the NO<sub>2</sub> concentrations in gas mixtures and cigarette smoke. Applying these to 9:1, 3:2 and 1:1 molar mixtures of NO and NO<sub>2</sub> resulted in good agreement between the predicted and observed quantities of gas in the two traps.

With this smoking and trapping system, individual puffs of cigarette smoke were analysed and the division of nitrogen oxides calculated by the equations. Table 1 summarizes data obtained on representative puffs from several 'king-size' filtered and unfiltered cigarettes. Measurements of initial, intermediate and final puffs on these and other cigarettes, and with and without the smoke filter in the system, give essentially the same one-sided division of these oxides. From these data, it is clearly indicated that essentially all the nitrogen oxides in fresh, normally obtained cigarette smoke are the less pharmacologically active nitric oxide, and that nitrogen dioxide is present at most only in trace quantities.

The almost complete absence of nitrogen dioxide in the mainstream of cigarette smoke may stem from several factors. One is that during the formation of nitrogen oxides in the hot reducing atmosphere close behind the glowing cone, conditions favour the formation of the lower oxides. Elkins's<sup>9</sup> and Tada's<sup>4</sup> data on the NO and NO<sub>2</sub> contents of fumes from various combustion sources show this to be the general case. Another factor tending to eliminate such NO<sub>2</sub> as might be formed is the presence of moisture and reactive organic materials in the smoke stream and tobacco column, which can absorb and react with NO<sub>2</sub>.

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## IMMUNOLOGY

### *In vitro* Studies on Homograft Sensitivity

THE migration of cells from capillary tubes in tissue culture chambers has proved valuable in studies on delayed hypersensitivity in guinea-pigs<sup>1,2</sup>. Using this technique, it was observed that inhibition of migration of peritoneal cells from guinea-pigs with delayed hyper-

sensitivity to tuberculin and other protein antigens occurs only in the presence of the specific antigen. On the other hand, peritoneal cells from guinea-pigs producing circulating antibody were not inhibited by the corresponding antigens.

With this information at hand we have adapted this technique of peritoneal cell migration to the study of the homograft reaction in inbred strains of mice. We find that peritoneal cells from mice with homograft sensitivity are inhibited when mixed with peritoneal cells obtained from the strain of mice that had donated the sensitizing skin homografts. Furthermore, peritoneal cells obtained from unrelated strains of mice, when mixed and placed in tissue culture chambers, migrate well together without inhibiting each other.

The mice used in this study (*A/Jax*, *CBA* and *C57BL/6*) were obtained from Jackson Memorial Laboratory, Bar Harbor, Maine, and were 3 months old at the time of the experiment. The donors of sensitizing skin grafts were *A/Jax* mice and the recipients were *CBA* mice.

The sensitizing skin grafts were suprapannicular, circular, 11 mm in diameter. They were fitted grafts and were applied to the lateral thoracic regions. The grafts were covered with non-adhering dressing and wrapped with latex bandage and adhesive tape. Peritoneal exudate cells were collected in cold Hank's solution 3–4 days after the intraperitoneal injection of 3–4 ml. of sterile light mineral oil. Sensitive peritoneal cells were collected from the homograft recipients following the rejection of the sensitizing skin grafts. The cells were suspended in Eagle's culture medium containing 15 per cent normal guinea-pig serum, then packed in capillary tubes and placed in tissue culture chambers as previously described<sup>3</sup>. When cells from two strains were mixed, equal proportions were used. Tissue culture chambers were incubated at 37° C for 24 h and the area of cell migration from each capillary tube was measured by planimetry. Percentage migration of cell mixture was calculated as follows: average migration of mixture of cells multiplied by 10 and divided by average migration of normal and sensitive cells separately.

Table 1. MIGRATION OF PERITONEAL CELLS FROM NORMAL AND SENSITIVE *CBA* MICE IN THE PRESENCE OF CELLS FROM THE SENSITIZING STRAIN *A/Jax*

No. animals pooled	Percentage migration		
	<i>CBA</i> Sensitive to <i>A/Jax</i> + <i>A/Jax</i>	Normal <i>CBA</i> + <i>A/Jax</i>	<i>CBA</i> Sensitive to <i>A/Jax</i> + <i>C57BL/6</i>
15	56		
15	55		
15	70		
20	55		101
15		106	
16		100	
15		97	

It can be seen from Table 1 that inhibition of migration occurred in all the experiments where cells from sensitive *CBA* mice were mixed with cells obtained from *A/Jax* mice (the sensitizing strain). The specificity of the inhibition observed is illustrated by the normal migration of cells from *CBA* mice with homograft sensitivity to *A/Jax* strain in the presence of cells from *C57BL/6* (unrelated to *A/Jax*).

In this system the populations of cells comprising the mixture are functioning as antigen and sensitized cells

in addition, both are viable and capable of migration. Thus, the observed inhibition of migration may be due to an effect on either of the populations in the mixture or a composite effect on both.

Since peritoneal cells from the donor of the sensitizing skin homografts have transplantation antigens, the observed inhibition of migration could be an effect of cellular antigens (residing in or on the donor cells) on sensitive lymphoid cells. In this context the inhibition may be similar in nature to the effect of tuberculin or ovalbumin on sensitive peritoneal cells obtained from guinea-pigs with delayed allergy to these antigens. The homograft model, however, differs from the delayed allergy system in the use of viable cells as antigen in contrast to soluble antigens such as tuberculin.

On the other hand, it is possible that the inhibition observed is due to the damaging action of sensitive peritoneal lymphoid cells on cells obtained from the sensitizing strain interfering with their migration. This possibility could place the reaction in the class of target cells affected by sensitive lymphoid cells in other systems<sup>3</sup>.

What cannot be excluded is the possibility that the inhibition of migration may be the result of the two mechanisms discussed above operating simultaneously. Thus peritoneal cells from the donor and recipient of the skin homograft may be affected simultaneously.

In its gross appearance and temporal behaviour the migration of mouse peritoneal cells is similar to the migration of peritoneal exudate cells obtained from guinea-pigs<sup>2</sup>. Furthermore, under the conditions of the present experiment (24 h observation) peritoneal cells from one strain of mice do not exert any inhibitory effect on the migration of peritoneal cells from a genetically different strain of mice (for example, *CBA + A/Jax*, *CBA + C57BL*). This is of interest since the cells are in close contact and both populations are immunologically competent.

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### Studies of Group Agglutinogens of *Staphylococcus aureus*

THE serological differentiation of staphylococci has been the subject of many investigations since the preliminary work of Cowan<sup>1</sup> which divided coagulase-positive staphylococci into three main groups according to slide agglutination reactions with adsorbed antisera. Most of the serological reagents appear to be active against various type-specific antigens, and strains are usually classified according to a pattern of reactions. Recently, Haukenes demonstrated that a number of antisera in Oeding's serological typing system<sup>2</sup> were actually complexes of different antibodies<sup>3-5</sup>. Moreover, by small changes in the

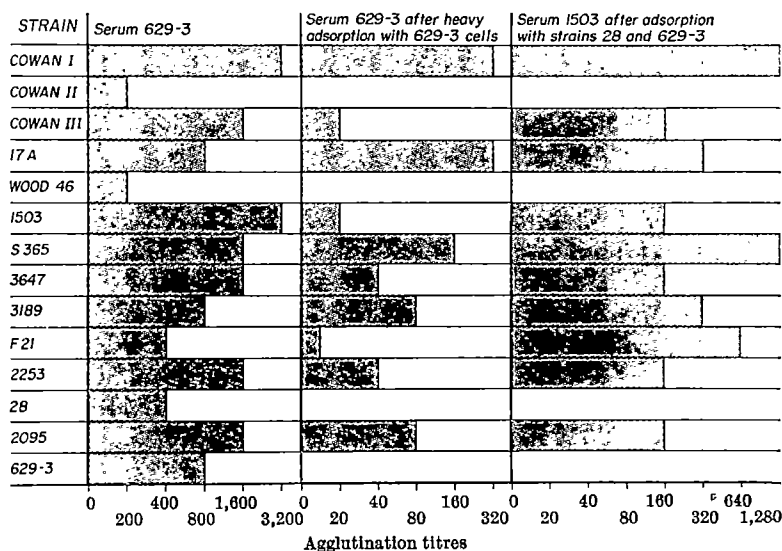


Fig. 1

adsorption schemes, Haukenes has succeeded in preparing some 15 or 20 serological reagents, most of which are useful for typing staphylococci.

Despite the new knowledge of type-specific antigens in *S. aureus*, means for the identification of group-specific antigens are still quite rudimentary. Most of the progress in this direction in recent years has been made by examination of so-called normal sera and by serum-gel diffusion. In 1958, Jensen<sup>7</sup> reported a group antigen from *S. aureus* which he called antigen A. Cohen *et al.*<sup>8</sup> identified another antigen (recently referred to as RL antigen<sup>9</sup>) by serum-gel diffusion with pre-immune rabbit serum. Antibody to this antigen has been found also in normal human, guinea-pig, hamster and mouse sera (including serum from germ-free mice). Recent work by Haukenes *et al.*<sup>10</sup> has resulted in the redefinition of polysaccharide A of Julianelle and Wieghard<sup>11</sup>. Polysaccharide A has been identified by serum-gel diffusion and appears to be immunologically identical with the main teichoic acid of *S. aureus*<sup>10</sup>.

When rabbits are immunized with a strain of *S. aureus*, the antiserum before adsorption usually reacts with most strains, and slide agglutination titres as high as 1:5,000 are not uncommon. The cross-reactions of a staphylococcal antiserum could be due to a single antigen present in the majority of strains or to a complex of antigens which vary from strain to strain. Results of a study of antiserum for strain 629-3 indicated that the so-called group agglutino-gen of *S. aureus* is actually a complex.

Strain 629-3 was isolated from a patient at the U.S. Public Health Service Hospital, San Francisco, California. The culture was submitted to the Communicable Disease Center for phage typing and was found to be phage type 53/83 and serotype *en*. Because of the apparent absence of other known typing factors, the culture was considered to be an interesting one for use as an immunizing strain.

The slide agglutination titres of serum 629-3 for the type strains are shown in Fig. 1 (first part). There was considerable variation in observed titre from strain to strain. Titres for some of the heterologous strains such as Cowan I were a good deal higher than that for the homologous strain. The low titres for strains Wood 46 and Cowan II are interesting because these strains are generally unreactive with 'normal' sera and appear to lack some part of the group antigens of *S. aureus*.

Cross-adsorption experiments were performed with some of the type strains. After serum for 629-3 was adsorbed with strain 28, considerable activity remained. Strain 28 has the serotype *aben*, and it had been predicted that this strain would clear 629-3 serum of all agglutinating



activity for *S. aureus*, since strain 28 has all the known typing antigens found in strain 629-3.

Further adsorptions were carried out with this serum (629-3-abs.-28; see Table 1). Strains Cowan II, Wood 46, and 28 did not remove any more agglutinating activity. The other type strains removed most of the activity. The most surprising result was that obtained when the serum was adsorbed with the homologous strain. Rather than clearing the serum, 629-3 adsorption caused little change in the reaction spectrum. After adsorption with the homologous strain, there was no further agglutination of 629-3, but heterologous strains such as Cowan I and S365 agglutinated strongly. Apparently strain 629-3 stimulated production, in rabbits, of an antibody with which it neither reacts in agglutination tests nor removes in adsorption tests.

Table 1. AGGLUTINATION ACTIVITY REMAINING AFTER ADSORPTION WITH STRAIN 28 AND FURTHER ADSORPTION WITH A SECOND STRAIN

Agglutination test strains	Slide agglutination reactions after further adsorption with	Slide agglutination reactions after further adsorption with	Slide agglutination reactions after further adsorption with	Slide agglutination reactions after further adsorption with	Slide agglutination reactions after further adsorption with
	629-3	Cowan I, F21, Cowan III, S365, or 2095	3189	Wood 46, Cowan II, 2253, 17A, or 28 again	1503
DA 629-3	-	++++	++++	++++	-
Cowan I	++++	-	-	++++	-
Cowan III	++	-	-	++++	-
17A	++	-	-	++++	-
1503	(±)	-	-	++++	-
S365	++++	-	-	++++	-
3647	++++	-	-	++++	-
3189	++++	-	-	++++	-
F21	(±)	-	++	++	-
2253	(±)	-	-	(±)	-
2095	++++	-	-	++	-

This experiment was repeated using twice as many cells for the adsorption, and then adsorbing again with the same number of cells. The central part of Fig. 1 shows the agglutinating activity left after this heavy adsorption with the homologous strain. Adsorptions with boiled and with autoclaved cells did not remove this agglutinating activity. The factor delineated here has been tentatively called the 629-G factor.

Pre-immune serum from both rabbits used for 629-3 immunization was tested for agglutinating antibodies with the type strains. Only strains Cowan I, S365, and 17A agglutinated. The titres were low; the activities were similar to those found in other non-immunized rabbits. The antibody present in 629-3 immune serum after adsorption with the homologous strain could not be accounted for by the antibody level in pre-immune serum.

When 210 other cultures of *S. aureus* were tested for agglutination in the 629-G factor, 65 were positive and 145 negative. Some of the cultures which failed to agglutinate could adsorb 629-G antibodies.

An attempt was made to prepare 629-G factor from one of the type strains. Serum for 1503, type *aemn*, was adsorbed with strains 28 and 629-3. The resulting antiserum did not agglutinate strains Wood 46, 28, Cowan II, and 629-3. However, when this serum was compared to 629-3 (by agglutination tests with miscellaneous *S. aureus* cultures), it exhibited a wider spectrum of reaction. When the adsorbed 1503 serum was diluted 1:400 it reacted predominantly with cultures which reacted strongly in the 629-G serum. Another sample of 1503 serum from the same rabbit, taken at a later bleeding, was adsorbed with strains 28 and Cowan II. This serum reacted in parallel with 629-3 serum adsorbed with 629-3 only, when 44 cultures were tested in both antisera.

The data indicate that: (1) 629-G factor is a part of the group agglutinin complex of *S. aureus*, and (2) other antigenic factors must be responsible for a portion of the reaction spectrum observed with unadsorbed staphylococcal antisera. These results suggest that the 'group' agglutinin of *S. aureus* is a complex, and that the competency of an adsorbing strain for the production of specific factor sera depends on its ability not only to adsorb those specific antibodies for antigens expressed in

its formula, but also to adsorb certain undesigned group agglutinins.

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## PATHOLOGY

### Role of Splenomegaly in Tumour-bearing Mice

MANY attempts have been made to explain the presence of splenomegaly in tumour-bearing mice. Early work by Andreini<sup>1</sup> *et al.*, in mice bearing homografted tumours, demonstrated splenomegaly which was interpreted as evidence of immunological activity against the tumour. When splenomegaly was found in the isologous situation the interpretation was made again<sup>2</sup> that this was a sign of a response of the mouse to a cancer-specific antigen in the tumour and this antigen might be lost on transplantation. Stuart and El Hassan<sup>3</sup> have recently shown the protective effect of isologous spleen cells from tumour-bearing mice with splenomegaly, injected locally with the Landschütz ascites tumour. In the experiments reported here I have attempted to demonstrate the protective influence of the splenomegaly by transferring spleen cell suspensions from tumour-bearing mice to isologous mice challenged with the same tumour.

An inbred colour of A strain mice was used. An A strain mammary tumour *AMT*<sub>1</sub> was transplanted by subcutaneous injection of a standard dose of tumour cells. Spleens were weighed from normal mice and from mice bearing tumours for different intervals and after injections of cell-free extracts of the tumour. The results shown in Table 1 confirm the presence of increasing splenomegaly with tumour growth. This splenomegaly has not been demonstrated after transfer of cell-free extracts of the tumour.

Table 1. SPLEEN WEIGHTS WITH TUMOUR GROWTH

Group	Spleen weight (mg)
Normal mice	120
Mice with 2-day tumour	128
Mice with 7-day tumour	229
Mice with 14-day tumour	249
Mice with 19-day tumour	450
Mice with cell-free extract	130

To investigate the apparently protective effect of the splenomegaly, spleens were taken from mice bearing tumours for 2, 7 and 14 days. Spleen cell suspensions were prepared as described by Woodruff and Symes. Cells were incubated in 0.05 per cent aqueous trypan blue for 10 min and, if less than 10 per cent of the cells stained, they were used for injection. The suspensions were injected slowly into the tail vein of the mouse at a dose of one spleen to a mouse in 0.5 ml. Medium 199. The spleen cells were injected 24 h after the subcutaneous injection of a standard dose of tumour. Groups of eight mice were used and control groups received tumour only. Tumour diameters were measured daily in two planes and the areas calculated. The measurement of the tumours showed clearly no difference in tumour size between the

experimental and control groups. Histological sections stained with haematoxylin and eosin showed no difference between the groups and no evidence of increased lymphocytic infiltration was seen. Finally, spleen cells and tumour cells were injected together subcutaneously in the same dose. Control mice received spleen cells from normal mice and the results again showed no difference in tumour size between treated and control groups.

Immunological attack on tumours depends on antigenic differences between cancer tissue and normal tissue. There is extensive evidence for cancer antigens in certain tumours<sup>4</sup>. However, in many experimental situations the cells have been sensitized to antigenic differences between tumour and host and not to any cancer-specific antigens. Woodruff and Symes<sup>2</sup> have proposed that splenomegaly seen in early transplants of spontaneous tumour was indicative of an antibody response to an antigen which is lost on subsequent transfer. If the splenomegaly seen here with transplantable tumour has the same explanation, then transfer of spleen cells from tumour-bearing mice should influence the growth of the tumour in new hosts. This has not been the case. This may be because this tumour may not possess, or has lost, a strong cancer antigen. Alternatively the splenomegaly seen may not indicate an immune response. Splenomegaly in mice is seen after injection of extracts of cancer tissue or toxohormone<sup>6</sup>, and this has been shown in certain cases to be due to bacterial or viral colonization of the tumour<sup>6</sup>. Old<sup>7</sup> has described splenomegaly after transfer of a virus particle in a cell-free extract of an experimental tumour, but this has not been seen in this situation.

Finally, the transferred immunologically competent cells may be in a state of immunological depression caused by the tumour, comparable to the depressed state seen in human cancer cases<sup>8</sup>. It is clear that splenomegaly in this situation does not indicate an effective transferable response by the host and further investigation is required to elucidate the true mechanism.

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## Origin of the Sickling Gene

SICKLING was first observed in an American Negro<sup>1</sup>. Many reports on the frequency of the sickle cell trait in various populations soon followed, and from them the conclusion was drawn that the black Negroes of the Central African Belt were the focus of this haemoglobin variant.

Recently, however, migrations from Asia to prehistoric Africa and not *vice versa* were thought more likely to have happened. This was based on: (a) the observation that the tall, broad-shouldered skeleton of the modern African Bantu appears only in Neolithic excavations, whereas the types found in older strata are more slender; and (b) on similarities in certain African and some Indian cattle and poultry<sup>2</sup>. It was later suggested that sickling was a Veddoid feature, and that it was from a

group of these people who lived in Arabia in Neolithic times that the sickling gene had spread both to Africa and to India<sup>3,4</sup>. The Veddoids are primitive tribes whose features have been traced in India, South Arabia, Persia, Egypt, Sardinia and Monaco<sup>3</sup>. In Arabia the Veddoids differ from the present-day inhabitants<sup>4</sup>.

In our opinion, the common single origin of the sickling phenomenon in the Near East remains to be substantiated. In the first place, sickling is rare in the pure Arabs or Bedouins<sup>4</sup>, and it is difficult to explain this finding unequivocally when these have been in a closer and longer geographical contact with the Veddoids in Arabia and their assumed sickle cell gene. Secondly, sickling is absent from some other Veddoid populations<sup>3</sup> and is not present in any outstanding incidence in other places where Veddoid remains have persisted, for example, in Egypt, Monaco and Sardinia. Thirdly, no spread of other genetically-determined Veddoid characteristic, for example, blood groups, has been reported parallel to that of the sickling gene. Fourthly, slavery introduced to the Near East great numbers of Negroes from Africa, whose role in the spread of the sickle cell gene has not been fully assessed.

It is interesting to note that sickling is not restricted to human beings only but is to be observed in the blood of rackitic white rats and experimentally in guinea-pigs<sup>5</sup>, and also in the white-tailed deer<sup>6</sup>. The distribution of different pigments is not unequivocally racial. A germane suggestion was recently made by Clément about the thalassaemic syndromes<sup>7</sup>. It is no longer conceivable that thalassaemia is an exclusive disorder of the Greco-Roman races, and haemoglobin variants have spared no race.

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## Assessment of Non-emergence of Drug-resistant Strains of Pathogens treated with Polynoxylin

THE difficulty of inducing drug-resistance of micro-organisms to polynoxylin has previously been reported<sup>1,2</sup>. Since July 1960, 360 bi-weekly sub-cultures of the organisms listed in Table 1 have been made.

Initially, attempts were made to render organisms 'insensitive' by the addition of small amounts of polynoxylin to large volumes of broth cultures. The mixture was thoroughly shaken and re-incubated and, after three days, large volumes of the treated broth were sub-cultured. Growth of sub-culture was obtained for the first month or two, but gradually 'tailed off'.

In the first experiments 100 ml. of nutrient broth was used; 0.4 mg of polynoxylin powder was placed at the

Table 1. TEST ORGANISMS FOR WHOLE SERIES

<i>S. aureus</i> , coag. positive	Sensitive to all antibiotics
<i>S. aureus</i> , coag. positive	Insensitive to penicillin
<i>S. aureus</i> , coag. positive	Insensitive to tetracycline
<i>S. aureus</i> , coag. positive	No. 7
<i>S. albus</i>	Nos. 2, 7, 8
$\alpha$ Haemolytic streptococci	Nos. 1, 3, 4, 5
$\beta$ Haemolytic streptococci	Nos. 2, 8, 9
'Indifferent' streptococci	3 types
<i>E. coli</i>	Nos. 3, 4, 6, 7, 8, 11, 12, 13
Pneumococci	No. 4
Mixed oral organisms	No. 2
<i>Pyocyanus</i> spp.	Nos. 1, 3
<i>Proteus</i> spp.	Nos. 2, 3, 4, 5
<i>B. subtilis</i>	No. 1
<i>Corynebact.</i> spp.	No. 1

bottom of a sterile culture bottle, to which the medium was slowly added so as not to disturb the powder. A standard loopful of a 24-h culture was added to the top of the medium without shaking and incubated at 37° C for three days, when a loopful of the growth from the 'top-layer' was plated and tested for sensitivity using the disk-method. Five drops were added to a similar PPI-treated broth. Eventually, in two or three months, the cultures died out.

A plate and disk-method was also used. The test organisms were plated out on solid media and a suitable polynoxylin sensitivity disk was placed on the moist media. After incubation for three days, standard loopfuls were removed from (a) the centre of the clear zone of inhibition; (b) the outer ring of the clear zone of inhibition; (c) the outer periphery of the zone, and (d) the junction of the zone of inhibition and growth. These were inoculated into broth and on to solid media.

No growth was ever obtained from inocula carefully taken from (a) and (b). In many cases inocula from (c) produced growth. Inocula from (d) always produced growth. No insensitive organism has emerged.

It has been reported that *Proteus* and *Pyocyanea*, when tested by the disk-method, show, on drying for periods longer than a week, some 'creep' of the organisms across the inhibition zone. I have observed this in four cases (two of each). The 'creep' colonies have been tested on repeated sub-culturing and are still sensitive to polynoxylin.

It is clear that polynoxylin is only active in the presence of water<sup>1-3</sup>. A neat demonstration of this is available by blowing pure polynoxylin powder through a 'Freon' aerosol. Cultures of *Staphylococcus aureus*, treated with the polynoxylin immediately after propulsion, show no inhibition when sub-cultured more than 5 min, up to 1 h. After this period of time, growth is somewhat inhibited and, after 85 min, has ceased altogether. In all, after 4 years serially sub-culturing of all these organisms, for 360 passages, no insensitive strain of any strain has emerged.

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## RADIOBIOLOGY

### Proteases and the Depletion and Restoration of Skin Responsiveness to Radiation

THE intravenous injection of a vital dye (pontamine sky blue, 1.2 ml. of 5 per cent solution/kg body-weight) immediately after local irradiation of a rabbit's skin (1,000 r., 60-140 kV) is followed within a few minutes by an intense blueing of the irradiated area, allowing the investigation of tissue events hitherto unnoticed in the so-called latent period. This circumscribed leakage of dye in the early phase of inflammation is abolished if the animals are pretreated with total or partial body irradiation (400 r., 230 kV, half-value layer = 1.8 mm Cu) or systemically with alkylating agents, and also with softer radiations used in order to avoid effects on deep-seated organs. This unresponsiveness is temporary, as the restoration of local reactivity occurs within six days in the experimental conditions described<sup>1</sup>.

In a further series of experiments with partial body irradiation and exposure of varying sizes of area in the pretreatment, it was shown that the abolition or reduction of tissue response is proportional to the extent of the pretreated area and that distant and local factors are involved in the degree of tissue responsiveness<sup>2</sup>.

These observations have a two-fold clinical importance. First, they indicate the possibility of achieving a potentiation of radiation effects, a form of radiosensitization, by fractionation in time in which the periods of reduced responsiveness to radiation are taken into account or by fractionation in space (sieve method) in which volumes of tissues protected from radiation afford anchorage and starting points for the complex phenomena that make up the radiation-induced inflammation. Secondly, they indicate that in grafting work, apart from the preliminary total body irradiation, local irradiation of the graft area at intervals of time dictated by the dose used in the pretreatment may prove advantageous regarding the rejection of grafted tissues.

Measurements with the technique of Grant and Rothschild<sup>3</sup> as modified by Miles and Niven<sup>4</sup> showed that the observed changes in vascular permeability were not related to a fall in blood pressure after total body irradiation.

The lack of response to local irradiation after total body irradiation appeared instead to be correlated with the number of circulating leucocytes, as an investigation of these in peripheral blood showed that the reappearance of the response coincided with the return of the number of leucocytes to their original level. An analogy could therefore be drawn with the observations made by Humphrey<sup>5</sup> and Pepys<sup>6</sup> on the role of leucocytes in the variation of the Arthus phenomenon and the abolition of the Mantoux reaction caused by alkylating agents.

While it is accepted that the very early changes in vascular permeability are mediated by histamine<sup>7</sup>, the delayed response, the subject of the experimental work here described, is independent of the histamine effect. Antihistamines, such as mepyramine and triprolidine, do not affect the delayed permeability response to local X-rays. Also 5-hydroxytryptamine (in the rabbit present in very small amounts only) has been proved not to play a part in these phenomena; its antagonist, lysergic acid diethylamide, has no effect on the degree of leakage of dye.

Further investigations have shown that soya bean trypsin inhibitor has an effect similar to that of total body irradiation. The results indicate that enzymatic processes are involved in the described phenomena of reduction or abolition and restoration of tissue responsiveness. This was confirmed in a series of experiments by the use of amino-*n*-caproic acid as the proteolytic agent. The action of this amino-acid in diminishing the capillary permeability supports the suggestion that this phase of inflammation is initiated by a protease system liberating proteolytic portions pharmacologically active on capillary endothelium.

However, the influence of the structure of these macromolecules has also to be considered, as was shown in another series of animals by the marked effect on the first phase of inflammation of meso-inositol hexanicotinate ('Hexopal', Bayer-Winthrop, Ltd.). It was similar to the effect of meso-inositol used by Brinkman<sup>8</sup> in his investigations of substances with radioprotective or radiosensitizing properties.

Without elaborating too much it can be stated that the reactions observed are due to an interplay or balance between permeability factors and their inhibiting enzymes. It is possible that the pretreatment at first depletes the amount of inhibiting enzyme and hence gives free way to the permeability-increasing factor until the inhibiting enzyme is restored (24-36 h) or the promoting enzyme is depleted.

There may be also an increase of pharmacologically active poly- or oligo-peptides due to tissue demolition products or the acid-alkaline phases in blood and tissue following irradiation, and these may influence the enzymatic balance, as it is accepted that acidosis in itself enhances capillary permeability and thus plays a part in inflammation.



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## BIOLOGY

### Diagrams of Changes in the Distribution of Plant Dry Weight or Other Variables

IN investigations of plant development it is helpful to be able to represent diagrammatically the changes which occur in the distribution of dry weight, total nitrogen or some other variable as the plant grows. It is usually desirable to distinguish at least four components of the plants of different physiological function, namely, leaf laminae, stems plus petioles, flowers plus fruits and roots, though data for the last-mentioned are often lacking. The values of the variable for each of these components should be plotted against a suitable independent variate which may be, for example, age, node number, leaf area or total dry weight. If the variable for the four components is plotted as four ordinary two-dimensional graphs, whether independently from the same base line or superimposed to build up the total, it is not easy to see whether the ratios of the values for the different components are constant or changing with growth of the plant. This can be seen by plotting logarithms of the values of the variable, but then their absolute magnitudes are obscured.

These difficulties have been partly circumvented by developing a substitute for a five-dimensional diagram. An example is shown in Fig. 1, which represents the distribution of dry weight in the barley plant during the second half of the growth cycle when ear development takes place (data for season 1938 from ref. 1). To plot such a diagram triangular graph paper ('isometric grid') is used and one of the three axes is selected for the independent variate (marked 'days from sowing' in Fig. 1). At any point on this axis a vertical line downwards gives the value of the variable for roots ( $R$ ), a vertical line upwards for flowers plus fruits ( $F$ ), a line to the right for leaves ( $L$ ) and one to the left for stems plus petioles and seedlings ( $S$ ). In the example chosen for Fig. 1 there are, of course, no petioles and the leaf sheaths have been included with 'stems'.

The ends of the lines for roots, leaves, flowers and stems may be joined to give four triangles. If the partition of dry weight has not changed with growth of the plant, the corresponding triangles will be similar and the corresponding lines all parallel. Changes in ratio of dry weights for  $R:S$ ,  $R:L$ ,  $L:F$  and  $S:F$  can be readily seen from the changes in shape of the appropriate triangles. It is a weakness of the diagram that this type of comparison is only available for four of the six possible ratios, which depend on the directions chosen for plotting the variable or the four components, and in Fig. 1 the changes in ratio of dry weight for  $L:S$  and  $F:R$  are not so easily seen. To assist in these last comparisons the  $L$  dry weight may be marked off on the  $S$  line and the  $F$  dry weight on the  $R$  line by short strokes (Fig. 1).

As an alternative to drawing the triangles the parallelograms may be completed, as has been done for one occasion (81 days) in Fig. 1. The changes in their shapes then indicate the changes in ratios. This method has the

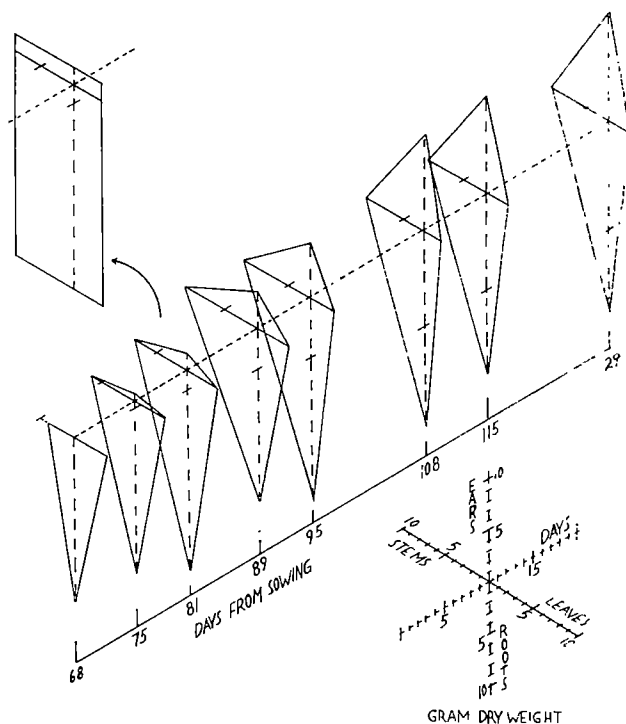


Fig. 1

advantage that it also indicates changes in  $R:(L+S)$ ,  $F:(L+S)$ ,  $L:(F+R)$ ,  $S:(F+R)$  and  $(F+R):(L+S)$ .

If desired the successive values of each component may be joined to emphasize the relations with the independent variate, and an example of this may be seen in ref. 2.

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### Ion Uptake and Protein Synthesis in Enzymatically Isolated Plant Cells

THE D-threo isomer of chloramphenicol is an antibiotic and a specific inhibitor of protein synthesis in bacteria<sup>1</sup>. It also inhibits protein synthesis (net synthesis or turnover) in higher plants<sup>2-6</sup>, including cell-free systems<sup>7,8</sup>. Suppression of the induced increase in oxygen uptake in potato disks by D-chloramphenicol<sup>9</sup> has been considered as indirect evidence of an inhibition of protein (possibly cytochrome oxidase) synthesis. Enzyme development during germination was also inhibited by D-chloramphenicol<sup>10,11</sup>. Recently, this chemical has also been used in several systems to relate ion uptake to protein synthesis in higher plants<sup>2,3,6,12-16</sup>. Others oppose this interpretation<sup>17-20</sup>. Meanwhile, the extrapolation of information on chloramphenicol from bacteria to higher plant systems has been criticized<sup>4,18</sup>. Ellis<sup>18</sup>, for example, observed that D-chloramphenicol did not affect incorporation of amino-acids into protein (trichloroacetic acid (TCA)-insoluble fraction) and yet it reduced ion uptake in higher plants. In contrast, the L-threo-isomer of chloramphenicol, which is neither an antibiotic nor an inhibitor of protein synthesis in bacterial systems, does inhibit ion uptake<sup>18</sup> and root growth<sup>21</sup> in higher plants. Thus, further elucidation was necessary as to the action of chloramphenicol as an inhibitor of ion uptake via an effect on protein synthesis.

The comparative effects of the D- and L-threo isomers of chloramphenicol on rubidium uptake and protein synthesis were determined on a mixture of palisade and

mesophyll cells enzymatically isolated from partially expanded, green tobacco leaves (*Nicotiana tabacum* L. Burley type). Cell separation procedures were modified after Zaitlin<sup>23</sup>. The separation mixture contained pectinase 0.2 per cent, peptone 0.2 per cent, glycerol 2 per cent, sucrose 0.1 M, ethylenediamine tetraacetic acid (EDTA) (pH 6.4) 0.02 M, *tris*(*tris*-(hydroxymethyl)amino methane) maleate (pH 6.4) 0.02 M, K<sub>3</sub>-citrate 0.01 M and Na<sub>2</sub>-succinate 0.01 M. Isolated cells were washed with cold 0.35 M sucrose containing 0.0002 M CaCl<sub>2</sub>, and suspended in ice-cold 0.35 M sucrose until utilized. Isolated cells retained only 25 per cent of protein (TCA-insoluble fraction) originally found in the leaf lamina of comparable weight. Further details of the methods for separating leaf cells and mechanisms of ion uptake by these cells will be published elsewhere<sup>23</sup>.

Investigations of the uptake of rubidium proceeded as the cells were suspended in solutions in 125-ml. Erlenmeyer flasks in a water-bath shaker in the light (about 500 ft.-candles) and at 25 ± 1° C. Each flask contained the following in 15 ml.: living cells equivalent to 133 mg dry wt., sucrose 1.76 mmoles, Na<sub>2</sub>-succinate 30 μmoles, NaHCO<sub>3</sub> 30 μmoles, MgCl<sub>2</sub> 1.5 μmoles, peptone 3 mg, casein hydrolysate 0.1 per cent, *tris*-maleate (pH 6.4) 150 μmoles, and 250 p.p.m. chloramphenicol as a variable. Cells were pre-incubated for 11 h in the aforementioned solution before the introduction of 0.5 μc. <sup>86</sup>Rb/1.5 μmole RbCl plus 'Tween-20' (polyoxyethylene (20) sorbitan monolaurate, final conc. 0.01 per cent). Treatments were duplicated, and the absorption period was 12 h. Cells in 1-ml. portions of incubation mixture were collected on filter paper (2.1 cm diam.), with mild suction, and washed with 1 ml. of 0.35 M sucrose. Air-dried samples were used for radio-assay.

Similar experimental conditions and procedures were utilized in investigations of the absorption of glycine-1-<sup>14</sup>C and its incorporation into the protein of suspended leaf cells. Each flask contained the following in 7.5 ml.: living cells equivalent to 50 mg dry wt., sucrose 880 μmoles, glycine-1-<sup>14</sup>C 1 μc. (specific activity of 249 μc./μm), casein hydrolysate at 0.1 per cent, peptone 1.5 mg, Na<sub>2</sub>-succinate 15 μmoles, NaHCO<sub>3</sub> 15 μmoles, MgCl<sub>2</sub> 0.75 μmoles, *tris*-maleate (pH 6.4) 75 μmoles, and 'Tween-20' at 0.01 per cent. At the end of an experiment the incubation mixtures were quantitatively transferred to centrifuge tubes. Cells were spun down by centrifugation, and supernatants discarded. They were then washed by resuspension and centrifugation with 5 ml. of 0.35 M sucrose. Cell pellets thus obtained were resuspended in 15 ml. of 70 per cent ethanol. Incorporation of glycine-1-<sup>14</sup>C into protein was ascertained by counting the radioactivity remaining in the alcohol insoluble fraction after 8 h extraction. This alcohol insoluble fraction has been designated as protein<sup>24-27</sup>. Total absorption of glycine was expressed as the sum of radioactivity recovered in the alcohol soluble and insoluble fractions.

Absorption of rubidium was significantly reduced by both D- and L-threo isomers of chloramphenicol (Table 1). The L-isomer was 77 per cent as effective as the D-isomer.

Similarly, both isomers of chloramphenicol inhibited the incorporation of glycine into protein (Table 2), with the D-isomer correspondingly more effective than the L-isomer. There were no significant differences in the radioactivities recovered in the alcohol soluble fractions. The activities were 134, 144 and 129 c.p.m. for the control, D-chloramphenicol, and L-chloramphenicol treatments, respectively. This suggests that the primary

Table 2. EFFECTS OF D- AND L-THREO ISOMERS OF CHLORAMPHENICOL (250 P.P.M.) ON GLYCINE-1-<sup>14</sup>C UPTAKE, AND ITS INCORPORATION INTO THE PROTEIN OF CELLS ISOLATED FROM GREEN TOBACCO LEAVES

Treatment	Glycine uptake (c.p.m./12 h)	Glycine incorporation (%) <sup>a</sup>	% Reduction of incorporation
Control (no treatment)	197	32.1	—
D-Chloramphenicol	157	9.2	71.3
L-Chloramphenicol	155	10.6	48.3

<sup>a</sup> Represents that percentage of total glycine absorbed.

effect of chloramphenicol was on protein synthesis, and not on amino-acid uptake.

The parallel inhibition by the D- and L-chloramphenicol isomers of uptake of rubidium and incorporation of glycine into protein suggests that absorption of rubidium is closely correlated with protein synthesis. The isolation and identification of a particular protein associated with uptake of rubidium remain to be elucidated.

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### Precision of Thermocouple Psychrometers for measuring Leaf Water Potential

INCREASING use is being made of psychrometric methods of measuring vapour pressure in biological systems as a means of assessing the energy status of water in the system, using a small thermocouple as a psychrometer to measure relative humidity under constant temperature. The choice of equipment is obviously related to the use to which it is to be put, but for laboratory estimates of soil water potential the simplest equipment described to date is that of Monteith and Owen<sup>1</sup>. This method has been modified, by greatly reducing the size of both thermocouple and chamber, to ensure the rapid equilibration needed with plant tissue samples, and has then been used for making an assessment of the precision of measurements of leaf water potentials. Full details of the equipment used are given elsewhere<sup>2</sup>.

In testing precision the interest lies in the relation between measuring and sampling errors, and in the sources

Table 1. EFFECTS OF THE D- AND L-THREO ISOMERS OF CHLORAMPHENICOL (250 P.P.M.) ON UPTAKE OF RUBIDIUM BY CELLS ISOLATED FROM GREEN TOBACCO LEAVES

Treatment	Rb uptake (μmole/mg dry wt. × 12 h)	Per cent inhibition
Control (no treatment)	0.42	—
D-Chloramphenicol	0.07	83
L-Chloramphenicol	0.15	64

f these errors. The equipment is calibrated against sodium chloride solutions of known molality, which give known vapour pressures at 25° C, and the precision with which relative humidity can be measured in the chambers is shown by the variation between measurements made with standard NaCl solutions. The assessment of error involved in measurement of leaf water potential is complicated by the fact that large numbers of leaves known to have the same water potential are not available, as it has been found that leaf water potentials may vary within a plant according to the position of leaves on the stem. For estimates of this error it therefore seemed advisable to regard only the two halves of a symmetrical leaf, lying on either side of the midrib, as having potentially identical water status. Estimates of precision were therefore based on an investigation of such paired samples.

Four pairs of thermocouples were used and the same pairings maintained for all determinations. Osmotic potentials were estimated on the same leaf samples, following snap freezing with solid carbon dioxide to release cell sap<sup>3</sup>. The species used was *Salvia patens*, originally elected for its convenience in making measurements of leaf expansion over short intervals of time. Twenty-four fully expanded leaves were used from plants that had been subjected to varying degrees of water strain (by allowing the soil to dry out), and two samples from each leaf were examined in turn for leaf water and osmotic potentials. Finally, measurements were made on NaCl solutions of 0.1 M, 0.2 M, and 0.3 M molality, covering approximately the same range of potentials as the leaf tissue determinations. Two samples of each solution were used with every thermocouple, giving a total of 48 determinations, as with the plant material.

Table 1. STANDARD ERROR OF DETERMINATIONS OF POTENTIALS (Metres of water)

Determination	Mean potential of each series	Range of potentials in the series	Within-chamber error	Sampling error
Water potential of tissue	-66.0	-23 to -127	±0.66	±2.51
Osmotic potential of tissue after freezing	-99.0	-60 to -118	±0.80	±1.71
Potential of salt solution	-94.0	-47 to -141	±0.90	±0.91

In order to assess the main sources of error, it was necessary to examine the variation between (a) successive determinations on a particular sample and (b) simultaneous determinations on twin samples taken from the same leaf or from the same stock solution of NaCl. Readings were taken 4 and 5 h after the samples were taken, by which time the system was at vapour equilibrium<sup>2</sup>, and the mean of these two readings was used in calculating standard errors for each series of determinations, that is, for the water potentials of tissue and salt solutions and the osmotic potentials of killed tissue respectively. The standard error of the mean of two successive readings taken for each individual sample can be regarded as 'within-chamber' error (Table 1), while the standard error of the mean of the pair of tissue samples for each leaf is the sampling error. Comparable values derived in the same way have been calculated for the determinations of osmotic potentials and salt solution potentials.

Within-chamber error for salt solutions, due mainly to limitations in temperature control, circuitry, and/or vapour leaks, was relatively small. The within-chamber error for leaf water potential is always likely to be greater than this, since it includes variations due to physiological activity of the tissue between the 4th and 5th h, but this effect was apparently small since the standard error was similar to that of the salt solution. Similarly, errors due to changes in osmotic potential between the 4th and 5th hours were unimportant. The sampling error for paired samples of salt solutions includes the variation introduced by handling the thermocouples and chambers in the preparation of each sample. Possible sources of error include contamination of the couples during exposure to the room atmosphere, inequalities of pressure between paired chambers arising during insertion

of the bungs (though precautions were taken to prevent these), and differing positions of the thermocouples relative to the evaporating surfaces. The measured error ( $\pm 0.91$  m) was much smaller than those associated with plant tissue samples ( $\pm 2.51$  and  $\pm 1.71$  m for water potentials and osmotic potentials respectively). It was therefore concluded that temperature control, circuitry and technique were adequate for the measurement of water and osmotic potentials of leaf tissue within the limits of accuracy imposed by sampling. The range of leaf water potentials which is of interest in irrigation research is that between full turgor and first signs of wilting, by which stage it is generally accepted that growth has been seriously affected. In *Salvia* this range extended from zero to about -100 m of water, and the method proved amply sensitive to cover the whole of this range, with a degree of accuracy that makes it a useful technique for agronomic work concerned with the water relations of plants grown under a variety of soil conditions.

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### The Bank Vole, *Clethrionomys glareolus* Schreber: a Mammal New to Ireland

ON August 20, 1964, one of us (A. J. M. C.), while trapping for small mammals near Listowel, County Kerry, caught an unusual 'mouse'. On subsequent examination it proved to be a member of the family Cricetidae, the bank vole, *Clethrionomys glareolus* Schreber—a family of mammals hitherto unknown from Ireland<sup>1-4</sup>.

The same area was again trapped during August 28-30 and a further 16 specimens were taken consisting of seven adult and three immature males, one immature and five adult females, in both breakback and Longworth live traps. These were all bank voles and were actively reproducing. Of the specimens caught by breakback traps, one female had three obvious foetuses (C.R. length 11 mm) *in utero*, and sperm production was evident in three of the males killed. The smallest animal (a male) weighed 11.2 g. Trapping was carried out in bramble and bracken banks and in dense grass and herb layers.

The density and ease of trapping of this species in this area is high: during the first 12 h (overnight) of trapping, one vole was caught per 3.83 traps. As the traps were not moved, this level dropped during the following 12 h (daylight) to one vole in 10.2 traps, excluding a *Mus musculus* (L.) caught during this period, and in the third 12 h (again overnight) only one vole was caught (1 per 36 traps). During this, the final trapping period, 4 *Apodemus sylvaticus* (L.), 1 *Mus musculus* and 1 *Sorex minutus* (L.) were also caught.

The replacement of the voles in this area, which was less than 1 acre in extent, might suggest that they are in active competition with these other species, especially *Apodemus*. However, despite this apparent competition it is more probable that *Clethrionomys* and *Apodemus* are not in active competition due to different activity cycles: *Apodemus* being essentially crepuscular and *Clethrionomys* diurnal, and because of the preference of voles for denser cover. Until extensive grid trapping is carried out it will be impossible to say with any certainty the habitat requirements of *Clethrionomys vis-à-vis Apodemus* in Ireland.

Preliminary examination of the skull suggests that the specimens are virtually identical with the British mainland and continental forms. A fourth inner loop is found on the third upper molar in some bank voles, which is regarded as a complex condition. This complexity occurs in 16 per cent of southern English bank voles, 33-41 per



cent in northern England and Scottish specimens, while Hebridean Island specimens show the complexity in c. 80 per cent of the cases<sup>4</sup>. Out of all the skulls examined (nine) in the present series, only one (11.1 per cent) showed the dental complexity of *M*<sup>3</sup>.

Comparisons of cranial characters were made with series of British mainland and continental specimens, by Dr. G. B. Corbet in the British Museum (Natural History). The characters compared were hind foot, condylo-basal length, zygomatic width, cranial width, interorbital width, anterior palatal foramina, length of maxillary tooth-row, relative lengths of *M*<sup>1</sup>, *M*<sup>2</sup> and *M*<sup>3</sup>, fourth inner loop of *M*<sup>3</sup>, length of nasals and width across condyles. The only detectable difference is that the nasals are on average shorter and the condyle width greater than British forms, but even here there is considerable overlap. From the evidence at present available it would appear that this Irish population is essentially similar to that in England. Trapping in neighbouring counties suggests that this population is restricted in distribution. Investigations now being carried out are aimed at establishing the present distribution of this species in Ireland.

We thank Dr. G. B. Corbet for making comparisons between the specimens and those in the British Museum (Natural History).

*Note added in proof.* Further specimens have recently been obtained from Co. Limerick, 50 miles from Listowel.

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### Environmental Acclimation in the Limpet *Patella vulgata* L.

THE differences in intertidal distribution of the two British species of prosobranch limpets *Patella vulgata* L. and *P. aspera* Lamarck have been well documented. *P. aspera* is limited to low shore-levels on shores exposed to wave action, while *P. vulgata* is widely distributed between high and low water levels on both exposed and sheltered shores<sup>1,2</sup>. During my investigation into the reasons for the differences in their distribution in the Clyde Sea area it was found that populations of *P. vulgata* from high shore-levels display physiological differences in several ecologically important characters when compared with those living at low shore-levels. Differences were observed in metabolic rate, the rate of loss of water under desiccating conditions and the ability to withstand loss of body water.

Metabolic rate, determined as the rate of respiration of whole animals in air, was used as an indicator of "internal physiological state" of the animals. Respiration was measured by means of a new type of constant pressure respirometer<sup>3</sup> and expressed as cubic mm of oxygen consumed/g wet weight soft parts/h. It was found that in summer the rate of respiration of high-level limpets is significantly lower than that of low-level limpets at temperatures of both 5°C and 15°C (Fig. 1). These differences in rate of respiration are not found during the winter months but reappear during the spring as the respiration rate of the low-level limpets begins to increase again. The respiration rate of the high-water limpets remains almost constant throughout the year.

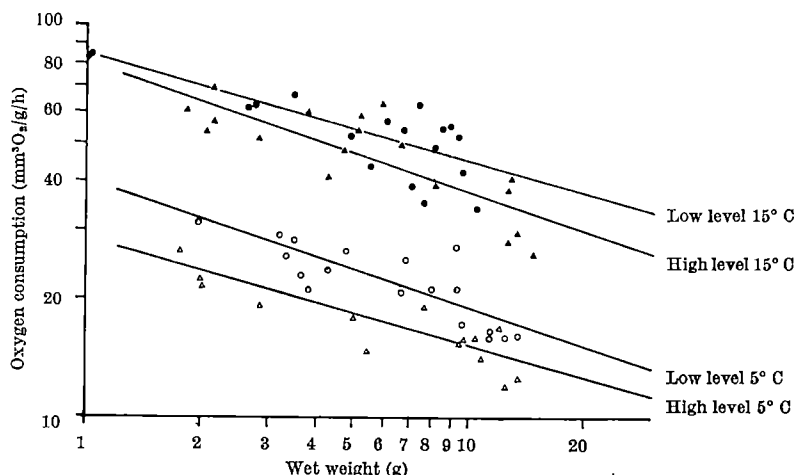


Fig. 1. Respiration rate of high-shore level ( $\Delta$ ,  $\blacktriangle$ ) and low-level ( $\circ$ ,  $\bullet$ ) *Patella vulgata* at 5°C and 15°C in July 1962. Regression lines are fitted by the method of least squares

For certain Californian molluscs, thermal acclimation has been postulated to explain differences in the rate of pumping of the gills in *Mytilus californianus*<sup>4</sup> and rate of heart beat of *Acmaea limatula*<sup>5</sup>, correlated with differences in tide level. In *Patella*, present results suggest that intake of food is also a causative factor, since in another series of experiments it was found that of all limpet populations on the seashore, the highest respiratory rate was recorded from those living beneath a cover of fucoid seaweeds and the lowest from a population living on rocks covered with barnacles. Reciprocal transplantation experiments demonstrated that these differences did not result from genetic selection, since after two months high-level limpets transplanted to low-level habitats had a higher respiratory rate than low-level animals which had been transplanted to high levels.

Under desiccating conditions similar to those experienced by the animals on the seashore when uncovered by the tide, it was found that the rate at which water is lost is related to the size of the limpets. In low-level limpets loss of water is proportional to the power  $-0.41$  of the body-weight, and in limpets from high water-levels, to the power  $-0.55$  of the weight. When compared at the same weight, low-level limpets lose water at a higher rate than high-level ones. In addition, there are differences in tolerance of loss of water during desiccation. In a sample of low-level limpets, 50 per cent succumbed to a loss of water of 50 per cent of their body weight while 50 per cent of a sample of high-level limpets were able to withstand a loss of water of 60-65 per cent of their body-weight.

It is clearly advantageous to limpets living under the adverse environmental conditions prevailing at high levels on the shore to maintain a lower metabolic rate, withstand greater losses of water and to lose water more slowly than those living at low shore-levels. Evidence is accumulating that ability to acclimate to wide ranges within the environment is of prime importance in the wide intertidal distribution of *P. vulgata*. From other studies, not detailed in this communication, it appears that this ability is not well developed in *P. aspera*.

This work was carried out during the tenure of a Department of Scientific and Industrial Research research studentship, and I thank Dr. C. H. Mortimer and Prof. C. M. Yonge for the provision of research facilities.

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## Peritrophic Membranes in the Phalangida

PERITROPHIC membranes have not been recorded in the Achnida although they are a widespread, but not universal, occurrence in insects. Phillipson<sup>1</sup> noted the occurrence of 'lamellated membranes' in the hind-gut region of *Itopus morio* and also in *Opilio*, *Platybunus*, and *Igolphus agrestis*, which he suggested were the source of the membrane of the faecal pellet. The purpose of the present communication is to record the presence of a peritrophic membrane in nymphal oligolophids and adult *Obunum rotundum* Lat. and *Phalangium opilio* Linn. though absent from the mid-gut diverticula it extends throughout the lumen of the alimentary canal and is composed of a series of concentric lamellae. It is therefore comparable with one of the two principal types found in insects.

After sectioning at 7 $\mu$ , measurement of the lamellae in photographs, at a magnification of  $\times 3,000$ , showed that each one was c. 0.5 $\mu$  in thickness. They were often situated close to the gut epithelium, are almost certainly homologous with the membrane of the faecal pellet noted by Phillipson<sup>1</sup>, and provided an interesting comparison with the membranes of insects. More detailed investigations are in preparation.

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## ENTOMOLOGY

## Batelli Glands of Cercopoid Nymphs (Homoptera)

Two major groups of theories have been proposed during the past 60 years or so to account for the formation of 'spittle' or foam by cercopoid nymphs. On one hand, the site of origin of the compound stabilizing the bubbles of foam is attributed to the glands of Batelli<sup>1-6</sup>, and the other to the Malpighian tubules<sup>7-10</sup>.

The Batelli glands have, from time to time, been supposed to produce a 'mucilaginous' compound<sup>2,5</sup> or a component or components of a 'soap'<sup>3,6</sup>. Such compounds, it is suggested, behave as surface tension depressants.

Batelli glands are swollen hypodermal glands, the precise location of which has been variously described. Batelli<sup>11</sup>, who first figured these glands, considered them to be on the last two abdominal segments. Gruner<sup>12</sup> and Porta<sup>13</sup> located them on the 7th and 8th abdominal segments, while Berlese<sup>1</sup> placed them on the 8th and 9th abdominal segments.

In the species of the family Cercopidae which I have examined, Batelli glands do not always appear to be present (Table 1). When present they are readily seen as ventro-lateral swellings on the 7th and 8th abdominal segments. The presence of 'wax plates', usually present ventrally at these sites, further facilitates identification. Although ventro-lateral swellings are not so readily discernible in adults, the presence of 'wax plates' in some adults serves to confirm the functional existence of the glands. As noted by Guilbeau<sup>2</sup>, setae appear to be absent from the cuticle in these regions.

In the species of the family Machaerotidae examined, Batelli glands are present on the 6th, 7th and 8th abdominal segments of nymphs of *Pectinariophyes stali* Grunberg and *Machaerota coronata* Maa. Maki<sup>14</sup> found Batelli glands on the 7th and 8th abdominal segments of nymphs of *Makiptyelus dimorphus* Maki; this observation was confirmed. Batelli glands also occur on the 7th and 8th abdominal segments of nymphs of *Chaetophyes compacta* Ulker. In these latter two species, however, the 6th

Table 1. PRESENCE OR ABSENCE OF BATELLI GLANDS IN CERCOPOID SPECIES

Species	Observed externally		Observed in sections	
	Nymph	Adult	Nymph	Adult
Sub-family cercopinae				
<i>Aenolamia varia saccharina</i> Distant	0	0	0	0
<i>Looris</i> sp.	0		0	
Sub-family aphrophorinae				
<i>Philaenus leucophthalmus spumarius</i> Linnaeus	+	+	+	
<i>Psophitus</i> sp.	+		+	
<i>Ptyelus</i> sp.	+		+	
<i>Bathylus albicinctus</i> Erichson	+	+	+	
<i>Clovina lineatocollis</i> de Motschulsky	+		+	

0, absent; +, present.

abdominal segment is modified ventrally to form part of an abdominal operculum.

The absence of Batelli glands in two species of the family Cercopidae suggests that these glands are not involved in the production of a bubble stabilizing agent in 'spittle'. This conclusion is supported by the presence of three pairs of glands in some nymphs of the family Machaerotidae, since the latter nymphs have very limited spittle-producing requirements and produce 'spittle' only at the times of ecdyses<sup>15</sup>.

It is of some interest that in the Cercopidae the two species lacking Batelli glands belong to the sub-family Cercopinae; the members of this sub-family are subterranean and produce 'spittle' masses on plant roots<sup>16</sup>. It has been suggested that the subterranean habit is a primitive adaptation to arid conditions<sup>17,18</sup>. Now Sule<sup>3</sup> and Gahan<sup>5</sup> both considered the secretion of the Batelli glands to be a wax, and Kato<sup>8</sup> detected a lipid which stains with sudan black B in these glands, an observation which I have confirmed (unpublished). Thus there seems little doubt that the Batelli glands produce a lipid substance which is possibly a wax. The production of such a substance may therefore be associated with the prevention of desiccation in above-ground dwelling cercopoid nymphs.

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Biochemical Changes in *Semiadalia undecimnotata* (Schneider) Adults during Diapause

THE function of food reserves during diapause is often discussed, but there are very few reliable data. The mass hibernation of adult *Semiadalia undecimnotata* (Schneider) (Coccinellidae) in accessible places allowed us to make analyses throughout the winter. Both sexes arrive at the winter quarters on warm, windless days from the end of July to the end of August, and leave from the end of April to the end of May, according to the temperature.

Samples were taken during three aestivo-hibernating seasons in the years 1957-59 in the Louny Hills in Northern Bohemia. Dry matter, glycogen and fat were determined<sup>1</sup>.

The absolute water content fluctuated considerably, from 6.8 to 13.5 mg in males, and from 7.9 to 16.2 mg

in females. During the eight-month period there was no general trend in either sex. The changes in water content may be attributed partly to the active uptake of water from the environment and partly to the production of metabolic water through fat metabolism. It seems probable that the Coccinellids drink water after their body water content has decreased during dry periods. The gradual increase in the relative water content is caused by the fall in weight of dry matter. In males the absolute water content is lower but the proportion of water is higher than in females.

Fat reserves are accumulated before migration. The digestive tract of both sexes is empty when they arrive at the winter quarters, and they do not feed until after they leave. A decrease in fat content during aestivo-hibernation is therefore to be expected, and was observed in all three seasons. At the beginning of the diapause (August 29, 1958) the fat content was as high as 6.4 mg in the males and 7.2 mg in the females, and this fell to minimum values of 1.2 and 1.7 mg in the males and females, respectively, at the end of aestivo-hibernation (May 9, 1959). Undoubtedly the actual decrease was even greater because the first sample for analysis was taken about one month after the Coccinellids had arrived at the winter quarters. The results (Table 1) show that the fat reserves are important as a source of energy during migration to the winter quarters and also during aestivo-hibernation. The ripening of ovaries in spring begins only after the Coccinellids have begun feeding.

The rate of decrease in fat reserves evidently depends on the outdoor temperature, which affects the activity and the metabolic rate of the insect (Table 1). The fall is very steep in the initial warm phase of aestivo-hibernation, whereas in the cold phase (November–March) there is only negligible daily decrease. The warmer spring weather increases the rate of fat metabolism during April and at the beginning of May.

The fat contents of the insects taken on similar dates in different years were similar. It is surprising that the trend of decrease in the fat reserves is essentially similar in both sexes despite substantial differences in the condition of their gonads. The female ovaries are in a state of undifferentiated germaria throughout diapause. In contrast the testicular follicles of the males show active meiosis except during the coldest period of hibernation<sup>2</sup>. Apparently the activity of testes requires relatively little energy and therefore does not make significant demands on the fat reserves. A similar situation has been described in *Pyrhocoris apterus* L.<sup>3</sup>.

The absolute as well as relative content of glycogen fluctuated considerably throughout aestivo-hibernation, although there was a general decrease. The highest absolute values were recorded at the beginning of the diapause, 388 and 432 $\gamma$  in the males and females, respectively (August 29, 1958), the lowest at the end

of the diapause, 94 $\gamma$  in the males (April 23, 1957) and 71 $\gamma$  in the females (May 13, 1958). Females always had more. In spite of many irregularities it can be seen (Table 2) that the environmental temperature affects the rate of decrease of glycogen. The amount of glycogen fluctuated much less than the glycogen reserves in samples taken at any one time as well as at different times.

A comparison of our results with data on other insect species shows that *S. undecimnotata* has the highest fat content of any so far described, whereas the glycogen content, compared with rather contradictory and inadequate data from other insects, seems to be relatively very low.

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### Multiplicity of Neurosecretory Cell Types and Groups in the Brain of the Saturniid Moth *Hyalophora cecropia* (L.)

As part of an extensive investigation of the anatomy and histology of the known endocrine organs of *Hyalophora cecropia* in the last 2 larval stages and in pupae and adults, we have examined the brain neurosecretor (NS) cells utilizing paraffin sections and the standard N stains, paraldehyde-fuchsin (PAF) with counterstains an chrome-haematoxylin (CH) with phloxine. Previous workers<sup>1,2</sup> had reported the existence of 26 apparently identical NS cells in the brain of this insect. Our results demonstrate the presence of at least 40 such cells and provide histological evidence for the occurrence of several distinct functional types.

The problems involved in correctly identifying NS cells are complex<sup>3</sup>. We have defined such cells in this animal as neurones which undergo alterations in their content of prominent stainable inclusions and which exhibit cytological evidence of axonal transport of the stainable materials to the corpora cardiaca. All the cell types discussed below fulfil these criteria.

Putative NS cells occur in 3 locations in the pars intercerebralis (PI) of the protocerebrum of this moth. They form the 2 major groups characteristic of insects (the pair medial and lateral nuclei) and also form a third pair clusters in the posterior PI. Eleven cells of 4 distinguishable types occur in each medial cluster, whereas the lateral nuclei each contain 7 cells of 2 distinct types. The posterior nuclei are each composed of 2 cells of a single type. Fig. 1 is a representation of the locations of the various cells and Table 1 lists their dimensions.

The NS cells of this insect fall into 2 major categories. One category, designated as 'A' cells, is characterized by cytoplasmic inclusions staining shades of purple with PAF and blue-black with CH. The inclusions of the second category of cells, the 'B' cells, usually stain shades green with the PAF counterstains and reddish with phloxine. The cells of each major category can be divided into sub-categories on the basis of size, location and number. In addition, each sub-category is characterized by periods of accumulation and depletion which are not identical to those occurring in cells of any other sub-category. Although the exact implications of these fluctuations are not as yet fully understood, they are interpreted as variations in the functional activity of the cells involved.

(1) The 'A' cells.

(a) The large medial 'A' cells. Cells of this type are the largest 'A' cells of the medial cluster in the larva

Table 1. DECREASE OF FAT CONTENT IN *S. undecimnotata* IN DIAPAUSE

Date	Temperature (pentad average °C)	Days	Decrease of absolute weight of fat per insect (mg)			
			Total	Daily average	♂	♀
27.3.-30.4.57	8-13	34	0.6	0.2	0.018	0.006
23.10.57-8.4.58	-2-+8	167	1.5	—	0.009	—
8.4.58-5.5.58	3-9	27	1.2	0.7	0.045	0.026
29.8.-25.9.58	15	27	2.2	1.8	0.082	0.067
25.9.-22.10.58	7-14	27	0.6	1.3	0.022	0.048
22.10.58-2.4.59	-3-+10	162	1.4	1.2	0.009	0.007
2.4.59-9.5.59	8-14	37	1.0	1.2	0.027	0.032
29.8.58-9.5.59	—	253	5.2	5.5	0.021	0.022

Table 2. DECREASE OF GLYCOGEN CONTENT IN *S. undecimnotata* IN DIAPAUSE

Date	Days	Decrease of absolute weight ( $\gamma$ ) of glycogen per insect			
		Total	Daily average	♂	♀
29.8.-22.10.58	54	136.5	148.7	2.5	2.8
22.10.58-2.4.59	162	132.2	108.9	0.8	0.7
2.4.-9.5.59	37	34.4	72.6	0.9	2.0
29.8.58-9.5.59	253	303.1	328.2	1.2	1.3



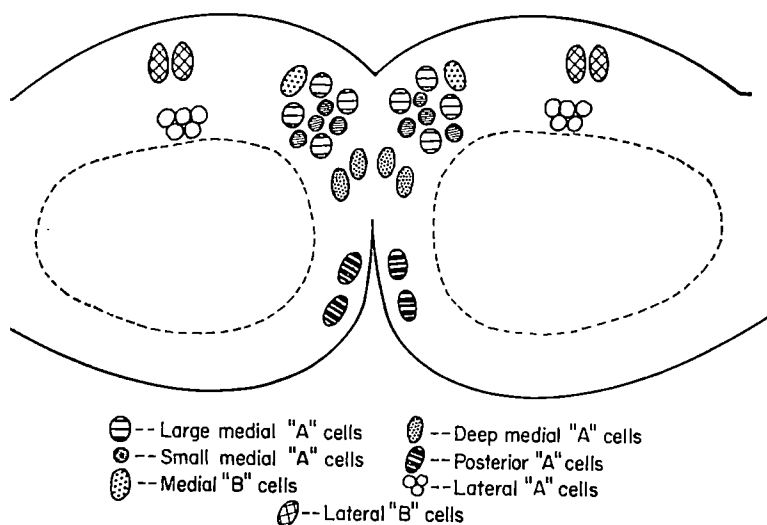


Fig. 1. A diagrammatic representation of the locations of the various types of neurosecretory cells in the brain of *Hyalophora cecropia*.

ages. Their inclusions are small and, in larval stages, are prominent than those of the small medial 'A' cells. These cells exhibit minor alterations in their content of inclusions in larval stages and major alterations in older animals. Four cells of this type can be readily distinguished in each medial cluster in larvae and young pupae; in older pupae and adults they are similar to the small medial 'A' cells and are less readily identifiable.

(b) The small medial 'A' cells. Four cells of this type are easily recognized in each medial cluster of larvae and young pupae where their inclusions are larger and more deeply stained than those of the large medial 'A' cells. Prominent variations in the number of inclusions in these cells occur only in pupae and adults.

(c) The deep medial 'A' cells. Two of these cells are conspicuous in the ventral region of each medial nucleus during the pupal and adult stages, but these cells cannot be easily identified in larvae. In adults they are frequently the largest of the several types of 'A' cells. They only maintain prominent inclusions in young pupae.

(d) The posterior 'A' cells. As with the deep medials, these cells are not conspicuous in most larval stages. In the fifth instar larvae and older animals they usually maintain prominent inclusions.

(e) The lateral 'A' cells. Five of these cells form a cluster on or near the dorsal surface of the neuropile. Typically the smallest of the 'A' cells, and possessing the smallest nuclei, they are devoid of inclusions during portions of each stage investigated.

## (2) The 'B' cells.

(a) The medial 'B' cells. One cell of this type lies in the dorsal region of each medial cluster. These cells exhibit fluctuations in their content of inclusions during each stage of the life-cycle, but such variations are most prominent in pupae and adults.

(b) The lateral 'B' cells. Two cells of this type lie at a short distance lateral and dorsal to the lateral 'A' cells. Unlike the medial 'B' cells, they contain both 'A' and 'B' type inclusions late in each larval stage. Like the medial 'B' cells their alterations are most prominent in later developmental stages.

Table 1. MAJOR DIAMETERS ( $\mu$ ) OF BRAIN NS CELLS AND THEIR NUCLEI IN *H. cecropia*

	Cells	Nuclei
'A' cells		
Large medials	25-40	10-13
Small medials	15-25	7-10
Deep medials	25-45	10-15
Posteriors	20-30	10-13
Laterals	15-25	6-9
'B' cells		
Medials	25-30	9-12
Laterals	20-25	8-11

The 'A' and 'B' cells are the only neurones in the brain of this species which meet the criteria for NS cells already mentioned here. Other large neurones are intermingled with the presumed NS cells, and such large neurones are also found in other regions of the brain cortex. In one 2-day-old developing adult a single cell was observed near one optic lobe which contained prominent 'A' type inclusions in both perikaryon and axon. The possibility therefore exists that other NS cells may occur in the brain of this moth.

Although the brain of *H. cecropia* has only been proved to produce a single hormone<sup>4</sup>, the occurrence of several apparently distinct NS cell types suggests that this brain may be the source of several hormones. Our findings are similar to those reported for other Lepidoptera<sup>5-10</sup> and appear to be duplicated in 2 other saturniids which we have investigated, *Antheraea polyphemus* and *Philosamia cynthia*. In particular, the posterior clusters appear likely to be characteristic of this order<sup>8-10</sup>.

The probability exists that the insect brain in general contains not only a multiplicity of NS cell types but also groups of NS cells located in other than the classical medial and lateral nuclei; support for this view has already emerged from investigations of representatives of such diverse orders as the Apterygota<sup>11</sup>, Dictyoptera<sup>12</sup> and Diptera<sup>13</sup>.

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## MICROBIOLOGY

### Microscopic Picture of Reversion of Glycine Spheroplasts into Rods in *Escherichia coli*

In recent years much attention has been devoted to the reversion of spheroplasts into normal cellular forms of Gram-negative bacteria from both the biochemical and morphological points of view.

Until now—so far as we are aware—only Jeaynes<sup>1</sup> has observed the evolution of spheroplasts prepared under the influence of glycine ( $\alpha$ -aminoacetic acid). He described the reproduction of *Vibrio cholerae* spheroplasts and also mentioned their reversion into cellular forms while decreasing the glycine content in the medium gradually;

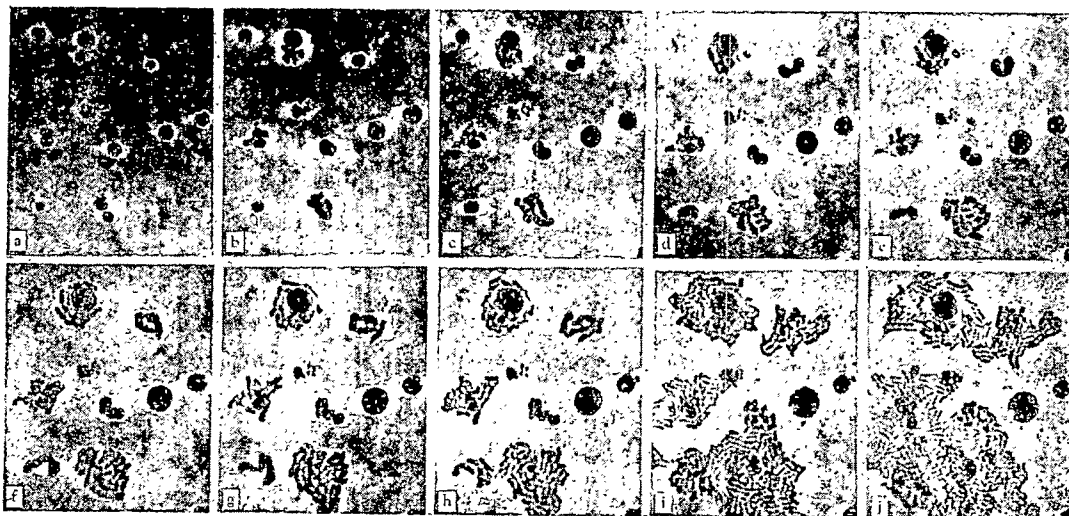


Fig. 1. Growth, division and regeneration of glycine spheroplasts of *Escherichia coli*: a, 1 h; b, 3 h; c, 4 h; d, 4.5 h; e, 5 h; f, 5.5 h; g, 6 h; h, 6.5 h; i, 8 h; j, 9 h ( $\times 750$ ).

he did not describe, however, through which morphological changes this reversion took place.

Our observations of the regeneration of glycine spheroplasts of *Escherichia coli* may be of some interest in this connexion. They were made in the course of control experiments when studying the morphological changes of the spheroplasts caused by colicin<sup>2</sup>.

We prepared spheroplasts from bacteria of strain I7 of *E. coli*<sup>3</sup> influenced by 3 per cent (w/v) glycine according to Jeynes<sup>4</sup>. Separated from the glycine medium and resuspended in broth, a drop of the suspension was placed on a low meat-peptone agar block on a slide, covered with a coverslip and framed by paraffin. These preparations were observed under a phase contrast microscope. Fixed fields of vision were photographed at different time limits<sup>4</sup>.

Part of the spheroplasts always lysed after having been transferred to the agar block; with the strain used in our case, however, the lysis did not exceed 20 per cent in the course of the first 3 h. About 50–90 per cent of spheroplasts grew and regenerated into rods. Growth of the majority of spheroplasts was conspicuously rapid—within the first hour—and regeneration of the rods began in 3 h.

Three kinds of regeneration were observed: (a) through a direct bipolar growth of the spheroplast into a rod, without previous division (this type of regeneration of spheroplasts has not been described so far); (b) after a single or double successive transverse division of the growing spheroplast (which corresponds to the finding of Nermut and Svoboda<sup>5</sup> on lysozyme spheroplasts); (c) after the fragmentation of a grown spheroplast.

We have not observed regeneration through growth and fragmentation of large long bodies, seen by Nermut<sup>6</sup> with penicillin spheroplasts.

After 7 h the regenerated rods covered more than half the preparation (Fig. 1).

In contrast, we have not observed any regeneration of spheroplasts in the *BI* strain of *E. coli*, in spite of the fact that even in this case about 30 per cent of spheroplasts grew and underwent many divisions in the preparations. It is obvious that the same factor in the same conditions can damage the cell wall to a varying degree in different strains.

In strain I7 glycine and penicillin spheroplasts presented a similar picture<sup>4</sup>. Both these types were on the whole constant, even in the broth without an osmotic stabilizer, and both rapidly regenerated into rods in a great majority of cases. In these qualities they differed from lysozyme spheroplasts.

Our observations accord with the view that glycine acts on the cell in a similar way as does penicillin, that is, it distorts the biosynthesis of its wall. The biochemical

nature of this inhibition in the two cases is, however, different.

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### Production of a Soluble Substance by *Corynebacterium ovis*

*C. ovis* produces zones of haemolysis when grown on nutrient agar plates containing blood from various animal species, but no soluble haemolysin has been demonstrated and it would appear that the haemolytic effect is dependent on the presence of living bacteria in the haemolysate system<sup>1–3</sup>. The purpose of this communication is to note the production of a diffusible antigen by *C. ovis* which in certain circumstances is capable of causing haemolysis.

Three strains of *C. ovis* were inoculated into digest broth (modified medium of Carne<sup>4</sup>), incubated 7 days at 37° C and centrifuged until the supernatants were sterile. Two strains each of *C. equi* and *C. renale* were treated in similar manner. The sterile supernatants were pipetted into sealed wells, 6.0 mm in diameter, in 5 per cent blood agar plates which were incubated at 37° C.

After 24 h, a 3-mm wide zone of partial haemolysis appeared round the wells containing supernatants of *C. ovis* though not round those of *C. equi* and *C. renale*. The zone became more distinct on further incubation or the plate was refrigerated. The *C. ovis* preparations were active at 1/4 but not at higher dilutions.

In a second experiment, a *C. ovis* supernatant was mixed with an equal volume of a potent *C. ovis* antitoxin and shaken at 37° C for 30 min before testing by the method described here; haemolysis was completely inhibited. The antitoxin had no inhibitory effect on a sample of staphylococcal  $\beta$ -haemolysin, and normal rabbit serum without effect on either preparation when similarly treated (see Fig. 1). Filtration of 15 ml. of *C. ovis* supernatant through a Seitz EK pad removed its haemolytic power, did heating at 75° C for 15 min.

The three aforementioned properties possessed by this diffusible haemolytic agent were also found to be possessed by *C. ovis* toxin, and such similarity suggests that both the toxin and the haemolysin of *C. ovis* might well be the

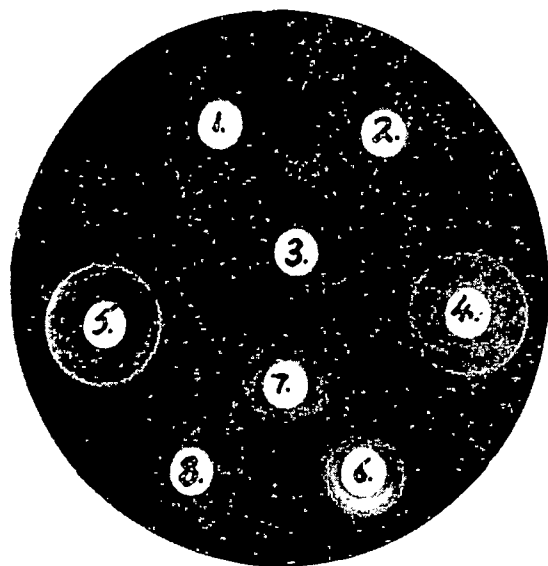


Fig. 1. Blood agar plate incubated at 37° C for 48 h (actual size). 1, *C. equi* supernatant; 2, *C. renale* supernatant; 3, digest broth; 4, a mixture of staphylococcal  $\beta$ -haemolysin, diluted 1/20, and normal rabbit serum; 5, staphylococcal haemolysin mixed with *C. ovis* anti-toxin; 6, *C. ovis* supernatant diluted 1/2; 7, mixture of *C. ovis* supernatant and normal rabbit serum; 8, mixture of *C. ovis* supernatant and the immune serum.

time; a similar relationship has been reported between the toxin and the soluble haemolysin of *C. pyogenes*<sup>5</sup>. A more detailed report of this work will appear at a later date.

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## GENETICS

### Effect of Mitomycin C on Early Phenotypic Expression in the Transformation of *Diplococcus pneumoniae*

CONCERNING the transcription of genetic information by DNA, recent investigations, using systems of the transformation of *D. pneumoniae*<sup>1</sup> and infection with some bacteriophages<sup>2-4</sup>, have demonstrated that the DNA molecule consists of an informational and a non-informational strand. Guild and Robinson<sup>1</sup> succeeded in obtaining actions of denatured transforming DNA in CsCl density-gradient centrifugation which seemed to correspond to each polynucleotide chain, and they examined the time course of the appearance of cells possessing the new phenotype. It was observed that the expression of the new phenotype by one fraction was retarded for about one generation period compared with the other fraction. The messenger RNA synthesized after infection with bacteriophages<sup>2,3</sup>, or the RNA synthesized enzymatically in an *in vivo* system<sup>2,4</sup>, were examined for hybrid formation with polynucleotide chains separated in CsCl density-gradient centrifugation. The data suggested that the messenger RNA produced *in vivo* can form a ribonuclease-resistant hybrid with the unique polynucleotide chain of the primer DNA.

During the course of investigations on the transformation of *D. pneumoniae*, we have found evidence which supports this hypothesis. In the transformation of *D. pneumoniae* by DNA marked with streptomycin-

resistance, the new streptomycin-resistant cells appear about 10 min after DNA fixation, then they develop very rapidly and their number reaches the level of transformable cells after about 90 min<sup>5</sup>. The latter level remains almost constant up to this time. When mitomycin C in sub-lethal concentration (0.03  $\mu$ g/ml.) was added to a culture at the end of DNA fixation, the number of newly developed streptomycin-resistant cells was found not to reach the level of transformable cells even after prolonged incubation, for example, 3 h (Fig. 1). The time of appearance of these cells, and the rate of their development, did not vary significantly from the time and rate in the absence of mitomycin C. At the mitomycin level adopted in these investigations, there was no cellular multiplication or extensive death and there was also no significant increase in the amount of DNA when checked by the incorporation of <sup>3</sup>H-thymidine into the DNA fraction.

In order to confirm that the observed difference in the two levels is due to the presence of mitomycin C, an experiment was performed to follow the two levels after washing the drug during the course of transformation. A result is given in Fig. 2. It is evident that the level of streptomycin-resistant cells, which is lower than the level of transformable cells at 90 min, reaches that level in the period corresponding to one generation time of the cell. Repeated experiments showed that in the presence of mitomycin C, the ratio of the level of transformable cells to that of streptomycin-resistant cells was 1.5-2.0, indicating that approximately half of the population of transformable cells cannot express the new phenotype in the absence of DNA replication.

These results can be explained satisfactorily in the following way. Since in the absence of new DNA synthesis, half the population of transformable cells can express the new phenotype immediately and the other half cannot do so unless at least one round of DNA replication takes place, the former half would have fixed the informational strand and can synthesize new messenger RNA without DNA replication. The latter half, however, must have fixed the strand which is comple-

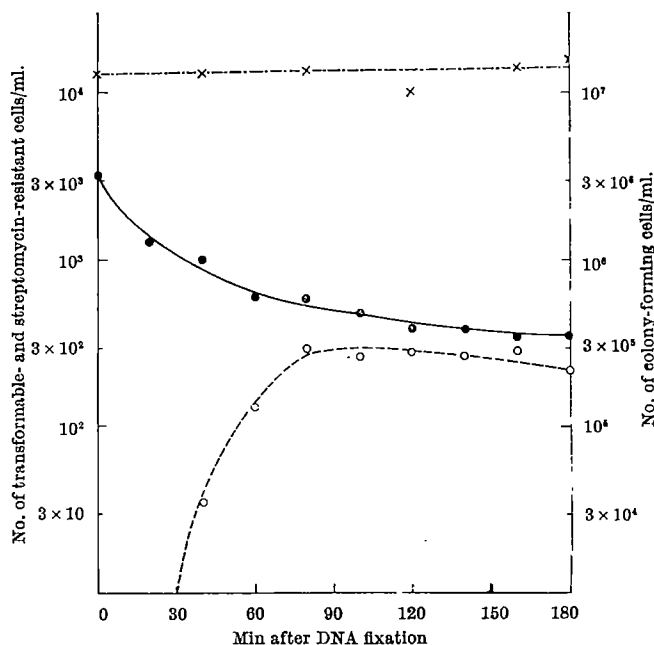


Fig. 1. Effect of mitomycin C on the development of streptomycin-resistant cells in the transformation of *D. pneumoniae*. DNA possessing streptomycin marker was added to a competent cell culture (0.5  $\mu$ g/ml.) and the latter was incubated for 7 min at 37° C. DNase (50  $\mu$ g/ml.) and mitomycin C (0.03  $\mu$ g/ml.) were added and the incubation was continued. At time intervals determinations were made in the usual way (ref. 7) with respect to the numbers of transformable cells (closed circles), streptomycin-resistant cells (open circles) and colony-forming cells (crosses). Selections were made at 100  $\mu$ g/ml. of streptomycin.



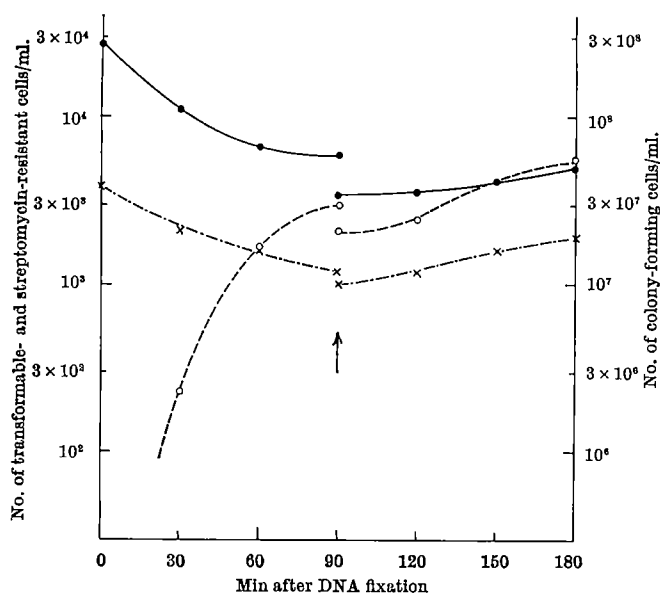


Fig. 2. Reversal of the effect of mitomycin C on the development of streptomycin-resistant cells in the transformation of *D. pneumoniae*. The experiments were performed in the same way as described in Fig. 1, except that the cell was washed after 90 min by centrifugation in the cold and reincubated in a fresh medium free from mitomycin C. In the figure the time of washing is indicated by an arrow. Symbols as in Fig. 1

mentary to the former half, and these cells come to be able to synthesize the messenger RNA after the informational strand is newly synthesized.

This view is based on two suppositions: (a) The recombination between the chromosomal DNA of recipient cells and the newly incorporated marker DNA must take place on a single-strand basis. This appears to be probable since Lacks<sup>6</sup> observed that a considerable part of the DNA comes to be single-stranded at the time of or immediately after incorporation into recipient cells of *D. pneumoniae*. (b) The genetic behaviour of both strands must be the same, so that both strands must be integrated with an equal probability.

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## PSYCHOLOGY

### Taste Perception and the Menstrual Cycle

SENSITIVITY to the taste of quinine and 6-*n*-propylthiouracil (PROP) has been determined throughout one or more menstrual cycles in a group of nineteen subjects. Taste tests were carried out at the same time each day, 3 days per week, for periods ranging from 4 to 9 weeks. The subjects were apparently healthy and normal females and all but two were student nurses. Their ages ranged from 19 to 27 (average 20.7). Subjects were requested not to take drugs during the experiment. Four additional

subjects were excluded from the analysis because of illness or medication.

Taste thresholds (that is, the lowest concentrations the compounds which could be distinguished from water) were measured by the method of Harris and Kalmus<sup>6</sup> modified by Fischer *et al.*<sup>4</sup>. The method involved separation by taste of sets of eight cups, four of solution and four of distilled water, into two groups. The mouth was rinsed with water between tasting each cup. Dilute solutions were first tasted, followed by solutions of increasing concentration, until the subject could correctly sort the cups.

Test solutions were prepared so that each whole number solution was twice the concentration of the preceding one. In addition, solutions were prepared at the midpoint in the series between the whole numbers. Solution No. 14 of PROP was made by dissolving 1.0212 g of the compound in 1 l. of distilled water. Solution No. 13.5 was made by diluting 282.8 ml. solution No. 14 with 400 ml. water. Number 13 was one-half the concentration of No. 14 and 12.5 was one-half the concentration of 13. Solution No. 13 of quinine, an almost saturated solution, was prepared by dissolving 1.1744 g of 1-quinine sulphate (U.S.P.) in 1 l. of distilled water, and diluting as for PROP. The molar concentration of a solution of a given number was the same for either compound. Solution No. 14 had a concentration of  $6.0 \times 10^{-3}$  M; solution 13.5,  $4.24 \times 10^{-3}$  M; 13,  $3.0 \times 10^{-3}$  M; 12.5,  $2.12 \times 10^{-3}$  M; etc. The most dilute solution used had a concentration of  $1.62 \times 10^{-8}$  M.

The days in the menstrual cycle were numbered forward and backward from the first day of menstruation. In those instances in which two cycles were recorded, the days were counted from the first day of menstruation in the second cycle. In the present report, sensitivity within three phases of the cycle have been compared: the pre-menstrual (days -9 to -5), menstrual (day -1 to +4) and post-menstrual (days +6 to +10). The average scores of each subject during each of these phases are shown in Table 1. The scores of the initial two tests of each subject have been excluded from the analysis, as several showed an improvement with practice. The variances of the scores are also given in Table 1. When calculating these values the scores on days -3 to +5 were excluded, so that the variances refer to the intermenstrual phases.

Several subjects continued to improve after the first two tests and therefore the most conservative comparison was between the menstrual and post-menstrual phases of the cycle. The final column of Table 1 shows the differences in scores between these two phases. In the majority of individuals thresholds tended to be lower (that is, more sensitive) during the menstrual period. Analysis by the Wilcoxon matched-pairs signed ranks test shows that these differences are statistically significant. In the two-tailed test, for PROP  $P < 0.05$ , for quinine  $P = 0.05$ , and for both combined  $P = 0.003$ . The average increase in taste sensitivity during menstruation was 0.68 threshold for PROP and 0.45 for quinine. When the scores for PROP and quinine were considered together, 18 per cent showed a decreased sensitivity during the menstrual phase compared with the post-menstrual, 16 per cent showed no change and 66 per cent increased in sensitivity. An increase of 0.5 thresholds or more was shown by 47 per cent of the total sample, 18 per cent had an increase of 1 or more and 8 per cent increased by 2 thresholds or more.

The responses of the subjects were markedly heterogeneous. The most extreme change was shown by subject No. 13. Six days before the onset of menstruation her scores for both compounds changed markedly and reached maximum sensitivity on day -3. On this day she could distinguish from water the remarkably dilute concentration of  $3.24 \times 10^{-8}$  M PROP and  $1.62 \times 10^{-8}$  M quinine. Her scores had returned to their usual values by day +1.

Table 1. MEAN TASTE THRESHOLD SCORES FOR PROP (P) AND QUININE (Q) DURING THREE 5-DAY PHASES OF THE MENSTRUAL CYCLE

Days -3 to +5 in cycle were excluded from calculations of variance		Difference (post-menstrual minus menstrual)			
subject	Variance (thresholds)	Pre-menstrual days -9 to -5	Menstrual days -1 to +4	Post-menstrual days +6 to +10	
2	P 0.88	10.0	7.5	8.25	+0.75
	Q 0.27	5.5	4.25	5.5	+1.25
3	P 0.09	4.5	4.25	4.25	0.0
	Q 0.18	4.75	4.25	4.5	+0.25
4	P 0.15	8.0	7.75	7.75	0.0
	Q 0.10	5.5	5.5	6.0	+0.5
5	P 0.25	10.0	9.0	9.83	+0.83
	Q 0.09	5.67	5.0	5.5	+0.5
6	P 0.47	8.0	7.5	7.25	-0.25
	Q 0.24	3.25	2.67	2.75	+0.08
7	P 0.73	10.0	8.5	8.75	+0.25
	Q 0.17	2.25	1.75	2.25	+0.5
8	P 0.28	10.75	8.75	10.25	+1.5
	Q 0.12	3.25	2.75	3.5	+0.75
9	P 0.56	8.75	8.25	7.75	-0.5
	Q 0.14	5.75	5.25	5.75	+0.5
11	P 0.34	7.75	7.0	7.5	+0.5
	Q 0.26	4.75	5.5	5.0	-0.5
12	P 0.37	6.25	5.75	5.0	-0.75
	Q 0.46	5.25	4.5	3.5	-1.0
13	P 2.96	3.25	0.75	7.17	+6.42
	Q 2.92	0.75	1.75	3.67	+1.92
14	P 0.03	6.5	6.83	7.0	+0.17
	Q 0.19	6.25	5.5	5.75	+0.25
15	P 0.07	12.25	12.5	12.5	0.0
	Q 0.07	5.75	6.25	6.25	0.0
16	P 0.12	8.0	7.0	7.75	+0.75
	Q 0.27	4.5	4.5	3.75	-0.75
17	P 0.15	5.25	3.5	5.5	+2.0
	Q 0.19	5.0	2.5	5.5	+3.0
19	P 0.64	10.75	10.5	10.5	0.0
	Q 0.38	6.0	6.0	6.25	+0.25
20	P 0.79	4.25	4.25	5.5	+1.25
	Q 0.65	1.25	0.5	1.0	+0.5
21	P 0.27	11.5	11.0	11.0	0.0
	Q 0.17	5.5	4.75	4.5	-0.25
22	P 0.16	13.25	12.25	12.5	+0.25
	Q 0.22	6.75	5.75	6.5	+0.75
mean (n=19)	P	8.36	7.53	8.21	+0.68
	Q	4.61	4.15	4.60	+0.45

Intermenstrual scores were equivalent to concentrations of  $3.31 \times 10^{-5}$  M PROP and  $5.86 \times 10^{-6}$  M quinine, representing a 1,024-fold change in PROP sensitivity and a 362-fold change in quinine. This subject was tested over two cycles and in both showed the same change immediately before the onset of menstruation. The change, if any, shown by other subjects was less dramatic and the majority reached maximum sensitivity after the onset of menstruation on days +1 to +5.

In large populations, taste thresholds for quinine follow a normal distribution, but the distribution for PROP is bimodal<sup>7</sup>. PROP and phenylthiourea (PTC) are related compounds and the taste responses to both are similar. Individuals can be classified as 'tasters' or 'non-tasters' of PROP according to whether their threshold lies below or above the antimode between thresholds 9 and 10 (ref. 3). Both tasters and non-tasters showed increased sensitivity at the time of menstruation. It is interesting that subject No. 2 was initially classified as a non-taster, but during menstruation she would clearly qualify as a taster. She changed from being unable to taste phenylthiourea paper (Carolina Biologicals, Inc.) to being able to taste it as bitter during menstruation. The degree of change associated with menstruation does not appear to be associated with the position of the individual's taste threshold on the population distribution curve.

Variations in numerous physiological and psychological characteristics have been correlated with the phases of the menstrual cycle, but reports on associated changes in sensitivity of the chemical senses are rare. Isolated instances of increased olfactory sensitivity during menstruation have been reported<sup>2,8</sup>, but no difference in taste threshold for PTC was observed in an investigation in which subjects were tested twice, once during the menstrual phase and again between periods<sup>4</sup>. Repeated testing under controlled conditions is a more sensitive method of examining constancy of the threshold. The wide variation in individual response shows that generalizations should be made with caution. Physiological and clinical changes associated with fluctuation in taste sensitivity in our sample are discussed elsewhere<sup>5</sup>.

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## ANTHROPOLOGY

### Seriation of Quantitative Features in Late Pleistocene Stone Technology

THE possibility that some or all lithic assemblages are structured in such a way that those which are closely related can be arranged in chronological order, as suggested by Mellars<sup>1</sup>, is of great interest. If trends do exist in all the Mousterian assemblages of the two traditions considered by Mellars, it would be reasonable to expect that similar systematic trends would exist in other, and perhaps all, well-isolated assemblages of other periods and areas. The only prerequisite for examining this is a group of comparably analysed assemblages, close in space and time; these are notably present in the Aurignacian of the Vézère Valley (Table 1). The eight assemblages considered in Table 1 exhibited very convincing trends. In addition, a level of frequency of other traits (not attained in non-Aurignacian assemblages) was maintained. From this it can reasonably be concluded that some social continuity must exist between the makers of the assemblages.

Sites with several culture strata and assemblages of the same tradition can indicate seriation by themselves; for example, the 4 Solutrean assemblages from the Fourneau du Diable at Bourdeilles (Table 2). The stratigraphy shows the direction of the trends through time, in Tables 1 and 2. The upper Solutrean is further instructive; its characteristic development is distinctively different from two broadly contemporary layers of Pech de la Boissière, also upper Solutrean, 60 km to the south-east; and from the upper Solutrean of Jean-Blanc, 55 km to the south. This could be explained on the grounds that separate regional development was increasingly characteristic of the post-Mousterian stage. That this is the correct explanation is confirmed by comparing the Vézère Aurignacian seriation-line (Table 1), which covers only a 15-km stretch of country, with Table 3, a probably parallel development or seriation-subline for 4 assemblages from near Brive, only some 30 km to the east, and equally with the seriation line of 8 assemblages of the Bourdeilles-Charente region (found at three sites called Chevre, Vachons and Rois) not reproduced here.

A close examination of the series of traits, the frequency of which is shown in Table 1, reveals three main trends: Burins and Nosed/all Carinate scrapers which rise, and Grattoirs on Aurignac blades which fall. Two features—Buskoids (all carinates and buskate burins) which remain constant, and Aurignacian retouch which fall a little—are not found in other traditions (that is, seriation-lines) of the Vézère in comparable frequency. These traits may be termed 'line-fossils'.

In following the foregoing trends through 8 assemblages, stages of development (or seriation-zones) can be distinguished; 4 may be postulated for Table 1—A, B, C, D. Sub-zones may also be postulated, notably C1, 2 and 3,

Table 1. VEZERE AURIGNACIAN SERIATION-LINE

The figures are all recalculated from D. De Sonneville-Bordes' *Le Paléolithique Supérieur en Périgord*, 1960, and the numbers in the top columns refer to the designation of the tool types

designation of the tool types											
Lithic-assemblages	No. tools		Traits							Seriation zones	
			Trends			Line fossils		Zone fossils			
			Burins (27-31) + (33-44) 1-68	6 (11-14) + 32 = Buskoids	Nosed Carinates All Carinates 11 11 + 13	Aurignacian Retouch 5, 6, 65-8 1-92	Buskoids (11-14) + 32 1-68	Aurignac Blades 6 + 67 1-68	Strangulated Blades 68 Buskoids		Buskates 32 Buskoids
	1-92 group	1-68 group									
Faurelle	520	497	25.4	2.5	67	9.2	23.4	1.2	0.9	24	D
Cellier-Ruth upper layer C	511	396	18.5	3	77	4.6	36.2	1.7	3.0	7	C3
Belcayre-Renne	576	500	15.6	3	51	14.6	28.6	1.8	0.7	13	C3
Lartet Gorge d'Enfer	699	660	15.1	13	57	20.8	22.9	8.1	0.7	0	C2
Belcayre-Metairie	393	329	11.6	14	40	13.9	22.2	5.1	0	4	C1
Cellier lower A	356	241	10.7	30	42	23.3	28.4	16.4	17	3.8	B
Castanet (Castel Merle) upper C	1,283	1,165	2.8	47	40	42.1	25.2	21.5	2.6	0.4	A2
Castanet lower A	1,824	1,712	2.8	125	32	46.6	23.0	35.7	3.8	1.2	A1

Table 2. SERIATION LINE  
Bourdellles later Solutrean

Bourdellies later Solutrean										
Lithic-assemblages	No. of tools in assemblage		Traits							
			Trends				Zone fossils			
			Grattoir	Double Grattoir	Borers	Line-fossil	Laurel leaf	Willow leaf	Shoulder Sol. pt.	ASSP
	1-15 1-72	3 R	23-6 1-72	Solutrean types 69-72						
1-92 Group	1-72 Group					70	71	72	56	
Bourdellies Fourneau du Diable III	1,430	1,283	8.2	8.5	4.1	53.4	2.2	4.8	45.2	24.2
Fd D upper terrace II	785	765	16.6	38.0	2.8	53.2	11.7	3.1	37.8	23.6
Fd D upper terrace I	1,110	919	42.9	45.5	3.0	37.6	19.6	1.4	15.5	5.3
Fd D lower terrace	1,010	972	46.1	50.5	0.8	28.2	26.9	0.9	0.1	0.3

Table 3. CORREZE (BRIVE) SERIATION-SUBLINE AURIGNACIAN

Table 3. CORREZE (DRIVE) SERRATION-SUBLINE AURIGNACIAN														
Lithic- assemblages	No. tools in assemblage		Traits										Zones	
			Trends			Line fossils		Sub-line fossil. Scaled pieces 76 1-92	Zone fossils					
	Burins (21-31) + (33-44) 1-68	Grattoir on Aurignac Blade Buskoids	Nosed Carinate 11 11 + 13	Buskoids (11-14) + 32 1-68	Aurignac retouch 5, 6, 65-8 1-91	Aurignac Blades	Strangu- lated Buskoids		Dufour + Font Yves 90 + 52 1-92	Buskate burins 32 Buskoids	Local	Vezere		
	Chanlat upper	1,360	1,193	31.4	2.1	75.9	33.3		8.3	5.9	1.3	0.5	0.1	7.5
Dufour	1,242	1,023	34.2	1.0	44.2	19.5	9.9	5.0	1.4	0.5	4.1	2.5	2b	C3
Chanlat lower	1,750	1,488	24.0	2.7	26.6	19.8	23.1	11.5	5.5	3.7	3.1	3.0	2a	C3
Font Yves	1,237	1,077	18.8	14.8	16.1	14.8	19.2	7.7	9.2	13.3	9.9	0.7	1	C1-2

but these are less clearly identified than the full zones. For example, zone A has a markedly lower burin and higher type 6 frequency than B-D; C1 is different from C2, but not so certainly outside its range of variation. These zones may be used as chronological scales. Each zone tends to have a different characteristic trait or zone-fossil of its own, rising parabolically during the opening of the zone and falling at its end: zone A, Aurignac blades; zone B, strangulated blades; zone C, possibly Dufour bladelets; zone D, buskate burins.

Broadly speaking, well-excavated assemblages appear to seriate better than less competently gathered examples. Assemblages in Table 1 are not particularly well excavated. The thinner the culture stratum from which they come, the better. Metairie and Renne both contain material from two distinct layers—hence there are some obvious discrepancies. Assemblages with more than 500 tools seriate better than those with less, and no doubt most discrepancies would disappear if only assemblages in excess of 2,000 tools in the 1-72 group were used.

Difficulties rapidly arise in seriating assemblages of Perigordian I and later date, if material from an area larger than the Vézère Valley is used. The true reason for this is probably that only a continuity of assemblages of a particular ethnic group will seriate. The seriation-line or sub-line, though normally local, could continue in a second locality if the ethnic group were to move. The Aurignacian V of Laugerie Haute appears to follow from an Aurignacian sub-line in west-central France, the latest rather widely spaced elements of which are

Vachons 2, Fontenieux and Laugerie D. The localisation of seriation applicability seems much less in the Mousterian and much greater in the Solutrean and late

It might be expected that further discrepancies would arise from particular economic specializations, or seasonal variants being represented among the assemblage. Actually there is no indication of this, and Bouchud work on cervid teeth suggests that all-year-round occupation was the rule. The discrepancies which do remain are gratifyingly small in most cases. They may be due to certain limited but inevitable randomness in human behaviour. However, it is equally probable that they would become negligible if a larger number of better gathered assemblages and more sensitive analyses of the traits within them were used—with more than 2,000 characteristic tools for each, and no great time between them.

In the meantime the available evidence seems quite adequate to support a hypothesis that lithic assemblages studied by quantitative seriation, are suitable for tracing ethnic continuity, where they are present in sufficient concentration; and that they can be used as a method of relative dating, along with more classic physical and geological methods, when suitable circumstances are present.

DESMOND M. COLLINS

"Evergreens",  
Gordon Ave., Stanmore, Middx.

<sup>1</sup> Mellars, P. A., *Nature*, 205, 626 (1965).



## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, March 1

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (in the Main Lecture Theatre, Department of Electrical Engineering, Exhibition Road, London, S.W.7), at 5.30 p.m.—Mr. L. Hix: "The Application of Automation to Railways".\*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Control of Room Storage Heaters" opened by Mr. K. B. Rowson.

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. Stewartson: "The Boundary Layer".\*

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Professional Engineer and Man-Management" opened by Sir Wm. Carron.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Microbiology Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. K. Mills: "Research and Brewing" (Jubilee Memorial Lecture).

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Dr. Audrey Butt: "Peoples of the Forest and the Rivers".

## Tuesday, March 2

UNIVERSITY OF LONDON (at Wye College, near Ashford, Kent), at 4.45 p.m.—Dr. H. Kern (Zürich): "Plant Parasitic Fungi and Their Toxins".\*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. D. A. Croney and Mr. J. A. Lee: "The Full-Scale Pavement Design Experiment on the A.1 at Alconbury Hill, Huntingdonshire".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. K. E. V. Willis: "Energy Storage".

FAUNA PRESERVATION SOCIETY (at the Zoological Society of London, Regent's Park, London, N.W.1), at 5.30 p.m.—Dr. David Snow: "Wildlife Conservation in the Galapagos".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. C. E. D. Taylor: "Diagnostic Applications of Immuno-Fluorescence". Fourteenth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.\*

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Applications of Gas Turbines in the Process Engineering Industry".

## Wednesday, March 3

GEOLOGICAL SOCIETY OF LONDON (joint meeting with the Exploration and Production Group of the Institute of Petroleum, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2 p.m.—Symposium on "The Geological Significance of Offshore Salt Basins Around Africa".

COLOUR GROUP (GREAT BRITAIN) (in the Large Theatre of the Physics Department, Imperial College, South Kensington, London, S.W.7), at 5.30 p.m.—Dr. W. A. H. Rushton, F.R.S.: "The Chemical Basis of Colour Vision and Colour Blindness" (Newton Lecture).

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, EDUCATION GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Discussion on "Teaching of Control Engineering".

INSTITUTION OF MECHANICAL ENGINEERS, LUBRICANT AND WEAR GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Lubrication and Wear Design Memoranda—What is Wanted and How to Present It".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Hugh Casson: "The Architecture of New Universities" (Alfred Borsom Lecture).

SOCIETY OF ENVIRONMENTAL ENGINEERS (at the Imperial College of Science and Technology, Exhibition Road, London, S.W.7), at 6 p.m.—Mr. E. Rivett: "Case Design for Service Equipment".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Northern Polytechnic Chemical Society, at the Northern Polytechnic, Falloway Road, London, N.7), at 7 p.m.—Prof. W. Klyne: "Optical Rotatory Dispersion".

ASSOCIATION OF THE WILLIAM PENGELLY CAVE RESEARCH CENTRE, in conjunction with IMPERIAL COLLEGE CAVING CLUB (in the Physics Department, Imperial College, London, S.W.7), at 8 p.m.—Mr. Colin Bristow: "The Resistivity Survey for Cavities in Devonian Limestone at Buckfastleigh, Devon".\*

## Wednesday, March 3—Thursday, March 4

ZOOLOGICAL SOCIETY OF LONDON (at Regent's Park, London, N.W.1), at 9.30 a.m. daily—Symposium on "The Cnidaria and Their Evolution".

## Thursday, March 4

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. L. S. Penrose, F.R.S.: "From Eugenics to Human Genetics".\*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. J. Owen: "The Determination of Exchange Interactions by Magnetic Resonance Techniques" (Review Lecture).

UNIVERSITY OF LONDON (in the Wright-Fleming Institute Theatre, St. Mary's Hospital Medical School, Paddington, London, W.2), at 5 p.m.—Prof. M. Singer (Western Research University): "The Tropic Role of the Nervous System".\*

INSTITUTION OF CIVIL ENGINEERS, ENGINEERING MANAGEMENT GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Management Training from an Employee's Point of View", introduced by Mr. A. M. Sowden and Mr. G. H. Oversby-Powell.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. J. H. Westcott: "Research and Development in Control Engineering".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. C. R. Twidale (Adelaide): "Problems of South Australian Geomorphology".\*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. R. Hobbs: "Disturbances of the Immunoglobulins". (Fifteenth of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation).\*

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. I. Asboe Jorgensen: "Refrigeration in Modern Abattoirs with Special Emphasis on the Chilling of Pig and Beef Carcases".

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Methods of Auxiliary Drive in Diesel Locomotives".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—M. Marcel Bruyere: "Modern Solutions of Coupling and Transmission Problems of Rotary Engines" (Eighteenth Louis Blériot Lecture).

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at West Ham College of Technology, Romford Road, London, E.15), at 7 p.m.—Prof. R. S. Nyholm, F.R.S.: "Metal to Metal Bonds in Complex Compounds".

## Friday, March 5

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Du Cane Road, London, W.12), at 10 a.m.—Mr. J. Corcoran: "Current Views on Genito-Urinary Tuberculosis in Ireland".\*

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 11 a.m.—Symposium on "The Experimental Taxonomy of Flowering Plants".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 2.45 p.m.—Annual General Meeting, followed by Dr. D. C. Garratt: "The Analyst in Pharmaceutical Industry".

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Fluid Mechanics Films".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. Stevenson Buchan: "The International Hydrological Decade".

## Saturday, March 6

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. J. D. Carthy: "Animal Communication".\*

## Monday, March 8

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 4.30 p.m.—Annual General Meeting. 5.30 p.m.—Dr. Mary Hesse: "The New Problem of Induction".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. Peter Scott: "The Population Structure of Australian Cities".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. H. E. M. Barlow, F.R.S.: "Screened Surface Waves and Some Possible Applications".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. T. Barnard: "Micropaleontology: Past, Present, and Future".\*

SOCIETY OF CHEMICAL INDUSTRY (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. M. Stanley: "Some Achievements in Petroleum Chemicals" (7th Castner Memorial Lecture).

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN ASTRONOMY—The Registrar, The University, Manchester. 13, quoting Ref. 28/65 (March 3).

LECTURER or ASSISTANT LECTURER IN MATHEMATICAL LOGIC—The Secretary, The University, Aberdeen (March 5).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/458 (March 5).

RANKINE CHAIR OF MECHANICAL ENGINEERING (Mechanics and Mechanism)—The Secretary of the University Court, The University, Glasgow (March 6).

ASSISTANT LECTURER IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, University College, Gower Street, London, W.C.1 (March 8).

SENIOR LECTURER or LECTURER IN STATISTICS—The Secretary, The Queen's University, Belfast, Northern Ireland (March 9).

LECTURER, SENIOR LECTURER or READER IN ANIMAL HUSBANDRY at Makerere University College, University of East Africa, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 10).

LECTURER (with interests in theoretical chemistry and/or spectroscopy) IN PHYSICAL CHEMISTRY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (March 13).

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER (graduate in biochemistry, or chemistry and biology, and a general interest in the use of physico-chemical methods in biology) to assist in the Department of Cell Biology—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (March 15).

POST-DOCTORAL RESEARCH FELLOW IN THE SCHOOL OF PHYSICAL SCIENCE to join a group working on the electron-microscopy of defects in solids, particularly at low temperatures—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (March 15).

PRINCIPAL LECTURER IN SOCIOLOGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (March 15).

SENIOR DEMONSTRATOR IN ZOOLOGY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 15).

LECTURER IN APPLIED MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (March 16).

LECTURER IN CLINICAL PSYCHOLOGY IN THE DEPARTMENT OF PSYCHOLOGICAL MEDICINE—The Secretary, The University, Edinburgh (March 17).

SENIOR LECTURERS OR LECTURERS IN PURE MATHEMATICS, APPLIED MATHEMATICS AND STATISTICS at the University of Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 21).

CHAIR OF ZOOLOGY at Makerere University College, University of East Africa, Uganda—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 30).

SENIOR LECTURER OR LECTURER IN BOTANY; a SENIOR LECTURER and a LECTURER IN CHEMISTRY; a SENIOR LECTURER and a LECTURER IN MATHEMATICS; a SENIOR LECTURER OR LECTURER IN MICROBIOLOGY; a LECTURER IN PHYSICS; and a SENIOR LECTURER AND LECTURER IN ZOOLOGY at Njala University College, Njala, Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (March 30).

CHAIR OF BOTANY IN THE FACULTY OF SCIENCE of the University of Malaya, Kuala Lumpur—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, March 31).

LECTURER/SENIOR LECTURER IN ANTHROPOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 2).

LECTURER IN ORGANIC CHEMISTRY—Secretary of the University Court, The University, Glasgow (April 16).

LECTURERS (2) IN THE DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Leicester (April 21).

AGRICULTURAL OFFICERS (nationals of the United Kingdom or the Republic of Ireland, with a degree in agriculture plus two years' postgraduate training or experience in tropical agriculture) in Malawi, to carry out the normal duties of an Agricultural Officer, which includes both European and African agriculture—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/134/02.

LECTURERS AND ASSISTANT LECTURERS IN CHEMISTRY—The Registrar, The University, Manchester, 13, quoting Ref. 29/65.

RESEARCH ASSISTANT (preferably with experience in animal behavioural techniques) IN PSYCHOPHARMACOLOGY—Prof. G. M. Carstairs, Department of Psychiatry, University of Edinburgh, 2 George Square, Edinburgh, 8.

RESEARCH ASSOCIATE (medical or non-medical graduate) IN THE DEPARTMENT OF MEDICINE, to join in a research project concerned with the metabolism of fat in arteries—The Secretary, Guy's Hospital Medical School, London Bridge, S.E.1.

SCIENTIFIC INFORMATION OFFICER (Abstractor) (with a degree in agricultural or natural sciences and ability to abstract from Russian)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berks.

TECHNICAL ASSISTANT (preferably with experience in a physiological laboratory)—The Director, Medical Research Council's Department of Experimental Medicine, Tennis Court Road, Cambridge, marking envelope "Technical Assistant".

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

The University of Leeds. University Studies in Applied Science and Technology: an Introduction to the Degree Courses in the Faculty of Technology. Pp. 88. (Leeds: The University, 1964.) [1112]

The Study of Enzymes. By Prof. K. S. Dodgson. (An Inaugural Lecture delivered at University College, Cardiff, 28th April, 1964.) Pp. 17. (Cardiff: University of Wales Press, 1964.) 3s. 6d. [1112]

Ambassade de France, Service de Presse et d'Information. Statement made by the Minister of Foreign Affairs in the National Assembly, 3rd November, 1964. Pp. 16. The French Press. Pp. 15. (London: Ambassade de France, Service de Presse et d'Information, 1964.) [1112]

The Electrical Research Association. Report No. 5026: Dielectric Effects of Flaws in Hydrogen Bonding III. By Dr. Vera Daniel. Pp. 13+9 figures. (Leatherhead: Electrical Research Association, 1963.) 12s. 6d. [1112]

Ministry of Housing and Local Government. Welland and Nene Rivers: Hydrological Survey (Hydrometric Areas 31 and 32). Pp. v+80. (London: H.M. Stationery Office, 1964.) 17s. 6d. net. [1112]

Discovery Reports. Antarctic and Subantarctic Mollusca: Amphineura, Scaphopoda and Bivalvia. By R. K. Dell. Pp. 93-250+plates 2-7. 100s. net. Larves de Ceriantales. By E. Leloup. Pp. 251-307+plates 8-21. 75s. net. (London and New York: Cambridge University Press, 1964.) [1112]

Thorium Limited: Fifty Years of Progress. Pp. 16. (London: Thorium Limited, 1964.) [1112]

The Institution of Electrical Engineers. 1964 Amendments to the Regulations for the Electrical Equipment of Buildings. Pp. 4. (London: The Institution of Electrical Engineers, 1964.) 1s. [1112]

The Library in the Hospital and Care in the Community. (Papers given at the Hospital Libraries and Handicapped Readers Group Conference and Weekend School held at Florence Boot Hall, University of Nottingham, July 1963.) Pp. 32. (London: The Library Association, 1964.) 9s.; (L.A. members 6s. 9d.) [1112]

Report of The Nature Conservancy for the year ended 30th September 1964. Pp. vii+173+10 plates. (London: H.M. Stationery Office, 1964.) 13s. net. [1112]

Science Teaching Techniques—XI: Simple Practical Human Physiology; Technique for the Preservation of Flowers; The Use of Animals in Teaching; Marine Plankton Studies. Pp. 68. (London: John Murray, 1964. Published for The Association of Teachers in Colleges and Departments of Education; The Association for Science Education; and The London Association of Science Teachers.) 4s. net. [1112]

Nickel in Wrought Copper Alloys. Pp. 20. (London: The International Nickel Company (Mond), Ltd., 1964.) [1112]

The Zoological Society of London. The Zoological Record, Vol. 98, Section 2, 1961: Protozoa. Compiled by Dr. R. A. Neal, Dr. R. S. J. Hawes, Pauline Curds and Sarah E. Bunney. Pp. 206. (London: The Zoological Society of London, 1964.) 20s. [1112]

National Lending Library for Science and Technology. List of Scientific and Technical Periodicals received from China. Pp. ii+54. (Boston Spa: National Lending Library for Science and Technology, 1964.) [1112]

The Acorn Lectures, Vol. 2. Edited by W. Banks. Pp. v+51. (Inverskirk: Arthur D. Little Research Institute, 1964.) [1112]

Office of Health Economics. The Pharmacist in Society. Pp. 32. (London: Office of Health Economics, 1964.) 2s. [1112]

### Other Countries

United States Department of the Interior: Geological Survey. Water-Supply Paper 1669-CC: Natural Sources of Salinity in the Brazos River, Texas, with particular reference to the Croton and Salt Croton Creek Basins. By R. C. Baker, L. S. Hughes and I. D. Yost. Pp. vi+81+plates 1-4. Water-Supply Paper 1671: Magnitude and Frequency of Floods in the United States. Part 1-A: North Atlantic Slope Basins, Maine to Connecticut. By A. Rice Green. Pp. x+260+plate 1. Water-Supply Paper 1779-B: Ground-Water Conditions in the Proposed Waterfowl Refuge Area near Chapman, Nebraska. By C. F. Keech. With a section on Chemical Quality of the Water by P. G. Rosene. Pp. v+55+plates 1-6. Water-Supply Paper 1779-X: Quality of Delaware River Water at Trenton, New Jersey. By Leo T. McCarthy, Jr. and Walter B. Keighton. Pp. iv+51+plate 1. Water-Supply Paper 1785: An Appraisal of the Possibilities of Artificial Recharge to Ground-water Supplies in Part of the Roswell Basin, New Mexico. By Ward S. Motts and R. L. Cushman. Pp. v+85+plates 1-7. (Washington, D.C.: Government Printing Office, 1964.) [1612]

India: Council of Scientific and Industrial Research. Central Building Research Institute Annual Report, 1963-1964. Pp. ix+62+15 photographs. (Roorkee: Central Building Research Institute, 1964.) [1612]

Annals of the New York Academy of Sciences. Vol. 116, Article 3: The Acute Inflammatory Response. By W. G. Spector, Albert S. Gordon and 39 other authors. Pp. 747-1084. 6 dollars. Vol. 119, Article 2: Problems of Cooley's Anemia. By Harold Fink and 76 other authors. Pp. 369-860. 8 dollars. (New York: New York Academy of Sciences, 1964.) [1612]

Union Radio Scientifique Internationale. XIV<sup>e</sup> Assemblée Générale, Tokyo, Sept. 9-20, 1963. Vol. XIII-4: Commission IV—Magnetosphere. Pp. 147. Vol. XIII-5: Commission V—Radioastronomie. Pp. 200. Vol. XIII-7: Commission VII—Radioélectronique. Pp. 169. (Bruxelles: Union Radio Scientifique Internationale, 1964.) [1612]

Japan. Meteorological Research Institute: Oceanographical Laboratory. Collected Reprints, Vol. 8. Reprints Nos. 132-153. (Mabashi, Suginami, Tokyo: Meteorological Research Institute, Oceanographical Laboratory, 1964.) [1612]

List of Papers published by the Members of the Research Institute for Iron, Steel and other Metals, Tohoku University, 1963. Pp. 25. (Sendai: The Research Institute for Iron, Steel and other Metals, Tohoku University, 1964.) [1612]

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# NATURE

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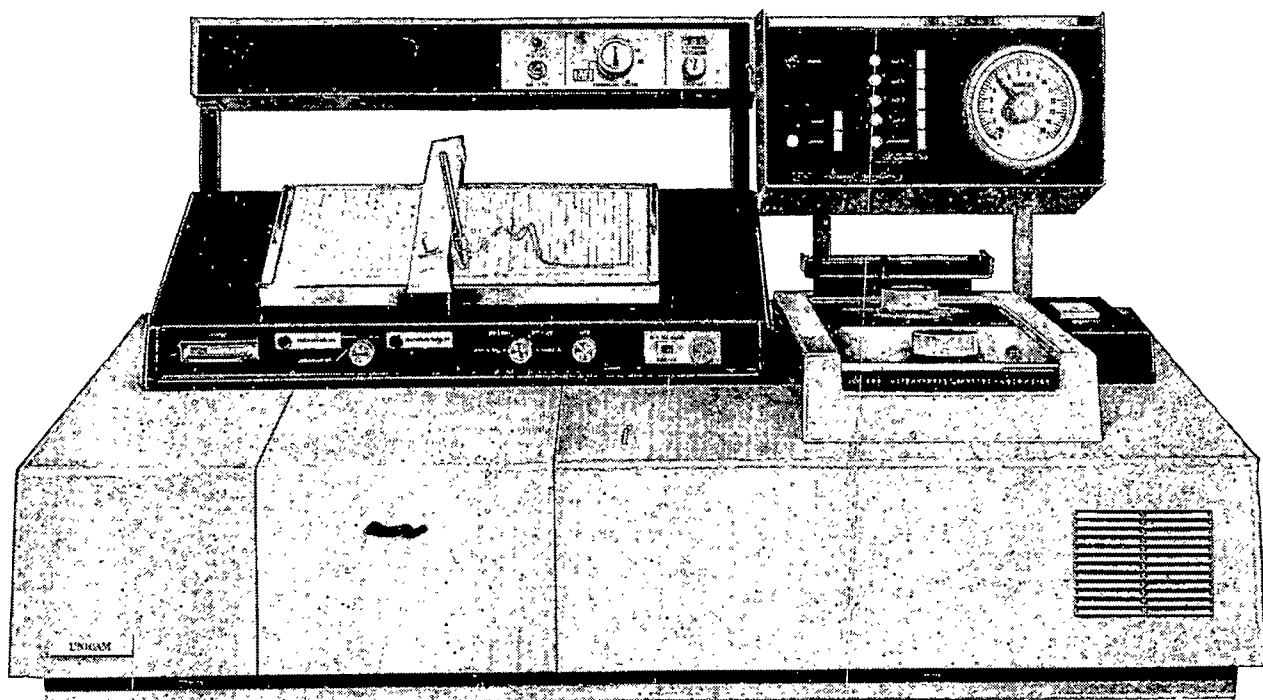
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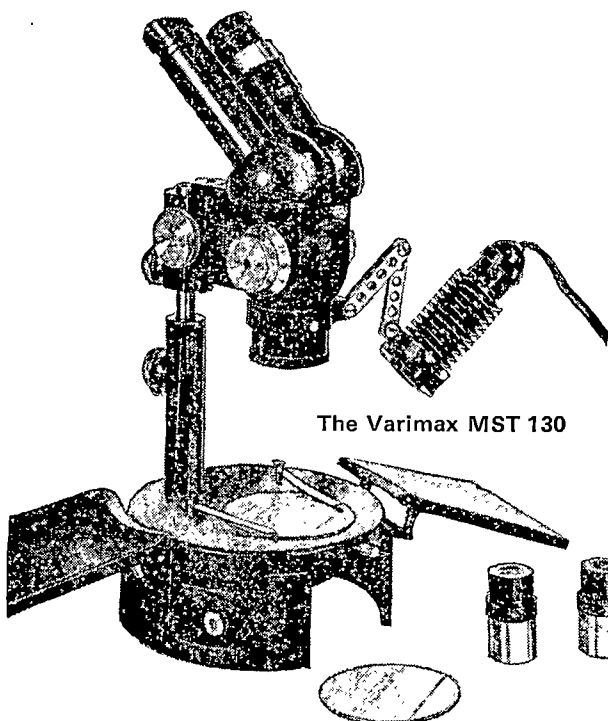
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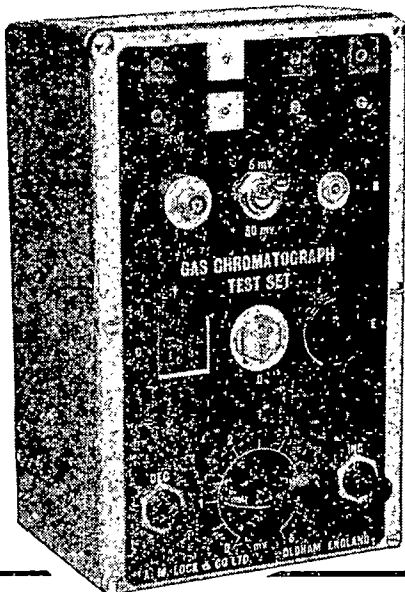
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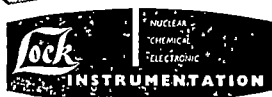


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## EDUCATING THE WHOLE SCIENTIST

THE autumn 1964 issue of *Daedalus* is devoted to the topic "The Contemporary University: U.S.A.". Some of the essays, such as Prof. W. C. Devane's "The College of Liberal Arts" and Prof. F. Heimberger's "The State Universities", are concerned specifically with conditions in the United States. Dr. Clark Kerr's "The Frantic Race to Remain Contemporary" is essentially an exposition of the argument for the multi-versity presented in his Godkin Lectures in 1963 and published under the title "The Uses of the University". Peter H. Rossi's essay, "Researchers, Scholars and Policy Makers: the Politics of Large-scale Research", is likewise concerned with the American scene, and almost exclusively with the effect of the development of research centres for social science within the university environment on the organization of a university, but in Dr. R. S. Morison's "Foundations and Universities" the wider implications are much more apparent.

Other essays have a bearing and interest that obviously extend far outside the United States. Dr. J. M. Ziegler's discussion of continuing education in the university is perceptive and highly pertinent to discussions on adult education which are current in Britain, and Dr. P. Weiss's essay on science in the university is of outstanding interest to the scientist, as well as to the university administrator and others. This issue also includes Dr. J. A. Stratton's commencement address to the Massachusetts Institute of Technology last June, which touches on the same broad theme of the purpose and function of the university in modern society.

For all his faith in and dedication to the work and methods of science, Dr. Stratton does not believe that we can any longer take for granted the thesis that technology is the driving force of progress. A blind confidence in the inevitable good of material progress can lead only to disillusionment. Technical advances have now such complex and indirect effects that it is only by an examination of their impact on society as a whole that we can judge the degree of progress from a particular innovation. Arguing in lines made familiar through the Buchanan Report, for example, Dr. Stratton urges that many of the problems of society to-day will yield only to planned collaborative attacks focused on clear objectives and based on concerted action.

It is from this point of view of the dependence of success on the joint contributions of physical and biological scientists, of economists and political scientists, engineers and architects, historians and philosophers, that Dr. Stratton turns to the university as the one institution capable of meeting this highest intellectual challenge of our time—that of stimulating or welding these components of learning into systems of understanding. Only within the framework of the university do we find the wide range of interests, a common ground for the exchange of ideas, a forum of discussion for scholars who draw on the arts and humanities as well as on science and technology. This is particularly true of institutions like the Massachusetts Institute of Technology, and Dr. Stratton believes that they cannot escape the responsibility of participating in the solution of problems touching deeply the total welfare of society.

This is manifestly recognized, for example, by the Imperial College of Science and Technology, but Dr.

Stratton goes on to urge that the changing role of science and technology, arising out of their penetration into every domain of human affairs, carries, in a free society, personal and individual responsibility, as well as that which properly falls on the institutions of society—on Government, industry and university. Dr. Stratton here addressed himself specifically to his own students, but his emphasis on personal and professional responsibility, like that on the responsibility of the university itself, is in harmony with Dr. Weiss's view of the place of science in the university. Dr. Weiss recognizes that examination of the growing impact of science on the universities is a continuous process, which involves finding ways for the university to accommodate the legitimate claims of science, and at the same time protecting the university against expansion of the scientific sectors beyond a stress limit at which the ideal of a university as a cohesive body would collapse. He deplores the antagonism inherent in the idea of 'two cultures' and seeks to eliminate such discords and contentions, while admitting the lack of symmetry in mutual relations between scientists and non-scientists which is the core of Lord Snow's argument.

Pointing out that no one scientist can any longer claim the authority to act as spokesman for the whole of science, Dr. Weiss nevertheless regards this staggering internal diversity of science as an asset, as an essential factor in the role of science in education. There the significant feature of science lies in the spiritual or cultural values it imparts. The scientific method teaches man to discipline his thinking and his actions based on thought. Broadly applied, it can clear away the underbrush of superstition and prejudice, correct or eliminate complacency and cocksureness, inspiring fresh resolves to search and strive rather than to conform, checking the deadening effect of mechanization.

Science, in Dr. Weiss's view, is thus a major integral factor of our culture, not just a service function; its spirit is of as much concern to man as its products. Science and the humanities are seen not as opponents but as partners in a common enterprise, and it is wrong to single out the scientist as specially benefiting from a broad liberal education, which aims chiefly to provide educated, responsible and judicious citizens in general. Nevertheless, this does not necessarily mean that the basis of general education should be the same for all, even for all who contemplate a career in science.

That point assuredly requires to be considered more carefully in the expansion of higher education in Britain, for it bears on the nature of institutions as well as the content of courses. Dr. Weiss believes that the conflict between the demand for early technical specialization and the demand for a broad general education to safeguard the creative scientist (as well as in the interest of adaptability) can be resolved if sufficient forethought is given to the specific needs of both streams for a broad general basis. Surrender to the growing demands for professional or vocational training at the expense of breadth and flexibility, however, would be fatal, above all to the maintenance of a supply of men of science of independence and integrity, vision and courage.

It is in this belief that Dr. Weiss proceeds to discuss the question not what is a university but who is 'the university', and urging the necessity for the university-

and the scientific establishment to accept the great variety of form which characterizes them both and to seek to effect the most congruous match between these respective patterns. He considers that only a collective integrated system of plural universities with diverse functions can fulfil the ideal overall function of 'the university'. He visualizes the university of the future as being a community of scholarly institutions instead of a community of scholars.

This idea is implicit in certain trends in Britain, for example in the way in which groups of universities are sharing particularly costly equipment, and in comments such as are to be found in the report on the organization and management of research regarding the location of research associations and research institutes or stations. It has clearly been taken into account in planning the specific activities of new universities, at least to the extent of recognizing that it is wiser for a new university to be selective in the fields of study it offers and not attempt to cover the whole field of knowledge. Dr. Weiss observes that every institution has certain areas of strength which it tends to cultivate more than others. The significant difference between this outcome of accident and necessity, which marks the present situation both in the United States and in Britain, and the orderly scheme he envisages for the future, is that in the past differentiation between the universities has been piecemeal and incoherent so that the various pieces do not fit together to form the harmonious whole which he advocates.

That ideal system cannot be achieved unless a certain number of universities come together to review their respective functions and capabilities and, with the minimum infringement of independence or autonomy, work out a collective plan so that collectively they supplement each other over the whole field of knowledge. Such efforts at integration would be guided by three main considerations: the filling of significant gaps; the correction of serious disproportions; and the lessening of redundancy, particularly where merging isolated units would lead to increased strength. Such measures would, he holds, restore the very principle of continuity, unity and universality of knowledge implicit in the idea of a university, and could also contribute to maintaining the highest possible standards of education for responsible leadership which remains a prime obligation of the universities.

Looking next at the functions of a university in this context before he discusses the place of science, Dr. Weiss distinguishes four essential major functions: the preservation, critical interpretation and synthesis of existing knowledge; furthering the acquisition of new knowledge and developing the necessary intellectual and technical tools; training in the application of existing knowledge to the practical needs of man; and transmitting and disseminating knowledge and its implications and applications. He believes that a community of institutions such as he advocates would be in a uniquely favourable position to give balanced attention to all these functions. In regard to the first, for example, greater concern with the history, structure and cultural values of science has created not only an opportunity but also a genuine demand for a far more active interplay between the sciences and the humanities in education, with benefit to the humanist as well as to the scientist.

The second function involves more difficult issues through the risk of disproportionate expansion of scientific research. Here Dr. Weiss emphasizes the risks of excessive concern with publication and of inflating the numbers of

research workers with those possessing little aptitude for or interest in, scientific research. He also stresses the need for the universities to resist pressure to undertake more sharply-delineated 'project' or 'contract' research than or may be useful for training purposes. He sees the main safeguard against the latter in the participation of experienced scientists in the formation of policy and evaluation of programmes, and stresses the importance of scientist accepting such administrative responsibilities for maintaining standards and efficiency. The main motive for scientific research in the university, Dr. Weiss maintains should be to turn out scientific personnel, not scientific products.

It is this, of course, that really links research with teaching, and Dr. Weiss, in a passage reminiscent of an early report from the University Grants Committee urges that every kind of institution of higher education including teacher training colleges, would gain from having some degree of scientific research conducted on its campus, motivated not chiefly by the need to train specialists but by the disciplinary value of research in developing critical thinking. That is a point of view to be given more weight than it appears at present to be receiving. In discussing the question of how research might figure more prominently in teaching and training those who are not destined for a career in research, Dr. Weiss commends the encouragement of elementary research in the schools and a critical revision, if not reduction, of the content of curricula. He does not suggest that there is any specific ratio or formula for the proportion of research to teaching, but reminds us that this depends alike on the stage reached by the student as well as on the aptitude of the teacher, and once again he joins issue with the tendency to relate academic status to the range or quantity of published papers, to the neglect of the quality of teaching.

Finally, Dr. Weiss emphasizes the vital importance of adult education both for the universities and the advancement of science, and the necessity for the universities to participate with real imagination and determination in extramural work to that end. He offers no specific recommendations in this field, and it is indeed the general character of his observations that makes them so pertinent for the discussions now proceeding on the expansion of higher education in Britain and the re-shaping of the relations between science and society as well as its organization within the civil administration. The main issues are clearly discerned, but the details and the particular measures to be concerted to resolve the problems depend on the special conditions of the country or society they are intended to serve. Both Dr. Stratton and Dr. Weiss give warnings and counsel that we in Britain would be wise to heed.

## THE WIND THAT SHAKES THE BARLEY

### Barley Genetics I

(Proceedings of the First International Barley Genetics Symposium, Wageningen, 26-31 August, 1963.) Pp. xxii + 387. (Wageningen: Centre for Agricultural Publications and Documentation, 1964.) n.p.

*BARLEY Genetics I*, the record of the first international symposium on barley genetics, contains 35 papers all in English, presented under eight main headings.

The origin and phylogeny of barley are dealt with in three papers. F. Kh. Bakhteyev (U.S.S.R.) reviews the

various hypotheses which have been put forward and suggests that an experimental test of existing as well as of new hypotheses is needed. R. Takahashi (Japan) presents the results of his survey of the geographical distribution of the genes for brittle ear, resistance to powdery mildew and spring habit and agrees with Bakhteyev on the monophyletic origin of barley. D. Zohary (Israel), after suggesting that both *Hordeum agriocrithon* and *H. lagunculiforme* are of hybrid origin, agrees that *H. spontaneum* may be the sole progenitor of cultivated barleys. The organization of international expeditions as suggested by Bakhteyev is under consideration and it is likely that material collected will be available to all countries.

Five papers are devoted to mutation. R. A. Nilan, C. F. Konzak, R. E. Heiner, and E. E. Froese-Gertzen (United States), in a paper on chemical mutagenesis in barley, discuss the problems of technique and interpretation in measuring the effects of mutagen treatment and review recent work with alkylating agents. Special attention is directed towards factors affecting mutagenic action of various chemical agents. A. Hagberg and G. Persson (Sweden) survey the application of induced mutations to barley genetics, taxonomy and plant breeding, with special reference to the results of Swedish mutation research. E. A. Favret (Argentina) compares the mutagenic efficiency of ethyl methane sulphonate (EMS) with that of ionizing radiations, and describes their interaction in combined treatments. A. Moës (Belgium) states that EMS before reaching lethality induces in the  $X_1$  leeper physiological damage than X-rays and induces a higher degree of sterility. U. Lundqvist (Sweden) describes the induction of mutations in barley pollen by means of ultra-violet and X-rays.

The section on chromosome aberrations deals with macromutations, aneuploidy and polyploidy. R. T. Lamage (United States) discusses the uses of translocations in genetic research and their application in the reduction of trisomies. T. Tsuchiya (Japan) describes his work on the production of trisomies from triploids and translocations in diploids. E. Reinbergs (Canada), in a paper on autotetraploids, emphasizes the high degree of fertility found and the few positive results obtained in attempts to reduce this by the introduction of structural rearrangements in the chromosomes.

During the symposium, a group of workers interested in linkage investigations attempted to produce a map of the chromosomes of barley. It is obviously incomplete and probably inaccurate, but useful. In this connexion, D. W. Robertson (United States) presents linkage data for many new genes together with their proposed positions on the chromosomes. Detailed linkage investigations are also given by S. B. Helgason (Canada) and G. Holm (Sweden). In order to provide international co-ordination of genetic investigations in barley, procedures for the nomenclature and handling of genetic stocks are proposed and the names and addresses of the proposed co-ordinators are given.

A single paper on "Interspecific and Intergeneric hybrids in *Hordeum*", by T. Rajhathy, J. W. Morrison and S. Symko (Canada), deals primarily with species relationships based on evidence obtained during the past ten years in Ottawa and includes a review of most of the published work on this subject.

One of the largest sections is that on disease resistance. Here eight papers are presented. J. G. Moseman (United States) deals with the complementary reaction between host conditioning and those governing the pathogenicity of the powdery mildew fungus. In two further papers on powdery mildew, Y. Koltin, R. Kenneth and I. Wahl (Israel) describe physiological specialization of the pathogen in Israel and E. P. Baker (Australia) presents further evidence for an allelic series conditioning resistance to mildew at the Algerian locus. C. W. Schaller (United States) discusses barley yellow dwarf virus and barley stripe mosaic virus and their relationship to other

hosts such as oats and grasses. J. W. Gibler and Ch. F. Krull (United States) present a short note on the inheritance of resistance to "enanismo", a virus or virus-like dwarfing disease causing serious losses in susceptible varieties of barley, wheat and oats. J. M. Poehlman (United States) gives a brief description of breeding for resistance to loose smut, and J. C. Zadoks (Holland) deals with the use of race nurseries in cereal resistance breeding. The section is completed by a second contribution from J. G. Moseman on the present status of plant pathological research in his country.

There are three papers on physiological and quantitative genetics. Ch. R. Olien (United States) discusses the problems associated with winter hardiness in barley and describes a new method of evaluating stresses. J. E. Grafius (United States) presents a geometric scheme for the construction of populations which may provide the maximum chance of success in an acceptable phenotype in breeding for yield when only a limited number of plants is selected on the basis of more readily observable traits. G. A. Weibe (United States) surveys the contributions of DDT and its analogues to the genetics and breeding of barley.

The final section of this symposium, on breeding techniques and malting quality, is the largest and contains nine papers. G. D. H. Bell (Britain) gives a comprehensive review of general breeding techniques. C. A. Suneson (United States) discusses the use of populations in breeding and possible economic uses of heterosis. Five papers are devoted to malting quality and cover the methods and achievements of breeders in Western Europe (A. Lein, Germany); methods of testing for malting quality and investigations into its inheritance (R. N. H. Whitehouse and E. T. Whitmore, Britain); the development of a system for evaluating malting quality of new strains, and methods of selection for malting quality (D. Glas, W. Wilten and M. L. Wijvekate, Holland); breeding for malting quality (A. R. Raw, Australia) and the production of improved malting varieties with increased resistance to lodging and mildew (J. Lekeš, Czechoslovakia). The phenotypic expressivity of genotypes in different environments is discussed by K. Gotoh (Japan), and K. W. Finlay (Australia) presents an investigation on the measurement and significance of adaptation in barley breeding.

The book is a valuable collection of papers with, in most cases, copious references. The discussion following each section is reasonably well reported.

E. V. B. WILSON

## ENVIRONMENTAL STUDY

### The Biology of the Living Landscape

An Introduction to Ecology. By Prof. Paul B. Sears. Pp. 176. (London: George Allen and Unwin, Ltd., 1964.) 21s. net.

FROM its title one might imagine this to be a book concerned specifically with the landscape of the author's native America—a biologist's book, having perhaps only a limited application to the landscape of Britain. But this is far from being the case. Prof. Sears has far wider aims: he sets out to trace the emergence of ecology as a science, and to bring home to his readers a realization of its importance to human happiness and prosperity. Indeed, it is Prof. Sears's theme that man himself is, willy-nilly, an integral part of the living landscape he describes, and so cannot afford to disregard its laws. Though knowledge of science and technology has given him a measure of control over his environment, there is grave danger in the popular fallacy that science enables him to outwit Nature—thoughtless exploitation of natural resources during the past century has left



clear enough evidence of this. The pressure of the rapidly expanding human population makes it more urgent than ever before that we should be deeply concerned about conservation and the utilization of land to secure its highest potential for sustained productivity. The attendant need that ecological understanding should be "built into the wisdom of the human race" is really what provides the stimulus for writing this book.

Accordingly, Prof. Sears has written for the non-biologist, conveying, in his easy, friendly style, a breadth of vision and understanding that will commend the book widely. He illustrates his discussion with well-chosen examples drawn largely from plant biology. The biologist may feel a little disappointed that the stimulating introduction does not lead to something more detailed and precise. By following up some of the examples, the reviewer feels, the argument might have been made more forceful, yet at the same time this could upset the balance of the broad picture Prof. Sears has set out to give. There is a refreshingly wide perspective in the writing which must appeal alike to the general reader and those biologists, victims of extreme specialization, for whom plants and animals in their natural surroundings are unfamiliar and perhaps something of an anachronism.

In the teaching of biology in schools and colleges Prof. Sears urges priority for the out-of-doors as the proper laboratory in which to begin, so that the student may be "helped to see—really to see—grasses and trees, dogs and rabbits before we begin to talk to him of the marvellous self-replication of DNA or the relation of the Krebs cycle to bioenergetics". It is not that Prof. Sears is in any way seeking to put the clock back, but rather to restore perspective and a reasonable balance of emphasis, so that we may better understand the picture as a whole.

After introducing his theme in the first two chapters, and showing its relevance to problems of modern civilization, the author takes us back to the very beginning and reviews the narrow range of physical conditions, existing on this planet, which make life as we know it a possibility. He then sketches in some of the landmarks of biological thought, tracing the early stirrings of ecological ideas and the pioneer work which led to the emergence of ecology as a science in its own right. There follows a consideration of energy transformations and entropy, their bearing on life as seen in the individual's quest for a supply of energy and the circulation of energy and materials within a natural community; next some discussion of the classification of living things and the individual; then a short review of the contributions of water, rocks and soil to the living landscape. In the final two chapters, entitled "The Historical Factor" and "Reading The Landscape", the author rounds off what is a scholarly book, leaving the reader much to think about.

MAURICE ASHBY

## ELASTIN AND ELASTASE

### Elastolysis and Ageing

By David A. Hall. (A Monograph in the Bannerstone Division of American Lectures in Geriatrics and Gerontology.) Pp. xiii+160. (Springfield, Ill.: Charles C. Thomas, 1964.) 6.75 dollars.

THE fibrous proteins of connective tissue comprise more than 40 per cent of the total body protein in mammals, and it is not surprising that the structure of collagen and elastin and its change with age have been investigated extensively. Over the past ten years or more many chemists and physicists have found it more profitable to direct their attention to collagen and much is now known of its structure, but recently there has been a resurgence of interest in elastin. As detailed investigations reveal more precise information about its structure, no doubt attempts will be made to relate this new information to the behaviour of elastin-containing tissues *in vivo*,

particularly to changes accompanying ageing and the development of pathological conditions in the cardiovascular system. There is, however, a substantial gap between results of *in vitro* investigations on highly purified elastin and its behaviour in its very complex natural environment. Dr. Hall's book, therefore, appears at a very appropriate time, for it deals primarily with the interactions between elastic tissues and some of the agents they might be expected to encounter *in vivo*, in particular with enzymes of the elastase complex and their inhibitors.

Dr. Hall and his colleagues have been active in this field for a number of years, and *Elastolysis and Ageing* is, to a large extent, an account of their efforts. He has been led to the view that several of the features of vascular degeneration and atherosclerosis may be attributed to the interaction of the elastase enzyme system with elastic fibres, and here he attempts the onerous task of presenting a unified picture of these diverse phenomena.

The book is divided into four main sections. The first deals with the structure of elastic fibres and includes discussion of the properties of solubilized elastins and their possible relation to elastin fibrogenesis. Most of this section, however, is concerned with the evidence for a two-component structure of the elastic fibre, consisting of a protein core surrounded by a 'sheath' consisting of protein, polysaccharide and also probably lipid. Much of this evidence arises from experiments in which elastase was used as a 'structural probe', and the second, and largest, section describes the preparation and properties of this pancreatic enzyme. Hall, and more recently other workers, have recognized that this enzyme system contains two distinct enzymes which interact with elastic fibres—a proteolytic enzyme (elastoproteinase elastase) and a second enzyme which has a synergistic effect on the first and according to Hall and his colleagues is a lipoproteinase. The evidence for their existence and mode of action is discussed in detail together with the similarity of the lipoproteinase and the lipoproteinase of post-heparin clearing factor. The third section is devoted to the nature and mode of action of elastase inhibitors occurring in serum and elastic tissues.

Dr. Hall has drawn together all these facets of an extremely complicated situation into a unified picture in which the normal functioning of the blood vessel walls depends on a delicate balance of the effects of the several enzymes and inhibitors. The way in which this balance may be disturbed in ageing is indicated in the final chapter. In achieving this he has put forward several controversial hypotheses which attribute a key role to the mucoprotein-lipid complex of elastic fibres. This is regarded as being responsible for the binding of elastoproteinase to the fibres, for the binding of lipid and as a substrate for elastolipoproteinase. The need for caution in interpreting results obtained with highly purified elastin in terms of functional changes is thus emphasized.

The standard of presentation of the book is high, though there are a number of typographical errors, particularly in the description of some of the tables and figures, and some references have been omitted. G. C. WOOD

## ULTRASOUND IN SURGERY

### Ultrasound as a Diagnostic and Surgical Tool

Edited by Dr. Douglas Gordon. (Based on the International Symposium held at the Royal College of Surgeons, London, December 5th and 6th, 1962.) Pp. xii+413. (Edinburgh and London: E. and S. Livingstone, Ltd., 1964.) 63s. net.

THE task of editing a symposium is not an easy one, since the individual contributions do not necessarily come together as a complete account of the subject, and may occasionally represent facets, the growing points in the estimation of some but not necessarily of others. Dr.

Gordon has managed to preface this record of the first international symposium on "Ultrasound as a Diagnostic and Surgical Tool" by an eminently readable account of the general physics of this subject. Since the book is aimed at the clinical research worker, the text is couched in general terms and does not include much mathematical treatment, some of which is, however, included in an appendix. An account of the properties of ultrasound, and of its transmission and biological effects, precedes an account of crystals and transducers—all of which are simply and directly propounded. The chapter on generators, however, is much more technical and might with advantage also have been placed in an appendix. The chapter on measurement of ultrasonic power stresses the need for more uniform calibration scales before there can be a uniform assessment of results. There is a greater need for metering the intensity of ultrasound energy *in vivo* at the tissue level than is commonly allowed for in *in vitro* calibration. This trend, if established, would achieve greater selectivity in the production of biological lesions. The description of pulse echo technique is a particularly clear exposition of the subject.

The remainder of the book reflects the advanced work on this subject done in many different centres. Reflexion techniques are described in several chapters for the exploration of the heart and cranial cavity. It is of interest that ultrasonic methods have been explored not only for pre-operative diagnosis, but that, through the use of probes at operation, greater localization and detection of lesions is being achieved. For example, an intra-operative application for neurosurgical use is described by contributors from Japan, but might well also be utilized by the cardiac surgeons in examinations conducted within the heart. De Viegler reviews extra-cerebral methods of detecting the excavations and displacement effects of blood clot in subjects who have extra-cerebral haematomas. Dr. Gordon's own chapters cover tomography, measurement of the performance of diagnostic equipment, and a chapter on the other biological and medical uses of ultrasound. The most firmly established use of ultrasound is still in the treatment of Ménière's disease, and the chapters by Arslan of Padua and by the Bristol group state well the position in this subject in the hands of these experienced workers. Other chapters cover such diverse entities as ophthalmic ultrasonography, a means of producing neurological lesions in the experimental animal by focused ultrasound, and the statistical evaluation of such effects. Unfortunately, the Glasgow group contribution to the original symposium, concerning the obstetric and gynaecological uses of ultrasound, has not been included. This seems a strange omission at a time when an alternative means to the use of ionizing radiation is being sought for the examination of the female pelvis and its contents.

The book concludes with a useful glossary and bibliography, and is well indexed.

ADAM N. SMITH

## PSYCHOLOGY OF HUMAN LEARNING

### Categories of Human Learning

Edited by Arthur W. Melton. Pp. xvi + 356. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 68s.

THIS book presents the revised version of seven papers given at a symposium on human learning at Ann Arbor in 1962. The authors form a distinguished group—Grant on conditioning, Underwood on rote learning, Estes on probability learning, Postman on short-term memory and incidental learning, Kendler on concept learning, Fitts on skilled behaviour, and Gagné on problem solving—and had the purpose of the symposium

been simply to review these areas, it is likely that a useful, if unadventurous, volume would have been produced. To some extent this has been done, and the chapters by Estes and Postman, and to a lesser extent (because they were asked to cover a wider field) those by Fitts and Gagné, provide valuable surveys of the literature. But the editor had wider ambitions. "The theme of the symposium", he says, "is the interrelationship of different categories of human learning." The participants were asked to consider such questions as: Are all forms of human learning similar? Can comparable principles be discerned running through the different areas psychologists have investigated?

It is difficult to formulate such questions clearly; it should not be surprising therefore that it is even harder to answer them. Those participants who make the attempt come up with three classes of answer. The most popular solution is to argue that if the same variables have similar effects in different situations, then the learning processes involved must be similar. This argument is used by Kimble in his discussion of Grant's paper to suggest that since partial reinforcement has different effects in classical and instrumental conditioning, the two must be distinct; and by Postman in an attempt to show that short- and long-term forgetting are similar because both are due exclusively to interference effects (a suggestion which underestimates the significance of Sperry's work). Both Underwood and Estes point out that the argument is invalid, since quite apart from differences in learning processes, apparently similar operations might have different effects in different situations because "the operations are not comparable, because the effects of the variable are different in the two situations, because measuring units are not co-ordinate, or because other factors modify the effect".

Estes's answer is a theoretical one. He argues that if a single theory can handle the results of experiments in different areas, then the areas may be regarded as similar, and illustrates the argument by applying statistical theory of conditioning to experiments on probability learning. The claims he makes here for statistical learning theory are notably more moderate than those he has made in the past, but Anderson is still able to make some fundamental criticisms. These are both justified and well-intentioned: Estes's theories are too interesting to be allowed to be discredited by their failure to handle the complexity of probability learning experiments. Thus, although Estes may be ultimately right in suggesting a theoretical answer to the symposium's question, the specific example he uses is not particularly successful.

The third and most interesting suggestion is made by Underwood. His major thesis is that the search for common processes has been conducted at too superficial a level. "To point out that retroactive inhibition occurs in motor learning, in concept learning, and in verbal learning would seem to be of little value. We need to know the comparability of the mechanisms involved in producing the retroactive inhibition for the various tasks; we need to isolate the subphenomena to see if the total effects are compounded in the same way." It is not retroactive inhibition that is likely to appear in common in different areas (and if it does, this tells us little about the identity of the underlying mechanisms), but the more molecular processes the effects of which we lump together when we talk of retroactive inhibition.

Underwood's article is valuable both because he makes a serious attempt to answer what is surely an important and difficult question, and because the answer he proposes suggests one cause of its difficulty, namely the belief that "conditioning is the simplest form of learning", a belief which Kendler defends at length in this volume. Many objections have been raised in the past to the suggestion that all more complex forms of learning can be derived from conditioning; in the present context the implication to which one might take exception is that

conditioning itself is a simple process. This attitude has been responsible for the superficial level of analysis to which Underwood objects. If a more realistic account of the behaviour of humans in conditioning experiments were provided (if we could uncover the more detailed processes at work), then there might be a chance of showing these same processes at work in apparently more complex situations, and thus at last of integrating the various areas of human learning.

N. J. MACKINTOSH

## LABELLED COMPOUNDS

Proceedings of the Conference on Methods of Preparing and Storing Marked Molecules, Brussels, November 13-16, 1963

Pp. xxiii + 1359. (Brussels: Euratom, 1964. Available from Central Sales Office for Publications of the European Communities, 2 Place de Metz, Luxembourg; and Presses Académiques Européennes, 98 Chaussée de Charleroi, Brussels.) 1,000 Belgian francs.

THE preparation of labelled compounds is an absorbing activity for those relatively few people who undertake it professionally; but for the multitude of research workers who use radioactive tracer methods, procurement of a compound labelled specifically for the purpose at hand is a means to an end. If the right compound can be bought at reasonable cost, it usually will be. Nevertheless, the diversity of the requirements of tracer users is now so vast that no organized supply can possibly satisfy them fully, and research workers are necessarily left to their own devices to fulfil their more specialized needs. So producers and users of labelled compounds are related more closely than just as supplier and purchaser: they have many practical problems in common.

These mutual interests were the foundation of the conference on "Methods of Preparing and Storing Marked Molecules", which was the first international gathering on this topic. It was attended by 150 people of varying interests, coming from a dozen or more countries in addition to those of Euratom, who were the hosts and organizers. Clearly it was a most successful conference, which fulfilled a real need for direct personal communication. The *Proceedings*, which provide a full record of the conference, comprising seven general surveys and sixty-three original papers, should be equally valuable.

The established chemical and biological approaches to labelled compounds are reviewed competently by L. Pichat and J. R. Catch. Many of the other papers break fresh ground, by describing original synthetic methods for particular compounds or groups of them, and syntheses of some forty substances are recorded here for the first time.

The products described are almost exclusively those of biological interest—for example, the tropane alkaloids, chloramphenicol, the actinomycins, gibberellic acid, proteins and peptides; and they are predominantly labelled with carbon-14 or tritium. The papers reflect the present buoyant interest in tritium compounds as biological tracers and the trend towards still higher specific activity and specific chemical labelling with this isotope. There are rather few references to other labelled substances of technical interest outside the biological field.

A useful review by W. Herr of radiochemical methods for marking organic molecules emphasizes the merits and the difficulties of these methods. Other papers demonstrate the usefulness of the Wiltzsch method when it is applied critically to otherwise intractable problems of labelling, and also its practical limitations.

With the rapid growth of provision and use of radioactive labelled compounds, and the constant trend towards higher specific activities and more critical

standards of purity, better control of radiolytic decomposition during storage has become a matter of urgent practical importance. This first open discussion of the subject is therefore of special interest. Excellent reviews of the radiation chemistry of amino-acids and proteins are given by B. M. Tolbert and co-authors, and show the difficulty of relating the results of academic investigations to the stability of labelled compounds observed in practice. An empirical approach to this problem is generally the only one that is immediately useful, and accordingly the practical experience of several observers given in this and other papers is particularly valuable.

The book is excellently edited and printed. It is a large volume of more than 1,350 pages, but roughly half is taken up by English translations of the original texts in other languages. The translations are not always perfect, but they are most serviceable. The editorial staff of Euratom, the publishers and the conference organizers are to be congratulated on getting these proceedings on record less than a year after the event.

W. P. GROVE

## MATHEMATICAL ANALYSIS

An Introduction to Mathematical Analysis

By Prof. Robert A. Rankin. (International Series of Monographs on Pure and Applied Mathematics, Vol. 43.) Pp. xv + 607. (London and New York: Pergamon Press, 1963.) 80s. net.

REAL variable analysis is a standard part of the undergraduate course in most university departments of mathematics. It has been so for many decades, and it might be thought that the details of the subject, including method of treatment, are so standard and crystallized as to be almost lifeless. However, this is not the case, and a comparison of Prof. Rankin's *An Introduction to Mathematical Analysis* with some recent American and German books reveals how wide the discrepancy can be. Prof. Rankin's book is based on lecture courses given over many years at the Universities of Cambridge, Birmingham and Glasgow. It deals rigorously with subjects such as limits, continuity, differentiability, integration and convergence (of series and products), and presents what might be regarded as the traditional British approach to the subject. This approach is a gentle one for the student who has had a good grounding in school and first-year mathematics. It is reasonably down-to-earth and part of the philosophy is the provision at numerous points of suitable examples and exercises. In fact, Prof. Rankin writes in the preface as follows: "These exercises have a threefold object, to test understanding of the preceding theory, to provide practice in carrying out the techniques described, and to encourage imaginative thinking by requiring the student to provide his own examples and counter examples".

There can be no doubt that for the great majority of students the approach of this book is the right one. But how sharp is the contrast with, say, the recent book by Maak on *Introduction to Modern Calculus*. The latter is a beautifully written book, but the approach is highly sophisticated. Let us trace one aspect of the contrast, namely, the introduction of the trigonometric functions. Both authors use an analytical approach, with no reference to triangles. Maak, however, who directs much attention to sequences, introduces a particular sequence, the limit of which is the arctangent function. He then introduces inverse functions, and thus defines the tangent function as the inverse of the arctangent. After that the sine and cosine functions emerge in terms of the tangent function (of half the angle). Rankin, while remaining strictly analytical, makes very little use of sequences, and introduces the sine and cosine functions directly in terms of



the appropriate infinite series. His approach is both rigorous and practical; that of Maak, while attractive, is so polished and lengthy that none but the chosen few in the mathematical class can take it. In trying to reconcile these two books, perhaps the proper view is to regard Rankin's book as a prior text, and a very good text-book it is, and Maak's book as one of reference by those who already know the subject.

One half of Prof. R. A. Rankin's book is given to integration and to convergence and uniformity. A few words about the former may not be out of place. Two points of view are adopted. In the first, integration is defined as the reverse process to differentiation, and this approach leads to the standard results of the theory quite quickly. In the second the Riemann-Stieltjes integral and the Riemann integral are introduced, the theory being based largely on the properties of interval functions and dissections that are studied previously. Once again the sound practical approach to the subject is evident.

Prof. Rankin's book is a first-class text for undergraduate classes. Not only are numerous exercises provided but also hints for the solutions of many of these are given. It is a gentle text and will form a most valuable adjunct to a course of lectures on the subject.

L. S. GODDARD

## STRUCTURE AND MEANING

### Structural Anthropology

By Dr. Claude Lévi-Strauss. Translated from the French by Claire Jacobson and B. G. Schoepf. Pp. xvi+410. (New York and London: Basic Books, Inc., 1963.) 52s. 6d.

ANY professional teacher of social anthropology (say, any one of the 200 or so members of the Association of Social Anthropologists) must be competent to write a conventional introduction to the subject, that is, to the work of other people, for if he could not so order the elementary instruction which he provides he should not occupy a university position. To receive *Structural Anthropology*, therefore, as yet one more text-book would be to encourage no special expectation. Prof. Lévi-Strauss, by contrast, is not only one of the extremely few scholars of sufficient distinction to be able to say, without presumption, what social anthropology is about; but he has also actually demonstrated, by numerous analytical articles and monographs, that he is pre-eminently worth listening to. He does not just talk about the subject, he works; he does not simply practise social anthropology, he makes exciting and original contributions to it.

Yet this collection of some of his papers, although the nearest thing so far published to a representative introduction to social anthropology, nevertheless shows that a text-book of the subject is not possible. It has, to begin with, no distinctive subject-matter, and it possesses no singular method of analysis. Lévi-Strauss demonstrates the former point by writing about a wide range of disparate topics such as sentiments, dual organization, sorcery, myth, symbolism, dialectics, and split representation, taken from a variety of both great and minor civilizations; and he makes the second by constant references to history, linguistics, communications theory, and physics. What is it, then, that he and the discipline of which he is the foremost theoretical proponent have to offer to human understanding?

The most concise response is that social anthropology is the structural analysis of meaning. Its focal concerns are social categories, relations, and values; its characteristic objects of examination are symbols. The source of the special authority of the social anthropologist in this extensive and general field of investigation is the universal scale against which he pursues his enquiries, so that when he tries to construct general propositions (as, of course,

do historians or economists) he alone may properly aim at complete generality within the territorial and temporal limits of mankind. Thus Lévi-Strauss deals, in one essay, with material and with periods as greatly dispersed as China in the first to second millennia B.C., the Amur region in the prehistoric era, Brazil in the twentieth century, and New Zealand in the fourteenth to eighteenth centuries. The social anthropologist cannot be a polymath to the extent of possessing a scholarly command of all that is known about every area and period, but the breadth of his comparisons and the abstract nature of his discipline as a general theory of relations permit him, nevertheless, to advance novel and empirical considerations.

*A priori*, it would seem unlikely that this result might be possible, but Prof. Lévi-Strauss is superbly capable of demonstrating that it is. He does so in the main not by concentration on substantive cultural particulars but by analysis in such relational terms as opposition, reciprocity, and homology, and it is through the structural models thus constructed that he is able to encompass such a mass of apparently incomparable evidence. How he does so is not a matter for report but for examination, and anybody who thinks such an enterprise worthy of attention should learn much from reading this volume. But even Lévi-Strauss cannot be taken as an exact exemplar in all respects, and the most prominent ground for demur concerns the relationship of his own work to linguistics, the subject to which he so frequently has recourse in his methodological observations. He makes many professions of illumination yielded by linguistics, and generously concedes priority to it as a field of more advanced structural analysis; but it is a question whether social anthropology has ever profited in one clear respect from the example of that admirable discipline. The parallels of concept and method to which Lévi-Strauss alludes are indeed noteworthy, but it is arguable that they are no more than parallels, for the principles implicit in his work are readily discernible in Dumézil also, in Hocart and in Rivers, and of course in Mauss and Hertz and Durkheim. The structuralist approach which these scholars have used, in a long and continuing tradition of ideas, owes nothing historically and particularly to linguistics; nor is there to be found in linguistics to-day, for example in the algebraic tautologies of the so-called "componential analysis of kinship", any more immediate or useful effect. We should instead take it, therefore, that Lévi-Strauss has merely found in the terminology of linguistics a technical idiom which appeals to him, rather as Dr. E. R. Leach (Lévi-Strauss's counterpart in Britain) has found it convenient to make a similar comparison with topology. This view is not merely a suggested qualification to be kept in mind when reading Lévi-Strauss's papers, but, if accepted, it has the result of at once making his thought more directly accessible to understanding, and placing him in a recognized tradition proper to his discipline.

What this discipline is, to return, may be illustrated—not catalogued—by the papers in *Structural Anthropology*. They are divided into five sections: language and kinship; social organization; myth and religion; art; and problems of method and teaching. The seventeen articles were originally published, all but four in French, between the years 1945–56 (the collection could be marvellously amplified with other papers published since), and have been translated into an exact and fluent English by Claire Jacobson, with the collaboration of Brooke Grundfest Schoepf. A random check reveals small uncertainties, or even a misrendering such as "generalized reciprocity" for the key term "*échange généralisé*", but in general the translation is excellently worthy of the author. There is a full bibliography and a good index. The volume is most handsomely produced, and it is only to be regretted that the American publishers (unlike the original French) should have found it expedient to put the footnotes exasperatingly at the end of each article instead of where they belong.

R. NEEDHAM

### Behaviour of Electrons in Atoms

Structure, Spectra, and Photochemistry of Atoms. By Dr. Robin M. Hochstrasser. (The General Chemistry Monograph Series.) Pp. xii+162. (New York and Amsterdam: W. A. Benjamin, Inc., 1964.) 4.50 dollars.

A STUDENT of chemistry needs to know, at the very start of a university career, something about atomic structure and atomic spectra. However, because the interactions between electrons are complex and intricate, it is extremely difficult to give an account which is both honest and at the same time easily intelligible to the newcomer. Moreover, it is to be hoped that such an elementary description will not only be intelligible, but will also give the student some feeling and understanding for the factors that govern the energy and other properties of atomic systems.

Prof. Hochstrasser has tackled this problem in a new and interesting little book of 162 pages. The presentation is qualitative in character and does not attempt to derive mathematically the various expressions that are presented in the text. The figures are extremely good, and the use that has been made of colour in the energy level diagrams adds greatly to their intelligibility. The material is well set out and clearly written, and the summaries at the end of each chapter are most helpful. There are also some good problems.

Most of the book is very successful, but it is somewhat doubtful whether the section in the neighbourhood of page 50 will give the young student any real feeling for orbitals and the corresponding probabilities. The same is also true in respect of the energies of atomic systems, which are dealt with in Chapter 4. However, in this chapter some features are brought out more successfully than elsewhere; Fig. 4-5 is a helpful one. With regard to energies, Fig. 5-2 is wrong because all the levels for the lithium atom should be below those for the hydrogen atom which have the same principal quantum number. In Table 6-1 it would have been helpful if an energy or frequency scale had been given. Chapter 7 is very interesting, but it is doubtful whether Chapter 9 was worth including.

The author started by linking his description closely with experimental data. In the middle of the book he unfortunately departed from this approach to a considerable extent but returned to it strongly in the last few chapters. He can certainly be congratulated on providing a useful little book which will help new chemistry students in a subject which they frequently find difficult. However, they will ultimately need to carry their knowledge further.

J. W. LINNETT

### Stress Waves in Anelastic Solids

Edited by Herbert Kolsky and William Prager. (International Union of Theoretical and Applied Mechanics Symposium, held at Brown University, Providence, R.I., April 3-5, 1963.) Pp. xi+342. (Berlin, Göttingen and Heidelberg: Springer-Verlag, 1964.) 67.50 D.M.

STRESS wave problems are among the most important, technologically, of the problems connected with deformable media. They may not be the centre of interest. Steady-state problems are likely to take pride of place so long as any important ones remain unsolved, and in recent years experiments at hypervelocities and questions about implosion have proved fascinating. But stress waves have never been far from the centre of interest, and during the past ten years solutions have been obtained to many wave problems in spite of theoretical and experimental difficulties.

The contents of *Stress Waves in Anelastic Solids* are the invited papers at a symposium attended by many, if not all, the experts in this field. That it should be solely concerned with anelastic solids and large strains emphasizes both the advanced stage of the theory for elastic

materials and the inadequacy of that theory when there is a concentration of stress. Even the paper by Goldschmidt, at the end, giving reproducible data for such a difficult material as rock, is concerned with the dynamic as well as the static characteristics.

The two main problems in the field are the discovery of the laws of flow with large and variable strains, and the analysis of the wave patterns which arise. It is clear that steady advances are being made in both problems, but it is also clear from the papers on shock waves and those on plastic waves that the laws of flow yet known are only just adequate to correlate experiment and theory. An excellent review by Hopkins on strain-rate effects is followed by a paper by Hunter and Johnson, who analyse the propagation of small plastic vibrations along a bar, and show that the dispersion due to the finite cross-section is so appreciable at low frequencies that it could explain the results without introducing any strain-rate effects.

There are a number of papers analysing the structure of the wave pattern and its development with time. There are two on spherical and plane shocks, one on transient wave and fracture, and many dealing with waves of finite amplitude and their decay. The more interesting papers are distinguished by a final selection containing conclusions. There is a challenge here to theorists, as well as experimenters, to solve problems even more difficult than those connected with shock waves in aerodynamics. Even if the properties of the material are known, and symmetrical arrangements are chosen to avoid awkward reflexions at boundaries, these wave problems in solids remain confused owing to the internal discontinuities due to change of structure, and the associated dispersion and anisotropy.

Meanwhile, practical problems must be solved and theoretical calculations continue. There are some excellent mathematical papers on plastic waves, including one on anelastic ropes, and several on viscoelastic solids, which deserve a more complete discussion than can be given here. This is a collection of individual papers which gain by being published together.

G. J. KYNCH

### Numerical Methods

By Dr. Ben Noble. Vol. 2: Differences, Integration and Differential Equations. (University Mathematical Texts.) Pp. viii+157-372. (Edinburgh and London: Oliver and Boyd, Ltd.; New York: Interscience Publishers, Inc., 1964.) 12s. 6d.

THE first volume of Noble's book dealt with iterative methods for solving algebraic equations, programming, linear equations and matrix methods. In this second part, the relevant parts of the calculus of finite differences are expounded and then applied to the interpolation, differentiation and integration of tabular functions. Then there is a long chapter on ordinary differential equations, with accounts of iterative methods (Runge-Kutta), predictor-corrector methods (Adams-Bashforth, Milne), and deferred-corrector (central difference) methods. In the final chapter, we have a good if brief introduction to finite-difference and relaxation methods for partial differential equations of the second order in two space variables. But the volume is no mere epitome of formal techniques; much attention is paid to the choice of appropriate practical methods, to error estimation and to the often subtle question of stability. There is in fact a surprising amount of information packed into this small book, for the author shows a happy skill in combining brevity with clarity. Fifty years ago, it was difficult to find a text on numerical analysis; to-day, the difficulty is one of choice out of a very large number, but the novice will get as good a view of the domain and its major routes from Noble's book as he can find anywhere. He will not get much direct help for further reading, but the author very wisely recommends for this purpose the good bibliography in *Modern Computing Methods* (H.M.S.O.).

T. A. A. BROADBENT

## SIR HENRY TIZARD (1885–1959)\*

By PROF. R. V. JONES, C.B., C.B.E.

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SIR HENRY TIZARD'S work and, above all, his part in our survival in 1940 have been the subject of many memoirs since his death in 1959. The biography commissioned by his family and written by Ronald Clark will shortly appear. This first Memorial Lecture of the Royal Aeronautical Society thus occurs when a full record of Tizard's life is about to be made available, and there is therefore little point in the plain reiteration of a condensed biography. Future Memorial Lecturers, as they inevitably grow more remote from personal contact with Tizard, may well choose to describe the advances of the future in those fields to which Tizard himself so notably contributed: science, defence, education and government. My purpose in this first Memorial Lecture is therefore to build a bridge between biography and those future fields in which Tizard's example, although unlikely to be excelled, will be emulated and extended.

As for my qualifications to do so, there are certainly men who knew Tizard more closely than I did. They were more nearly his contemporaries. But I was fortunate in my generation in being brought into his orbit at an early stage; and his effect on my own career was profound. When I first met him in 1937, he was at the height of his work and I was, at twenty-six, half his age. Age or youth was, however, no barrier with Tizard—he could talk easily with either; he was always keen to know what the young men thought—and he was always ready to give them the benefit of his experience, and to stimulate them with challenges. This was to be my good fortune over a period of twenty-two years.

## Westminster, Oxford and Berlin

This period, however, covered only the last third of Tizard's life, and I cannot claim first-hand knowledge of his earlier life except for a few chance sidelights that arose from comments that he made to me. Instead, I have to depend heavily on Sir William Farren's biographical memoir for the Royal Society and the biography that Mr. Clark has very kindly allowed me to see. From these sources you can find that the Tizard family came as Huguenot refugees to Dorset, with a tradition of connexion with the sea. Over the centuries they are said to have become "more English than the English". Henry Tizard once remarked, "With a name like mine, you have to be!" His father, Captain T. H. Tizard of the Royal Navy, was assistant hydrographer of the Admiralty, and it was taken for granted that the son would in due course follow into the Navy. In the summer of 1898, however, when Henry was nearly thirteen, a fly flew into his left eye; when he closed this eye he found that he could no longer see—and thus realized that his right eye had a blind patch. This incident changed his life, for his prospect of entering the Navy was shattered. Recovering with characteristic resilience, he won an Exhibition to Westminster School in 1899; and from there, feeling in the meantime the appeal of science, he won a Demysip in chemistry at Magdalen College, Oxford. He was thus set on a career in natural science. His 'First' in chemistry was backed up by another 'First' in Mathematical Moderations; and having run the gauntlet of a classical education,

and with a naval background that showed itself academically in a study of the works of Mahan, Tizard was admirably poised for the contributions that he was to make.

At first, it seemed that these might primarily lie in pure research. After his degree at Magdalen in 1908, he went to Berlin to work under Nernst. There he met Frederick Lindemann, working in the same laboratory. The story of their subsequent friendship and quarrel is near high tragedy. Much has been said and written about it, and I shall have little to add here, beyond saying that Mr. Clark has done a service in setting out the facts with an impartiality that Tizard himself would have applauded. The quarrel cannot be ignored, although its importance can be exaggerated; it is not a subject for vindictive anecdote, but it is a study in human relations between two men, each greater than most of us, who, with some astonishing similarities in experience and outlook, nevertheless became antagonized at the crisis of their country's survival. As such, it may with value be studied by any of us who are concerned with science and Government, not with the view of deciding who was right and who was wrong, but as a background against which to guide our own conduct should we be faced with our own crises. That the quarrel was between men of science was—contrary to outside opinion—immaterial. It could have arisen between two men in any field, and it is perhaps the common delusion that men of science are above emotion that has made the story such an occasion for popular remark.

At the time that Tizard met Lindemann, their different personalities were obvious, but their similarities were substantial. Both came from refugee families, both were men of science; their births coincided within a few months, and they each had defective vision in one eye. At a time when such views were unfashionable, both believed that science merited a fuller place in education and that good technology was a foundation of national well-being. Both were men of high courage, and in the War that followed in 1914 both flew aeroplanes with conspicuous understanding and skill.

In the years between 1909, when Tizard returned from Berlin to Oxford, and the outbreak of the First World War, he worked first on the sensitivity of chemical indicators, and later on electricity in gases. He was elected to a fellowship of Oriel College, Oxford, in 1911, and it appeared that a pleasant life stretched indefinitely before him. He afterwards told how he was contemplating this happy prospect over the comforts of hot anchovy toast and tea beside a blazing fire in his rooms in college after an afternoon walk: "What a lucky fellow I am—everything I want!", he thought—and immediately followed by "I shall stagnate—I must get out of this at once!"

A fortunate vacancy on the party of the British Association for the Advancement of Science going to Australia in 1914 was offered to Tizard at the last moment. This he afterwards described as his "greatest piece of good luck", and he sailed on July 1, 1914, in company with many eminent men of science. In particular, he met Rutherford, and formed a friendship which lasted until Rutherford's death, and which he most happily described in a notable broadcast of 1945. By the time the party reached

\* First Tizard Memorial Lecture of the Royal Aeronautical Society, delivered on October 28, 1964, at Boscombe Down.



Australia the War had broken out, and in October he returned to England with H. G.-J. Moseley, then aged twenty-seven, who had already "called the roll" of the chemical elements with his X-ray spectra; Moseley was killed while serving as a signals officer at Gallipoli in 1915.

### Bombsights and Test Flying in the First World War

Tizard joined the Royal Garrison Artillery at Portsmouth in November 1914 and was allocated to anti-aircraft gunnery; but, finding that he had too little to do, he secured a transfer in July 1915 to the Royal Flying Corps at Upavon as a second lieutenant and assistant equipment officer. In the meantime, in April 1915, he married Miss Kathleen Wilson, whom he had met five years before at Oxford. Robert Bourdillon was their best man, and it was the work of Bourdillon and G. M. B. Dobson on bombs and bombsights that attracted Tizard to Upavon. But again he encountered frustration: aeronautical research in Britain was being conducted by three separate bodies. The Royal Aircraft Establishment, then at Farnborough, had originated at Woolwich in 1878 as the War Office Balloon Equipment Store; the Advisory Committee on Aeronautics (now the Aeronautical Research Council) directed the Aerodynamics Department of the National Physical Laboratory; and in addition there was the Experimental Flight of the Central Flying School at Upavon.

The primitive state of knowledge and the thoroughly experimental approach of the Central Flying School are illustrated in a story afterwards told by Tizard: "Some young officer argued one day in the Mess, that as the resistance to motion got greater the further anything dropped under gravity (which is true) it followed that if you dropped an egg from a sufficient height, say 3,000 feet, it would not break when it hit the ground. This outrageous statement was received with hilarity by the young scientists present, but the Commandant was not convinced by their scepticism and decided to make the experiment".

A pilot was duly ordered to take a *Maurice Farman* up and drop the eggs one by one. Tizard went on:

"We all waited patiently while the machine climbed laboriously to this dizzy height, but no one saw anything dropped. When the pilot came down he said that he had dropped all the eggs; so a search was made for them. No trace of them was found and so the question had to remain open".

Tizard himself worked on a bombsight, the first the Royal Air Force ever had. He devised a photographic method of recording bombing runs from the ground, and his observations led him to suspect that sighting errors were in fact much less than flying errors. "I then came to the conclusion", he wrote, "that it would be difficult for any scientific man to do really important relevant work unless he himself learnt to fly." An argument with the War Office followed. The Central Flying School was held to train operational officers: Tizard was not an operational officer, and so he could not be trained. As a compromise, it was agreed that Tizard could be trained if his training took place on days which were too bad for training operational officers. And so he learnt to fly.

Many of the details of this period of Tizard's career can be read in Ronald Clark's biography—they will ring true to anyone who has worked in that fascinating region between science and the services, characterized by mutual admiration for each other's achievements and amusement at each other's peccadilloes. In this atmosphere, in which the enthusiasm of the pioneer was enhanced by the urgency of war, Tizard gained that deep understanding of the interplay of the scientist and the serving officer that was going to be so valuable in 1940.

The frustration experienced by Tizard arose partly from the fact that it was not easy to test experimental

armaments at a training aerodrome. Fortunately the situation changed sharply with the appointment of Bertram Hopkinson, professor of engineering at Cambridge as Director of Aeronautical Equipment in the War Office. Hopkinson quickly realized the difficulties, and moved the armament work to Orford Ness in June 1916, and the remainder of the Experimental Flight, now renamed the Testing Squadron, from Upavon to Martlesham at the end of 1916. Tizard was appointed as Captain in Charge of Experiments at Martlesham early in 1917. His work was concerned with the development of test flying, and as quickly as March 1917 he read a characteristic paper on "Methods of Measuring Aircraft Performance" before the Society. In it he described the system he had introduced in the Testing Squadron for accurately establishing the performance of an aircraft despite variations of altitude and of weather. He had great faith in the value of direct observations by the pilot, and relied on recording instruments only as a check on these observations. The quality of the pilot's work was, according to him, what mattered more than anything else:

"In the end the accuracy of the results really depends upon the flier, who must be prepared to experience a care and patience unnecessary in ordinary flying. Get careful fliers whose judgement and reliability you can trust, and your task is comparatively easy; get careless fliers and it is impossible".

Tizard had placed his stamp on the character of test flying. Before him, skill and bravery were the attributes sought in test pilots; after him, they had in addition to be patient, objective observers.

On one of his own test flights in a *Sopwith Camel* in July 1917, a formation of *Gothas* was reported to be making for London. Tizard, whose plane had untested guns succeeded in intercepting the formation, twenty strong at 17,000 ft. His guns jammed after less than 100 rounds, and so he shadowed the formation taking note of its aerodynamic performance.

Towards the end of the year, Bertram Hopkinson invited him to go to the Ministry of Munitions as his deputy. After Hopkinson was killed in August 1918, Tizard took over in his stead, finishing the War as a lieutenant-colonel and being awarded the Air Force Cross in recognition of his test flying. He returned to Oxford and became reader in thermodynamics in February 1920.

### Aircraft Fuels

Among the interests that he carried back to Oxford as a result of his war experience was that of aircraft fuel. In 1917 special aircraft fuel was running short because of sinkings by German submarines. This oil came from Pennsylvania and consisted almost entirely of aliphatic hydrocarbons. Tizard decided that tests should be made at Martlesham of diluting the Pennsylvania fuel with benzol from gasworks. This mixture turned out to be even better for aircraft engines than the Pennsylvania fuel alone. While the benzene in the mixture tended to freeze out at low temperatures, the other aromatic contents in the benzol did not. This work brought Tizard into contact with H. Ricardo, who invited his co-operation in a major programme of research for the Asiatic Petroleum Company. Tizard brought in David Pye, and they worked between Oxford and Ricardo's laboratory in Shoreham. They confirmed that the incidence of detonation was the most important single factor limiting the performance of the petrol engine, and Tizard suggested that this factor should be expressed in terms of a 'Toluene Number', which would be the proportion of toluene (the least prone of any known hydrocarbon to detonate) that had to be added to heptane (the worst) to match the detonation quality of the fuel under examination. Some years afterwards the Americans replaced toluene by iso-octane, resulting in the standard 'Octane Number' definition of the anti-knock properties of a fuel.

# The Department of Scientific and Industrial Research and Aeronautical Research

While this work was going on, Tizard received in 1920 an invitation from Sir Frank Heath to join him at the Department of Scientific and Industrial Research, then four years old. Such a change of career involved much thought. His correspondence with Heath shows that he realized very well the difficulties that the new Department would find in making its way, largely because it had been set up at a relatively late phase in the development of Britain. For lack of much central thinking about research, even more so in technology than in science, a number of independent organizations had been set up to deal with specialized aspects of applied research, and it was not clear, for example (and the example was Tizard's), what the part of the Department of Scientific and Industrial Research would be in co-ordinating aeronautical research, which was already under way at Farnborough and the National Physical Laboratory. We are still not free from the difficulties that Tizard foresaw, although, with the deliberations of the Trend Committee, some of them are beginning to be faced.

There were several reasons that determined Tizard in coming to the conclusion that he ought to leave Oxford and take up the burden of the Department of Scientific and Industrial Research. Among these was his realistic assessment of his prospects in pure science, where he had lost five of his most creative years in the War, and where younger men of great ability were coming up. The more important reason was, as he recorded: "My war experiences had taught me the value, and interest, of the application of science. It was a neglected field in England".

Tizard's work as Assistant Secretary of the Department of Scientific and Industrial Research was control "of the group of Boards and Committees entrusted with the co-ordination of research for the fighting services". This represented an early attempt to straighten out the tangle of research around the Armed Forces, and if anyone could have done it, it was surely Tizard. But the task, difficult even under the urgency of war, was impossible in peacetime. Too many independent bodies were individually too well set to be merged into effective combination. The Germans faced this same problem later when the clandestine research organizations set up before 1933 came into the open; because they were clandestine they had had to be small, and even a single Service like the *Luftwaffe* had several of them. And when they became overt, they were individually too strong to be welded into a really effective organization. Despite these lessons, we are still in the same kind of difficulty in Britain to-day. There may not be a complete solution: one mammoth research organization for all problems, both military and civil, is not the answer, nor is a large number of *ad hoc* and uncoordinated research institutes. Somewhere between may lie the optimum compromise.

Anyhow, by 1927, Tizard had seen that the boards and committees were not really effective, and they were all dissolved except for the Radio Research Board. In the meantime his interest in aeronautics had remained; he had been a member of the Glazebrook Committee on Education and Research in Aeronautics in 1918 and 1919 which had recommended that there should be a Board of Aeronautics run jointly by the Air Ministry and the Department of Scientific and Industrial Research. The Service did not like this proposal, and it was not implemented. Tizard himself in 1924 suggested to the Air Member for Supply and Research (Sir John Salmond) that the actions should be separated at director level, and a director of scientific research appointed to take charge of the research activities of the Air Ministry. This suggestion was accepted by Salmond and backed by Tizard, who had been consulted. They were clear out who the first director of scientific research should

be: Tizard. However, Tizard refused the post because, to judge by the salary offered, the Air Ministry was not recognizing its status adequately; finally, the Treasury was persuaded to make Tizard personally a special offer, but he rejected it because he thought that it ought not to be personal, and that it would be unfair to another director in a comparable post if Tizard were to be paid more than he was. After some delay, H. E. Wimperis was appointed instead.

At the time when these negotiations were going on, Tizard delivered the Wilbur Wright Lecture to the Royal Aeronautical Society. The text of it appears in the *Journal of the Royal Aeronautical Society* for 1924, and the immediately following paper is that of his inaugural address as chairman of the Society later in the year. He chose as his title "Commonsense and Aeronautics", and in it he gave the new Directorate of Scientific Research the benefit of the doubt that he privately felt about the scope that it would be given by the Air Council:

"We look for the closest co-operation between the executive and the scientific branches; between, in short, the user and producer. The war showed us the value of that; but we seem to have been forgetting the lesson. Our little aeronautical world has tended to be divided into so-called scientists (a small, slightly troublesome, and wholly incomprehensible sect) and practical men (who do the work). Placed on close but parallel lines, they only meet at infinity. Four years of experience on the Aeronautical Research Committee has taught me the danger of this; but we hope that the new organization will avoid it".

The 'commonsense' of the title Tizard explained as that of "the intelligent thoughts of the ordinary man" and he ranged over civil as well as military aeronautics, making a powerful plea for reliability and safety as the foundation of a thriving civil aviation industry. The address is well worth reading in entirety to-day. Let me quote a little to convey something of its spirit and foresight:

"One often gets the impression from debates in Parliament, and from articles in the press, that military research is believed to result solely in the evolution of new methods of destruction, and to have no real value in itself. Nothing, surely, could be further from the truth. . . . In scores of ways, direct and indirect, the results of research undertaken primarily to meet Service requirements are continually filtering through to, and benefiting, the industries of the country. . . . Few, if any, industrial organizations in this country could afford to carry out the kind of work that is pursued in the Services experimental establishments, even if they were convinced of its value; and yet it is just this kind of work that leads in the long run to important industrial advances. . . . There is only one danger that I see in this instance of the value of scientific work. It is that the scientific worker may continue to be regarded, as he undoubtedly has been in the past, as a peculiar being, to be segregated from his fellow men and left to the evolutions of his inner consciousness. . . . But most of us, it must be admitted, need the inspiration of practical aims to call forth our best work. In applied aeronautical research there is room for everybody. A vast amount of important work waiting to be done needs no deep scientific knowledge; scientific method and a logical and unprejudiced mind will carry one far. There are officers in the executive branch of the Royal Air Force, the value of whose judgement, criticism and experimental skill is gratefully recognized by the scientific world. To such a one, the Society has awarded its Silver Medal tonight".

Incidentally, the recipient of the medal was Squadron-Leader R. M. Hill who, as Air Chief Marshal Sir Roderick Hill, was later to follow Tizard in the Rectorship of the Imperial College of Science and Technology.

In 1927, Tizard succeeded Heath as Permanent Secretary of the Department of Scientific and Industrial Research; but in 1929, in a move that had something of accident

about it, he left the Department to become Rector of the Imperial College of Science and Technology. There was, of course, nothing accidental about his fitness for the new post: his natural interests in technology and in education, coupled with his administrative experience, qualified him admirably. And while his new duties absorbed much of his energy, he was well able to continue national work as a now independent member of Committees and Boards. In a special place among these was the Aeronautical Research Committee, of which Tizard had been a member, in one capacity or another, since 1920. Tizard was chairman of the Engine Sub-Committee. Against protests he insisted on a policy of testing all new Service engines on fuels of higher octane rating than those then generally available: he argued that under the stimulus of war such fuels would be produced, and therefore that the engines should be designed to operate at higher pressures and so take advantage of them. As a result of this policy, when improved fuels were produced in 1940, our existing engines could be used with confidence. This example seems to me to be 'vintage Tizard', showing his faith and foresight at their best—not so extravagant as to make it impossible to carry others with him, but showing an extra anticipation that others lacked.

### The Jet Engine

Tizard was faced on the Engine Sub-Committee with a basic invention of outstanding importance—the jet engine. In 1926 A. A. Griffith put up a paper on the aerodynamic theory of turbine design, with the view of an axial-flow turbine driving a propeller, and Tizard proposed that the theory and its implications should be examined at Farnborough. As a result, an experimental rig was set up, and Tizard presided over a panel in 1930 to consider the results. Although Tizard himself felt that experiments should be pressed further, carrying the panel with him on this point, he agreed with the other members of the panel that there was as yet not enough ground for large-scale financial support; misgivings had been expressed about the abilities of known materials to withstand the operating conditions. There is some evidence to suggest that Griffith was discouraged by this equivocal verdict, and his work at Farnborough thereby lost its impetus. In the meantime, independent of all this, F. W. Whittle was working on his own ideas of a jet engine, and met Tizard at the Cambridge University Air Squadron Dinner in 1936. Tizard, after some months of thought, was convinced; and by February 1937 had carried the Engine Sub-Committee with him to the extent of recommending that the Air Ministry should explore the possibility of supporting Whittle's development. In retrospect, this appears leisurely progress, but it had had to be made against substantial opposition; and Sir Frank Whittle has said of this vital stage "Our best friend was Sir Henry Tizard". The subsequent history of the engine will be well known to many members of this Society, and can be read in Sir Frank Whittle's book *Jet*. He records how Tizard saw a successful demonstration run on the experimental engine in January 1940 and commented: "A demonstration which does not break down in my presence is a production job".

### The Committee for the Scientific Survey of Air Defence

In the early years of Tizard's Rectorship of the Imperial College of Science and Technology, it became increasingly obvious that the policy of Nazi Germany was very likely, sooner or later, to lead to war, and that Great Britain would have to defend herself against the attack of the German Air Force. As a result, despite Mr. S. Baldwin's counsel of despair in 1932, that "the bomber will always get through", a renewed scientific appraisal of the problems of air defence would almost certainly take place in one form or another—the national need was far too strong

and too widely felt to be ignored. This situation brought about two trains of events that were between them to give Tizard the opportunity for his greatest work, and to give him his greatest source of pain. In the Air Ministry A. P. Rowe, scientific assistant to H. E. Wimperis, began an informal survey of the air defence problem; this led Wimperis to propose that a committee should be formed for the scientific survey of air defence, and that Tizard should be chairman. Tizard was formally invited on December 12, 1934, the other independent members being Prof. A. V. Hill and Prof. P. M. S. Blackett.

While these steps were being taken, and perhaps even before, Prof. Lindemann in Oxford had been pressing with Mr. Winston Churchill, for a scientific investigation of the air defence problem to be made, most of the pressure being applied at the political level, and particularly to Mr. R. MacDonald, then Prime Minister, and Mr. Baldwin. When Lindemann discovered that in the meantime the Air Ministry had formed its own committee under Tizard, there was obviously scope for misunderstanding. Lindemann particularly wanted a committee independent of the Air Ministry, which he blamed for having given Baldwin such poor counsel in 1932, and had therefore suggested that the new body should be a sub-committee of the Committee of Imperial Defence. It looked to him as though the Air Ministry had brought Tizard in as part of a conspiracy to frustrate a truly independent investigation. There is no evidence whatever that this was in fact the case, but the unfortunate suspicion may well have coloured Lindemann's attitude. I do not propose here to recount the subsequent story of the differences, which have been described in varying ways by—among others—Prof. Blackett, Sir Charles Snow and myself. Lindemann's side of the picture has already been given by Lord Birkenhead in his biography, *The Prof. in Two Worlds*, and a thoroughly objective account will shortly be available in Mr. Clark's book. The fact to be remarked here is that despite the complications caused by the existence of two bodies (for the Committee of Imperial Defence did later form a sub-committee to deal with air defence, and appointed Tizard as a member), and despite the dislocation of the Tizard Committee's discussions caused by the attitude taken up by Lindemann, this latter Committee proved to be one of the most important and successful in our history.

There is absolutely no doubt that Tizard was the best possible choice as chairman. He had all the desirable experience, both as a scientist and as a serving officer; he had had acute experience of armament development; he had inherent faith in technology; and he was an understanding and skilful chairman. The difficulties with Lindemann apart, he was fortunate in his opportunity. He had on the one side a considerable potential of scientific knowledge and enthusiasm to draw on, both in the Government research establishments and in the universities; and on the other side he had a cadre of serving officers in the Royal Air Force who realized that in the difficult problem of air defence their Service was 'up against it' and who were therefore particularly ready to try out any device that might offer promise. As the ultimate stroke of good fortune, Watson Watt had produced, just as the Tizard Committee met for the first time on January 28, 1935, the idea that it might be possible to detect an aircraft by the energy that it reflected from a radio-transmitter or the ground.

### Radar and the Biggin Hill Trials

It is one thing to be presented with good fortune; but to take full advantage of it, it is necessary to have the appropriate background and to visualize the possibilities. Tizard had both these qualities in high degree, and in the next four years they were applied with brilliant devotion to the threatening problem of German air attack. He rightly foresaw that attack by day was the major threat



and the original radar system was built up to deal with this threat. It was also generally realized that, although the limited accuracy of this system would be sufficient for daylight operations, it would not by itself be adequate to solve the problem of putting a night-fighter into the necessary visual contact with a bomber in darkness. Tizard himself appears to have been the first to suggest that a small radar set might be made to go into a night-fighter, but for some time it was not clear whether such a set would fulfil its aim, since there was difficulty in making short enough pulses to give the necessary minimum range. In the meantime, the development of the original Chain Home System was proceeding, and it was an almost fantastic achievement by Watson Watt and his team that they should have converted the fitful radar sets of 1936 into a reliable operational system by 1939, especially at a time when 'Made in England' was no longer a synonym for reliability.

Tizard's part in all this was to have the judgement and faith to convince the Government to spend the necessary money. In retrospect, this may not appear to be a particular achievement; but in 1935 and 1936, with many of the technical uncertainties still in prospect, especially those of converting an unreliable laboratory demonstration into a reliable system that could be handled by the Service, it demanded much courage and persuasion.

It was not, however, on the administrative side alone that Tizard made such an important contribution. Just as he had earlier insisted that aircraft engines should be tested on fuels more advanced than those generally available in peace, so now he saw that other factors, beyond the technical performance of the radar equipment, would have to be pursued if the whole system was to be made ready in minimum time. Above all, the Royal Air Force would have to develop tactics and a control system designed to get the best out of the radar data. He therefore proposed in 1936, before the radar defence chain was built, that it should be assumed to exist and to be capable of providing the appropriate plots on raiding aircraft. The system could be simulated by flying an intruding aircraft along the predetermined raiding course, and feeding its 'plots' to the defence system. Practical trials could then be made to evaluate whether the data were sufficient to promote successful interception, and whether one interception technique was more suitable than another. The trials were conducted in 1936 from Biggin Hill, and the serving officers and scientists concerned still retain warm memories of Tizard's personal impact while the interceptions were being carried out. He would talk to anyone, civilian or serving officer of whatever rank, if he could thereby gain contact with first-hand experience, or find a new suggestion for solving a problem. As a result of the trials, Britain not only had the radar equipment on which to base her air defence system in 1939; she also had the whole system manned by a body of men who knew how to use it.

### Infra-red

It was at this point that I met Tizard for the first time. Almost by accident, I had been drawn into the air defence problem, because of my work in infra-red spectroscopy and in particular on infra-red detectors. This was in February 1935, when an American inventor giving a demonstration of infra-red detection at Farnborough asked me to make him a replacement thermopile for his own, which had broken down. Lindemann, finding that one of his young research workers had been drawn into the air defence problem in this unofficial way, then pressed the Tizard Committee to support the work. The Committee had already considered infra-red and dismissed it as unlikely to be useful; but it later realized that there might just be some hope that it would provide a method of detection which might enable a night-fighter to close to within visual range of its target, since the nearer the approach, the bigger the infra-red signal from the heat of the

bomber's engine. I have recounted the details of the story elsewhere. In brief, we had an infra-red detector flying in an aircraft at Farnborough in 1937, and it might if necessary have been developed, albeit with difficulty, to the required performance; but, by that time, airborne radar looked distinctly more promising, and the Tizard Committee thought of closing the infra-red work. Before they did so, however, I was invited to appear before them, and so met Tizard for the first time. Tizard was not sure about closing the work, but was not happy about its proceeding at Oxford, still in Lindemann's laboratory, and he invited me to lunch a few days later at the Imperial College of Science and Technology to discuss the possibility of moving the work there. Lindemann, when I told him that I was going to lunch with Tizard, warned me of the dangers I was letting myself in for, even by accepting an invitation to lunch. I therefore went up to London in some apprehension, and was shown into Tizard's room. His first remark to me was: "I don't suppose that you can remember the First War". My reply was that I could remember the First World War very well. I told him that I had been through all the London air raids and, remembering my own experiences as a child in them, I was wanting to do whatever I could to stop them happening again. Not only that, while I could not remember in detail August 4, 1914, I could certainly remember November 11 of that year, when my father went to France, and there were incidents that I could remember in the spring of 1913 when I was not more than 18 months old. Tizard said: "That's very interesting. You have a longer memory than anyone I know—except myself. Do you know. I can distinctly remember having had a bottle!" This, coming from the ogre that Tizard had been painted to me, was a most unexpected start to a quite unexpected friendship. He went on to talk to me about "this silly quarrel" between Lindemann and himself, and we had a cordial discussion of what might be done with infra-red and, for that matter, with radar.

I did not see much of him for the next two years, because the infra-red work, which was of minor importance, was transferred to the Admiralty Research Laboratory, and I went there with it.

### Scientific Intelligence

My next contact with Tizard was closer, and arose through the realization, in the discussions of his Committee in 1938 and early 1939, that we really knew very little about what the Germans were doing in the fields of air defence and offence technology. Tizard persuaded the Intelligence authorities to accept a scientist for a trial period to see whether he could find why it was that we knew so little, and suggest ways of improvement. Again, to some extent by accident, the choice fell on me, very largely due to the suggestion of Mr. A. E. Woodward Nutt, then secretary of the Tizard Committee, that I might do the job. I had, between Oxford and the Admiralty, spent a few months in Air Ministry headquarters in 1938, and Woodward Nutt had found that I was very interested in Intelligence.

Tizard came into the Air Ministry in September 1939 as scientific adviser to the Chief of Air Staff, and I began to see him fairly frequently, since I had been attached to the Intelligence Directorate of the Air Staff. I can remember him telling me: "Get a big map and stick it on your wall. You will have nothing to put on it now, but sooner or later you will have plenty". I felt rather ostentatious when I followed his advice, but that map finished the war with 740 German radar stations pinned to it.

Lindemann was, of course, in the Admiralty with Mr. Churchill. Since I was attached to the Air Staff, and felt a moral responsibility to Tizard, I kept right out of Lindemann's way through the winter of 1939. I used, however, to have lunch with his assistant, James Tuck, from time to time, and one day in early 1940

Lindemann found a message on Tuck's desk saying that I would telephone again. He sat at Tuck's desk until I called, and immediately wanted to know why I had not been to see him. I explained that I personally felt some difficulty, but that if he particularly wanted to see me I would certainly respond. Immediately afterwards, I went to Tizard and explained that I had done my best to keep out of the controversy, but faced with this direct invitation from Lindemann, my old professor, I felt that I ought to go but that he, Tizard, ought to know that I was going. Almost to my surprise he said: "I am glad. I think that you ought to go, and as a matter of fact you could do some good. Please tell Lindemann from me that if he is prepared to bury the hatchet, at least for the duration of the War, I shall be very glad to do so. We have got enough on our hands fighting the Germans, without fighting one another". I went off, naturally delighted. I explained to Lindemann what Tizard had said, but was much rebuffed by his comment: "Now I am in a position of some power, a lot of my old friends have come sniffing around". So, most unfortunately, no headway was made.

Tizard found his position increasingly difficult, but he continued to advise the Air Staff on all aspects of air warfare, and to cover other border matters, including the possible applications of nuclear energy. Although he was far from convinced about the possibilities of a practicable nuclear weapon, he had a year before tried to stake a British claim on the uranium output of the Katanga mines. Now, in the winter of 1939-40, he instituted a sub-committee of the Committee for the Scientific Survey of Air Warfare (which had succeeded the original Committee for the Scientific Survey of Air Defence together with a parallel committee for air offence, formed in 1937 under Tizard's chairmanship), under Prof. G. P. Thomson, to look into the question of nuclear weapons. This was the famous 'Maud Committee'.

When Mr. Churchill became Prime Minister, Tizard's position became rather more uncertain because, while he advised the Chief of the Air Staff, Lindemann was now directly advising the Prime Minister. The detailed story of what then happened is being described by Mr. Clark—and so there is little need for me to comment. Tizard in effect vacated his post with the Air Staff; it was an ironic loss because this was the time when we realized, in June 1940, that the German Air Force presented us with a night threat the accuracy of which was enhanced by radio aids such as the beams, which had hitherto been neglected in Britain. The fact that we were able to spot them in time was the first success of the Scientific Intelligence effort and, as I recorded at the time, it had been "fostered by Sir Henry Tizard, Mr. Pye, Wing-Commander Winterbottom and Mr. Woodward Nutt (and opposed by the Treasury)".

### The Tizard Mission

At the end of June 1940 Tizard resigned from his post as Scientific Adviser to the Chief of Air Staff; but he was persuaded to retain his chairmanship of the Aeronautical Research Committee. Some time before, he had suggested that scientific co-operation with the United States should be advanced by attaching a scientific adviser to the British Ambassador in Washington, Lord Lothian, who had already suggested to the British Government that an interchange of scientific information and service experience should take place between the two countries. A. V. Hill therefore went out as an Attaché to Washington in May 1940 but, finding that he had an insufficient mandate to release information, he came back to London to press for stronger action. As a result, Tizard was asked to head a mission; Tizard wrote his own terms of reference, which were approved by the Prime Minister.

"To tell them what they want to know, to give all assistance I can on behalf of the British Government to

enable the armed forces of the U.S.A. to reach the highest level of technical efficiency."

Other members of the Mission included J. D. Cockroft and R. H. Fowler. They carried with them many technical secrets, including the 10 cm magnetron and the proximity fuse. They also carried, perhaps without specifically realizing it, the great secret of co-operation between science and the Services. The impact in September 1940 of the Tizard Mission on military and scientific thinking in the United States was immense. The revelation of so many secrets without guarantee of an adequate return was an act of balanced courage, in which Tizard's judgement must have been a predominant factor. Thereafter there remained, except perhaps in the field of nuclear energy an exemplary co-operation between the two countries on scientific matters throughout the War.

One permanent effect on American organization of defence science arose almost incidentally from the Tizard Mission, and resulted in a substantially different organization in the United States from that which has developed in Britain. In Britain we have grown accustomed to defence research (and much civil research) being done primarily in Government establishments. This system has obvious advantages, but also one serious drawback. It results in a dichotomy between much of our scientific research potential and the teaching duties that have to be undertaken in order to bring on the next generation. The history of the dichotomy is a long one, going back into the nineteenth century. In the United States, in 1940, defence science was more rudimentary than in Britain, and there were few strong government establishments. When the Americans realized, as a result of what Tizard showed them, how much they had to catch up, they appear to have decided that there was no time to set up new Government establishments, but that the best thing would be to build defence laboratories on to existing institutions like the Massachusetts Institute of Technology. The Radiation Laboratory there was a paramount example. It worked very well and, as a result, the pattern was perpetuated in post-war America. We in Britain used to have doubts about the wisdom of this course, since it seemed that the teaching and pure research at universities might be unduly influenced by the large amount of contracted research for defence. There are undoubtedly dangers in this, and they have not all been avoided; but, on balance, it is questionable whether we have done as well as the Americans by building up our Government establishments at the expense of the universities. Much could be said on both sides of this topic, on which Tizard's own views would have been particularly interesting now that there has been time to watch the developments. We can only note here that the quite substantial difference in pattern on the two sides of the Atlantic arose, and that the universities of America seem to have benefited a great deal.

### The Air Council

Tizard's advice continued to be sought and listened to by the Air Staff, much as Smuts's was by the War Cabinet. I have several recollections of his being called in when urgent problems arose, such as the wanton loss of one of our prototype 'Gee' receivers in an attack on Hanover in 1941, and the exploitation of the Bruneval raid in 1942. There was another occasion in 1941 when a scheme had been advanced to Sir Charles Portal for the co-ordination of all scientific work in the Air Ministry under one head; this would include in particular Operational Research and Scientific Intelligence, and there was little doubt, from the way in which the scheme had been proposed, whom the proposer intended to head it. Fortunately, Sir Charles Portal called in Tizard almost as an inspector general, to investigate the scheme and advise on its feasibility. The first thing Tizard did was to make a reconnaissance of all the relevant activities that were going to be absorbed

into this empire. He telephoned me, telling me what he had to do, and asking whether he could come and see the work of my office. At that time, the total staff of Scientific Intelligence was three, but we did not want to be absorbed into the new empire. We therefore decided that we must put on our very best show of efficiency and have our one room really tidy when Tizard arrived.

Again, as with our first meeting, things did not go at all the way that I had expected. The first thing that Tizard did when he walked in was to pick up a book that was lying on my desk, largely because there had been nowhere else to put it. It was the first volume of Gibbon's *Decline and Fall*, which I was reading in the train travelling to and from the office. Immediately Tizard said: "Hullo—who's reading this?" I replied that I was, and he said: "You know, there is some very good stuff in this". He went on: "I wonder whether this is the right volume. Let me have a look". A few moments later he said: "Yes, here it is. Listen to this!" He then proceeded to read part of what Gibbon had said about the younger of the two Gordians:

"Twenty-two acknowledged concubines, and a library of sixty-two thousand volumes, attested the variety of his inclinations, and from the productions which he left behind him, it appears that the former as well as the latter were designed for use rather than ostentation".

After this hilarious start, we settled down to a serious exposition of what we did, and I showed Tizard how we spotted radar stations on stereo-reconnaissance photographs. We had no really suitable place for an eminent visitor and so we sat him down at the desk of my colleague, Charles Frank, and let him look through the stereoscope. He continued to sit at this desk for the rest of the time. He was very interested, and we thought that we were doing rather well, and giving the impression that we were such an efficient unit that we required no co-ordinating direction from above.

Our impression of efficiency was shattered in a manner that we could never have foreseen. The door of our room was pushed open and a girl's head appeared. She was a very pretty and high-spirited cryptographer who had married one of my assistants, a flight lieutenant. She was in search of her husband, and thought that he might be in our room. She called out to me: "Hullo, Doc., have you seen Harold?" I replied as coldly as possible that I had not, and hoped that she would take the hint and disappear. Quite the reverse happened. She was, as luck would have it, very short-sighted but refused to wear glasses which would spoil her looks. She usually bluffed her way happily through the world without anyone knowing it. Not realizing our tense situation, she proceeded to come skipping into the room, and set about teasing Tizard, thinking with her blurred sight that he was Charles Frank. Ultimately she sensed that something was wrong, and to clear her view went right up and looked into the face of the man at the desk, while I and my colleagues wished that the floor would open and swallow up either her or ourselves. When her face was about two feet away from Tizard's she burst out: "Good God, it's Henry Tiz!" I can recall few more miserable moments. In that flash, it was quite obvious that we would have to be co-ordinated, and that such an irresponsible unit had no hope of survival. To my amazement, Tizard said: "Hullo, Maggie, what are you doing here?" By an almost impossible coincidence, it turned out that the girl and her family had lived some years before next door to the Tizards in Wimbledon, and Tizard was known to all the children as "Henry Tiz"—and he knew it. I can still remember my intense surprise and relief, for up to that moment I had thought that she had gone completely off her head. After that, of course, it was all plain sailing. On Tizard's recommendation, no empire was built and our independence was assured.

### President of Magdalen

After 1940, Tizard's appointment had been in the Ministry of Aircraft Production rather than in the Air Ministry itself, but he continued to see much of the Royal Air Force through his membership of the Air Council. Unfortunately, he did not have the scope that he merited, largely because of the great interest taken by Lindemann, quite naturally, in air matters. Since Lindemann was advising the Prime Minister directly, and since he had won the respect of the Air Staff through his actions on several important matters such as the establishing of the inaccuracy of our night bombing, Tizard's views could in effect be overruled. This was unfortunate, because there were important occasions when Tizard was right and Lindemann wrong. In the end, Tizard decided to get out and he accepted the Presidency of Magdalen in 1942. He resigned as chairman of the Aeronautical Research Committee in the following year. Even so, he was continually called back, notably to head an enquiry into the future of weapons in 1945.

### The Ministry of Defence

With the political changes of 1945, Lindemann himself returned to Oxford, and the new Government invited Tizard to re-enter the administration in London, as Chief Scientist at the new Ministry of Defence. I remember him telling me about this in his Lodging in Magdalen, and saying: "They don't know what they are asking me to give up". It was obviously going to be a very tiresome time in London, and would contrast sharply with the pleasant satisfaction of the headship of one of the most beautiful Colleges in Oxford. Nevertheless one could be quite sure that, having been called, Tizard would go. The next six years were full of frustration and exasperation, because the Ministry of Defence had no clear idea of what it was trying to do. The Service Ministries viewed it with much suspicion and, apart from presiding over the run-down both in British influence abroad and in our defence resources, the Ministry had to face the new situation created by nuclear warfare—in so far as that situation was clear through the veil of secrecy over atomic developments that screened some of these even from officers as high in the Ministry as Tizard himself.

### Science and the Services

Not long before he left Magdalen, Tizard lectured to the Royal United Services Institution on "Science and the Services" surveying his unique experience. Of the Tizard Committee he said: "The first time, I believe, that scientists were ever called in to study the needs of the Service, as distinct from their wants, was in 1935, and then only as a last resort. The Air Staff were convinced of the inadequacy of existing methods and equipment to defeat air attack on Great Britain, and a committee was established for the scientific survey of air defence. I want to emphasize that this committee, although it consisted on paper only of scientists, was in fact from the first a committee of scientists and serving officers, working together.

"When I went to Washington in 1940 I found that radar had been independently invented in America about the same time as it had been invented in England. We were, however, a very long way ahead in its practical applications to war. The reason for this was that scientists and serving officers had combined before the War to study its tactical uses. This is the great lesson of the last War."

Here he was saying again the things that he had said twenty years before in his inaugural address to this Society; but this time he could point to the great successes which had been achieved by putting into practice what he had previously advocated. The points that he made deserve all the emphasis that we can give them to-day, particularly because, while the urgency of war forces their wisdom on us, we are apt to drift from them in the



apparently less-pressing conditions of peace. He concluded:

"What is needed is a scientific staff attached to the central Chiefs of Staff organization that will have no executive duties, nor be overburdened by administration, but will devote its whole time to the study of the influence of advancing scientific knowledge on the problems of defence".

I do not know whether we have yet gone as far as he would have liked, but the present organization of science in defence is certainly better, I think, under the last reorganization than it has been at any previous time. There still remain some of the basic difficulties that Tizard himself had encountered after the First World War, in the way of attaining the wisest co-ordination of research effort, which nobody has yet mastered.

Perhaps we should not be too discouraged, because even a man of Tizard's flair and insight could not always succeed so well as he did in air defence. The parallel pre-war Committee on Air Offence, in particular, was nothing like so successful, despite his chairmanship. Of this Committee he himself noted: "It did not meet with such enthusiastic welcome from the Air Force. As a result, its influence before the War started was only small". The lesson here is that even Tizard could not succeed unless an Armed Service was prepared to face its own defects and call in help to deal with them. That our Armed Services are now much more prepared to do this than they were before the War is very largely due to Tizard's example and influence.

#### Higher Education and Research

The other difficulty that he had faced in the 1920's was that which I had already mentioned, of having to co-ordinate a number of independent organizations. I should much like to have discussed this problem with him during the past few years, because this is a problem that we face increasingly to-day. Starting at school education, for example, we argue that school and university education are part of the same process, and therefore that schools and universities should come under one Minister. Many of us also say that teaching and research go best together. Therefore research also should come under this Minister. Then we say that there has been too much separation between pure and applied research, and therefore this Minister should cover all pure and applied research. We also say that in the early stages of research, even applied research, it is difficult to say whether the end-product is going to be of more use in the military or in the civil field; in addition, we can point to the disadvantages ensuing from the separating of research from development, and development from the user. Therefore all military and civil research should stay together. In the end, we decide that everything from school education to military research, and from the most academic arts study to the most down-to-earth technology, are all part of one entity and are inseparable—and so, in some senses, they are. But it is obviously impossible to run them all under a single administrative department, and yet it is undesirable to have too rigid a division. The problem of finding the optimum compromise is extremely difficult, as can be evidenced by some of the better of Parliamentary debates in recent years. I have in mind, for example, the debates on the Robbins Report, and those discussing the relation between the Ministry of Aviation and the Ministry of Defence, and on the Trend Report.

#### President of the British Association

Whatever uncertainties we might have about how Tizard would now view our administrative problems, he made it pretty clear in his Presidential Address to the British Association for the Advancement of Science in 1948 what he would think of our present expansion in higher education:

"Whatever may be done, in a burst of public enthusiasm to support and promote schools of research at universities, nothing should be allowed to lower the quality; and size beyond a certain point, undeniably does. . . . When a young graduate tells me, as one did recently, that he was going to do research because it got him off military training, it makes me wonder whether we are not already over-populating the research departments of universities. . . . It is not the general expansion of research in Britain that is of first importance for the restoration of its industrial health, and certainly not the expansion of Government research remote from the everyday problems of industry. What is of first importance is to apply what is already known. The fact is that all really new developments of industry are the product of the work of very few men".

These views may conceivably sound rather old-fashioned now, but I personally believe that there is a great deal in them, and we miss the voice of Tizard in checking the near panic for higher education at all cost.

In passing, we may note that Tizard, for all his faith in science and technology, was not thrown off balance by the spectacular development of nuclear energy, for in his Presidential Address to the British Association he said:

"I shall certainly assert that the production of power from uranium cannot bring such economic benefits to Britain within twenty years as would the practical application of known methods of economizing coal".

This statement looked peculiarly old-fashioned to a scientific world overflowing with enthusiasm for the newest technology, but Tizard appears to have been right. The improvement in the efficiency of conventional power stations is one of the reasons why the nuclear power station has not been so widespread as then appeared likely.

#### Higher Education

As for what he said in 1948 about education, this was not the first time he had spoken on this subject to the British Association, for he had been president of the Education Section in 1934. His address then is well worth reading. It is, as usual, full of wisdom, and reads as topically to-day as it did thirty years ago. I can only quote two passages:

"No scientific man desires to see scientific education pushed to the neglect of literary studies; all of us recognise that a properly balanced diet for the mind is as important as for the body: what we do think is that science, well taught, can supply all that is best in the classical tradition; can 'teach accuracy and exactness; can give a discipline in clear thinking; can teach boys to recognise differences in things which seem alike; can brace with its difficulties minds that are not afraid of difficulties; can inspire with its beauty minds not insensitive to beauty'—to quote the recent words of the Headmaster of Rugby in praise of Greek".

And of who is fit for university education:

"Where shall we draw the line? There are many students who occasion no misgiving. They are those who are capable of teaching themselves, given the opportunity. To them, and ideally to all, the attitude of the university should be this: We give you here the opportunity of learning, if you wish to, from masters of their subjects; we give you access to well-equipped libraries and laboratories; and opportunities for learning from each other. We help you to help yourselves. What use you make of these opportunities depends upon yourselves. If we find you do not, or cannot, make good use of them, you shall go, and make room for others. Broadly speaking, I believe that is the right attitude. In such an atmosphere, learning, individuality and self-reliance flourish; and public expenditure is worth while. Judged from this standpoint, I have little hesitation in saying that universities are too full. As a result the tendency is towards over-organisa-

tion, too little latitude, and too much spoon-feeding. The more distinguished the teacher, the more he is tempted away from teaching and research: his presence is required on committees. In London we elderly gentlemen even organise students' athletics; and official debates take place on such important questions as the site and finance of a university boat club for women. The wider we fling open the doors to a university, the more will such organisation be necessary, and the worse will be the conditions for the best teachers and students".

When we remember that Tizard was speaking when the proportion of the population at universities was substantially less than it is now, that he was always on the side of the young and the progressive, that he wished to encourage the spread of education in science and technology, and that he was one of the most experienced and wisest educationists of our time, are we not forced to wonder whether the present expansion of higher education is well founded?

### The Messel Lecture, 1952

Tizard elaborated his point of view in the Messel Memorial Lecture which he gave in 1952 on "The Strategy of Science". Here he concluded with:

"It is more important now to strengthen our technology than to expand our science; more important to do things than to write about how they might be done. Science is not enough. We haven't any money, so we've got to think".

Incidentally, that lecture was given in Aberdeen, and was the principal event of the annual meeting there of the Society of the Chemical Industry. My wife and I gave a cocktail party for various members of the Society and its guests, including, of course, Tizard and also Niels Bohr, who happened to be staying with us. The party was immediately before the main Society dinner, and we were all in full evening dress with decorations. The party taxed our limited domestic arrangements, but these we had successfully negotiated or, as in the case of Tizard's visit to my office of ten years before, thought we had. Perhaps memories of what happened then led me to keep an involuntary eye on the door. Well, it happened again. This time, however, the young lady concerned was not a pretty cryptographer, but my small daughter, not then two years old. She had been banished and bribed to her bedroom for the duration of the party, but she had decided that she must help with the entertaining, and slipped in wearing her nightdress. It was not easy to follow her through a party of about forty people; in fact, for some time no one else noticed her, until she surfaced in front of the chairman of Imperial Chemical Industries, offering him a rather sticky handful of potato crisps. Tizard, who was obviously very fond of children, immediately took charge of the situation. The up-shot was that all the guests were shepherded to the periphery of the room while Tizard joined up with Niels Bohr linking hands behind their backs, and my small daughter drove these two eminent gentlemen around the room as a pair of horses, steering them with one hand grasping the Order of the Elephant and the other the Grand Cross of the Bath, the one on the left being commanded as 'Borie' and the one on the right as 'Tizzie', to the general applause of the spectators. Never, when I had first read Niels Bohr's name in my school books, did I think that I would be privileged to see such a human incident.

### Retirement

This happened at the stage when Tizard was relinquishing his work at the Ministry of Defence, but I saw him in London from time to time, since I myself had work to do there. One Monday evening, about three months after the cocktail party incident, he was in the chair at a discussion dinner at the Athenaeum, where Sir Stephen King-

Hall was leading a discussion on "Delusions in Defence". Sir Stephen was making points that were both provocative and wise, the main one being that we might well be in danger of losing the 'cold war' against Russia through undue concentration on a hot war, which he did not believe that the Russians intended to wage. The discussion went on for some time, but appeared to be in danger of drying up when Tizard in a characteristic manner got up and said: "Well, all this has been very interesting, but we have only heard so far from the older men, who would not be directly involved in fighting the next hot war, if it should come. What I should like to hear [and here is the true Tizard] is the views of some of the younger men—someone under forty". He looked around the dining tables and, spotting someone in this category, said: "You, Sir, over there. Please get up and tell us what you think".

By a fluke, I happened to know the young man he had picked on, because I had met him for the first time two days previously at a wedding in Cambridge, where he had been best man and I was giving the bride away. I saw him stand up, swallow hard, and start to speak. I thought, from my short earlier contact with him, that he would do pretty well, because, as a barrister, he was accustomed to speaking, and this had been borne out by his performance as best man. He made a slightly hesitating start, but got on very well, explaining that he had commanded a motor torpedo boat during the War, and going on to describe his reactions at having to serve again. Everyone was very pleased, and the discussion finished shortly afterwards. Later that week I had been invited by the young barrister and his wife to a cocktail party, and I greeted my hostess by telling her how well her husband had done, impromptu, at the Athenaeum dinner. She surprised me by saying: "It wasn't quite impromptu. He went along all prepared to speak". I was puzzled, because Tizard's challenge had been very naturally delivered, and the surprise on the barrister's face had been quite evident. His wife then went on to explain that he had attended as a guest of a psychiatrist member of the Athenaeum who had said to him some weeks previously: "You ought to come along one day to one of our discussion dinners at the Athenaeum. I have got a list of the topics here, so let's pick one that we know something about". The first one on the list was on "Delusions in Defence". The psychiatrist knew perfectly well what delusions were, and the barrister knew what defence was. They therefore deduced that the dinner was going to discuss the plea of insanity in murder trials, which was topical on account of the Straffen case, and the application of the M'Naghten rules. In preparation each had briefed himself thoroughly; it was only when the discussion started that they realized that the delusion was theirs. Tizard was delighted when I told him what had happened.

This story, showing Tizard in retirement but as ready as ever to encourage youth to expression, must close my account. I have rambled between national events and personal anecdotes in the hope of drawing a warmer and more faithful picture than a formal memoir could portray. Even so, I have by no means measured the breadth of his interests and achievements. His prescient administration of the Imperial College of Science and Technology, and his work in the Commonwealth, for example, deserve notice that I have had to omit.

Integrity, physical courage, moral courage, energy, humanity and humour were all ingredients in his character to a quite unusual degree. Among the newer fields of human experience, aeronautics gained especially from his enthusiasm and foresight, and it has therefore been a high privilege for me to make this personal salute before this Society. Intense patriotism combined with common sense and deep wisdom made him the architect of understanding between science and the Services exactly when this understanding was vital to our survival; and he championed the full part that science and technology could play in human life throughout the world. May we always

remember, by the practical examples that he set, the lessons that he so effectively taught.

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## ROYAL SOCIETY VOLCANOLOGICAL EXPEDITION TO THE SOUTH ARABIAN FEDERATION AND THE RED SEA

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UNDER the aegis of the Volcanological Expeditions Committee of the Royal Society, and financially supported by the Royal Society and the Universities of Leeds and Edinburgh, a three-man expedition spent three months in the South Arabian Federation and the Red Sea examining the recent volcanics of that region. The aims of the expedition were to study the coastal volcanoes between the southern entrance to the Red Sea and the Aden peninsula, the western part of the extensive coastal volcanic field to the east of Aden, and the volcanic islands of the Red Sea. These aims were achieved and, in addition, a brief visit was made to the recent volcanic cones near Ataq, 175 miles north-east of Aden.

Published geological data on the area are mainly petrographic, describing specimens collected by individuals and expeditions, little attempt having been made to map or to elucidate the volcanic history of the area concerned. Significant contributions to the volcanology of South Arabia and the Red Sea have been made by Vellain<sup>1</sup>, McMahon<sup>2</sup>, Raisin<sup>3</sup>, Prior<sup>4</sup>, Bier<sup>5</sup>, Manasse<sup>6</sup>, Vredenburg<sup>7</sup>,

Teilhard de Chardin *et al.*<sup>8</sup>, MacFadyen<sup>9</sup>, Wissman, Rathjens and Kossmatt<sup>10</sup> and Shukri and Basta<sup>11</sup>.

### Central Volcanoes on the South Arabian Coast between Aden and the Red Sea

Along the south coast of Arabia, between Aden and the southern entrance to the Red Sea, there are six geologically recent volcanoes. From east to west these are: Aden, Little Aden, Ras Imran, Jebel Um Birka, Jebel Khariz and the Perim-at Turbah mass. All are central vent volcanoes characterized by the intimate interstratification of basic and intermediate lavas with acidic tuffs, some of which have comenditic affinities. So far as can be determined, the later stages of activity have, in all cases produced large central calderas, which have since partially or wholly been infilled with horizontal ignimbrites and lavas. It seems most likely that the calderas were created by explosive activity although formation by collapse cannot be precluded. Palaeomagnetic data<sup>12</sup> and the morphology of the cones suggest that the volcanoes were active about 1 million years ago.

**Aden.** The Aden peninsula, an area of about 12 square miles, consists entirely of volcanic rocks, the oldest of which belong to a radially inclined series of basic and intermediate lavas interbedded with lensoid masses of rhyolitic composition and layers of coarse volcanic agglomerate which formed the original cone. Although most of this extrusive material was derived from a central vent, there are parasitic cones on the flanks from which basaltic lava and scoria were emitted. A subsequent phase removed the central part of the volcanic pile leaving a near-circular caldera some two miles in diameter. This cavity was filled by horizontal rhyolitic extrusives and trachyandesitic lavas. At a later stage, further, probably explosive, activity formed a second caldera slightly smaller than the first, and centred a little farther to the

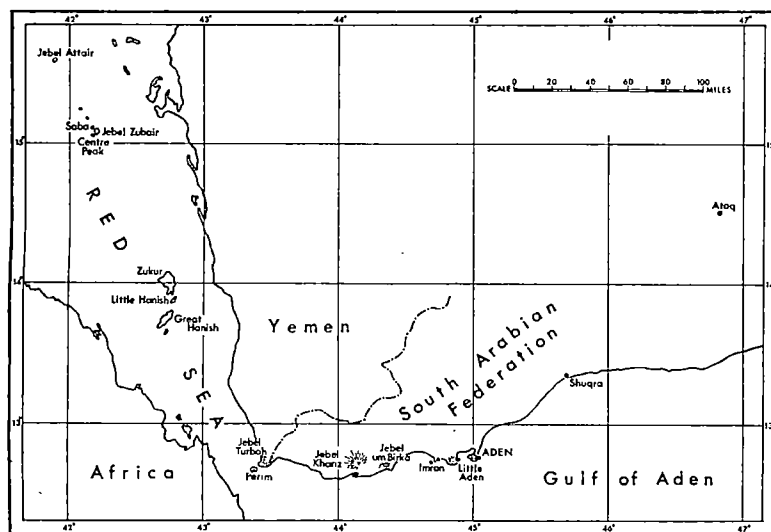


Fig. 1. South Arabian Federation and the Red Sea

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east. This, in turn, was partially filled by volcanic agglomerate and pumiceous tuff. Dykes are abundant and are radially disposed. Differential erosion has removed much of the radially-inclined sequence so that the horizontal rocks that filled the earlier caldera are now topographically eminent and form the horse-shoe range of hills known as Jebel Shamsan.

*Little Aden.* The peninsula of Little Aden has an area of about 16 square miles and, although the volcano is considerably more eroded than Aden, sufficient rock is exposed to make it quite evident that the two areas are structurally comparable. As in Aden, the oldest rocks are part of a composite cone of interstratified basic and intermediate lavas, rhyolitic effusives and agglomerates. Substantial remnants of this radially inclined sequence are preserved around the periphery of the area while the central and eastern part of the peninsula is partially occupied by horizontal basaltic lavas and rhyolitic ignimbrites that have filled in a large caldera. The dyke pattern is less obviously radial than that of Aden and has a marked east-north-east-west-south-west component. Erosion has removed much of the Little Aden volcanic pile, and large areas, particularly in the centre, consist of low plains covered with blown sand.

*Ras Imran.* Some 10 miles west of Little Aden there is a group of small hills forming the promontory of Ras Imran and the island of Jazirat Aziz. These are formed of basaltic pahoehoe lava overlying yellow volcanic agglomerate. As at Aden and Little Aden, there are both aphyric and markedly feldspar-phyric basalt lavas. Isolated lenticular masses of rhyolitic composition occur at Ras Imran and also on Aziz island, but they form less than 10 per cent of the total rock outcrop. All units have a northerly dip, and it is thought that they represent the northern flank of a central-vent volcano, the centre and southern flank of which have been removed by marine erosion.

*Jebel Um Birka,* 45 miles west of Aden, is the largest of a cluster of about 10 hills which rises to a height of 784 ft. Many of the hills, including Um Birka, are so covered by blown sand that the rock outcrops are scarce and confined to the leeward flanks. Rhyolitic ignimbrites and agglomerates are the most abundant rock types with black aphyric basalts occurring high in the sequence. All the extrusive units appear to have a radial dip away from a centre lying to the south-west so that from west to east the dip changes from northerly through north-easterly to due east. At Ras Ghawa, 3.5 miles to the south-east of Um Birka, there is a lone hill formed mainly of feldspar-phyric basalt lavas dipping in a south-easterly direction. All these hills are probably remnants of the northern and eastern parts of a central vent volcano of the Aden type.

*Jebel Khariz* lies 60 miles to the west of Aden and, with the exception of Aden itself, is the best preserved of the coastal central-vent volcanoes. It covers a roughly circular area of about 100 square miles, rises to a height of 2,766 ft. and is characterized, as are all the volcanoes of this group, by the close association of rhyolitic ignimbrite and basaltic lava. In this case, acid ignimbrites and agglomerates form more than 90 per cent of the radially inclined cone sequence while lavas of aphyric and feldspar-phyric basalt are found only on the south-east and northern flanks. The central caldera is exceptionally well preserved as a huge amphitheatre some 5 or 6 miles in diameter. Within this caldera, remnants of a later stage of volcanic activity which partially filled the caldera with horizontally bedded rhyolitic ignimbrites, basaltic lavas and agglomerates form a spectacular central tower.

The *Perim-at Turbah* mass lies on the south-western promontory of Arabia that forms the eastern margin of the entrance to the Red Sea. Most of this volcanic mass lies within the Yemen and was therefore not accessible. Examination of the area from the sea and from existing maps and photographs strongly suggested that this is another volcano of the Aden type with interstratified rhyolites and basaltic lava. The only part of this mass

that was examined was Perim Island, which is part of the western flank of the volcano and is separated from the rest by the eastern, narrower straits of Bab el Mandeb. On Perim, black aphyric and feldspar-phyric lavas are the only rock types present, and, although no rhyolites were identified, the lavas examined have petrographic affinities with those collected from the other volcanoes of this group.

All six of these central volcanoes lie close to a line which trends just north of east, near to this part of the coast of South Arabia. It seems probable that the zone of weakness delimited by the line of volcanoes forms the northern boundary of the crustal depression of the Gulf of Aden.

So far, the only chemical analyses available are of Aden and Little Aden specimens, although preliminary petrographic examination has been made on samples from all these volcanic centres. Chemical analyses of specimens from Aden given in Shukri and Basta<sup>11</sup>, and of specimens collected by Prof. W. Q. Kennedy and Dr. von Knorring from Little Aden<sup>12</sup>, show that the comendites are characteristically acidic rocks with silica ranging up to 72 per cent, relatively low alumina and high total alkali content. cursory examination in thin section reveals that the majority of the comendites consist of partly resorbed phenocrysts of sanidine and occasional smaller crystals of aegirine in a fine-grained mesostasis of perthitic feldspar, quartz, skeletal riebeckite and glass. The lavas range from olivine basalts through basalts to trachyandesites and trachytes. Olivine basalt and trachyandesite appear to be the dominant rock types and mineralogically the association has alkaline affinities. The two basalts that have been analysed are atypical, feldspar-phyric rocks highly charged with ore; this is reflected in the analyses which show high alumina, high combined iron ( $\text{Fe}_2\text{O}_3 + \text{FeO}$ ) and low  $\text{MgO}$ . In view of the wide range of rock types present in the volcanoes of this group, and in the unusual chemistry of individual types, the petrogenetic problems posed by this association are likely to be of considerable interest.

### The Shuqra Volcanic Field

In the Shuqra volcanic field, 70 miles north-east of Aden, very recent volcanics are widespread and consist of numerous cinder cones, many still perfectly preserved (see Fig. 2), associated with lava flows of black aphyric, feldspar-phyric and olivine-phyric basalts that have filled the irregularities in the pre-existing landscape. The pre-volcanic rocks consist of Upper Jurassic sediments resting on schists and gneisses of the crystalline basement. The area had been deformed into a spectacular, strongly faulted monocline parallel to the coast of the Gulf of Aden, before the basaltic lavas were poured out.

Isolated specimens from the Shuqra field that have been studied under the microscope consist of euhedral phenocrysts of olivine (c.  $\text{Fa}_{15}$ ), clinopyroxene and plagioclase (c.  $\text{An}_{65}$ ) in an intergranular groundmass of plagioclase laths (c.  $\text{An}_{60}$ ), granular clinopyroxene and granular iddingsite after olivine. Minute euhedral crystals of magnetite are scattered liberally throughout the rocks examined. These lavas appear to have mineralogical affinities with the alkali olivine basalt association.

*Ataq.* The town of Ataq, 175 miles north-east of Aden, lies in a sandy plain flanked to the west by granites and gneisses of the crystalline basement and to the east by an escarpment of Cretaceous and Lower Tertiary sediments. Three low hills formed by recent volcanic activity lie to the north of the town and are aligned in a north-westerly direction. These hills probably mark the northernmost extension of the Shuqra volcanic field. Two volcanic centres just to the north of Ataq were visited.

The southern volcanic mass is formed, for the main part, of black aphyric basalt which has flowed northwards from a vent near the southern margin of the mass. Two to three miles further to the north, there is a low eminence, some 200 ft. high, formed of volcanic material which is now partially covered by blown sand. This hill, locally



Fig. 2. Typical topography of the Shuqra volcanic field showing a well-preserved volcanic cone (centre) and an outlier of Upper Jurassic sediments (right)

known as 'Kirsh', consists of a well-preserved, circular crater some 200 yd. in diameter surrounded by a low rampart of volcanic agglomerate and basaltic lava spatter. To the north, a small lava field appears to have issued from the flanks of the cone. This centre is particularly notable for the agglomerate, which, in addition to the blocks of olivine basalt, contains abundant non-volcanic xenoliths. The non-volcanic xenoliths include schists and granitic gneisses, undoubtedly derived from the crystalline basement, and dunitic nodules which possibly originate in the upper mantle. The ultrabasic nodules are up to 9 in. in length in the agglomerates, but also occur in the lavas, where they rarely exceed 1 in. or are represented by solitary olivine xenocrysts. The dunites are completely fresh and show no sign of reaction at the margins; preliminary examination in thin section reveals that they consist mainly of forsteritic olivine, with chrome diopside, amphibole and spinel as minor constituents.

### The Red Sea Volcanic Islands

Volcanic islands are present only in the central southern part of the Red Sea and occur in three separate areas. These, from north to south, are Jebel Attair ( $15^{\circ} 33' N$ ), the Zubair group ( $15^{\circ} 12' - 15^{\circ} 00' N$ ) and the Zukur-Hanish group ( $14^{\circ} 05' - 13^{\circ} 31' N$ ). Many of the islands are single or double cones formed entirely of yellow basaltic agglomerate, while on the larger islands black basaltic lavas are abundant. Volcanic forms are well preserved (see Fig. 3) and all the islands are the products of geologically recent volcanic activity.

Jebel Attair, a solitary island measuring 2.5 by 1.5 miles, is centrally placed in the Red Sea trough and rises to a peak 800 ft. above sea-level. Radially inclined lavas of mainly aphyric and feldspar- and olivine-phyric basalts are the dominant rocks. Black and red cinder cones are numerous and lie on well-marked open fissures. These fissures are concentrated in three directions,  $310^{\circ}$ ,  $342^{\circ}$  and  $013^{\circ}$ , while the elongation of the Red Sea trough in this area is  $330^{\circ}$ . The vent at the summit gives off small quantities of steam and sulphurous gases and is partially encrusted with sulphur.

The Zubair group, between 24 and 29 miles south-south-east of Jebel Attair, consists of 10 islands with numerous offshore rocks and shoals. The smaller islands consist of single or double cones of yellow basaltic ash and agglomerate in various stages of erosion, while Saba, Centre Peak and Jebel Zubair, the largest islands, have

extensive lava fields as well as numerous pyroclastic cones. The lavas, like those of Jebel Attair, are mainly feldspar- and olivine-phyric basalts. The structure of the largest island, Jebel Zubair, is dominated by a median rift that trends at  $342^{\circ}$  and is marked by gaping fissures which cut across cinder and spatter cones and basaltic flows which have issued from them. From the morphology of the cone it is apparent that all the islands of the group are of geologically recent age, while on Zubair a distinct sulphurous smell can be detected near some of the vents suggesting that they were active in historically recent times.

South of the Zubair group the median trough of the Red Sea loses its identity and the Zukur-Hanish group of islands is surrounded by shallow water of less than 5 fathoms. As in the Zubair group, 75 miles to the north-north-west, the smaller islands of the Zukur-Hanish group consist of cones of yellow basaltic agglomerate in various stages of erosion, while the larger islands, Zukur and Great and Little Hanish, have numerous volcanic cones and extensive fields of black basaltic lava. Eruptive centres on these larger islands lie along fissures which have a north-easterly trend. This is oblique to the Red Sea median trough and does not appear to be related to the major structural lineaments of the adjacent African mainland<sup>14</sup>.

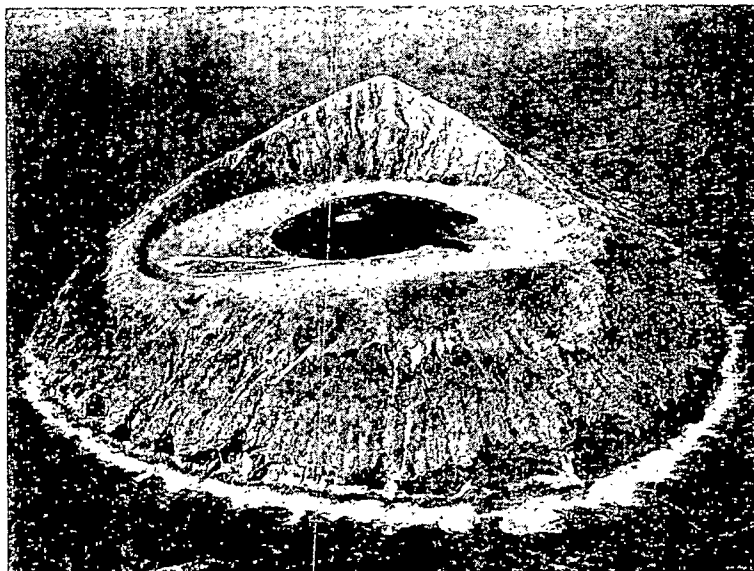


Fig. 3. Haycock Island in the Zukur-Hanish group; a near-perfect volcanic cone of yellow basaltic ash and agglomerate some half-mile in diameter with a small cone of basaltic spatter within the main crater

So far only isolated specimens from the Red Sea volcanic islands have received preliminary examination under the microscope. All the specimens examined were olivine basalts, but there is considerable textural variation from strongly porphyritic to aphyric types but with a relatively uniform mineral content. The main rock-forming minerals are olivine (c.  $\text{Fa}_{12-20}$ ), clinopyroxene and plagioclase (c.  $\text{An}_{50-65}$ ). When present as phenocrysts, the olivine is euhedral and commonly zoned from a forsteritic core to a more fayalitic margin, while plagioclase phenocrysts, although within the labradorite range, are often progressively zoned from a calcic core to a more sodic rim. The ground mass is intergranular with minute, sub-hedral crystals of olivine and clinopyroxene occurring between laths of plagioclase. Volcanic glass, or its alteration products, is rare. Sufficient work has not been undertaken to establish whether there is any significant petrological variation between the rocks of the three island groups, but specimens examined indicate affinities with the alkaline rather than the tholeiitic association.

Petrographic investigation of the specimens collected in all areas is in progress at present.

We thank the chairman and members of the Volcanological Expeditions Committee of the Royal Society for their support and encouragement and the Royal Society and the Universities of Leeds and Edinburgh for financial assistance. We also thank the various branches of H.M. Forces for their assistance and hospitality during our stay in South Arabia, and members of the British Petroleum Company, Aden and Little Aden, and the South Arabian Federal Government for their generous assistance.

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## OBITUARIES

### Prof. Hans Halban

PROF. HANS HALBAN, who died on November 28, 1964, at the age of fifty-six, will be widely remembered among scientists for his major contributions in slow neutron physics before the Second World War which led up to the successful realization of the nuclear reactor.

Halban was born in Leipzig on January 26, 1908, and after education in Austria he went to the University of Zurich, where he gained his Ph.D. in 1935, working in the laboratory where his father was professor of physical chemistry. He then moved to Mme Curie's Institut de Radium in Paris, and it was at this time that he started his research on slow neutron physics. In 1937 he went with a special scholarship to Niels Bohr's laboratory in Copenhagen and carried out neutron experiments with Frisch and Koch, which included a measurement of the neutron magnetic moment and a study of the internal field in a ferromagnetic substance using neutrons. He returned to Paris in 1938 to become Chargé de Recherches in the Laboratoire de Chimie Nucleaire in the Collège de France of which F. Joliot-Curie was director and where he continued his neutron studies. This culminated in his great work in 1939 along with Kowarski and Joliot-Curie on the neutrons from the fission of uranium. It was these experiments involving the moderation of neutrons in uranium solutions that first demonstrated the possibility of a chain reaction and, in a classic sequence of papers in that year, Halban, Kowarski and Joliot-Curie discussed the process together with methods for its control. This work was interrupted by the War and the fall of France in 1940, but Halban succeeded in escaping to England, bringing with him under dramatic circumstances the precious churns of heavy water. These 180 litres formed the greater part of the world's stock and had been purchased to extend the moderation studies in Paris. Under the auspices of the MAUD Committee this research was continued in the Cavendish Laboratory where Halban was in charge of the slow neutron team. As the effort on the project developed, so the active centre of research moved to Montreal and in 1942 he took charge of the joint British-Canadian team which included several distinguished French scientists and was concerned, among other matters, with the development of reactors. It was this work which finally led to the construction of the heavy-water reactor at the Chalk River Laboratories.

He resigned this appointment in 1945 and a year later went to Oxford at the invitation of Lord Cherwell, who

was then head of the Clarendon Laboratory. He quickly built up a team of research students which was set to work on problems both in neutron and nuclear structure physics. Many of these research students came from overseas and had been drawn to him through his wide contacts abroad and his international reputation as a physicist. Under his lively and imaginative influence the group flourished and produced many original papers on a wide range of subjects. The research was often hampered by shortage of funds and equipment, and with characteristic generosity he would contribute from his own resources to enable the work to continue. It was in 1951 that a co-operative effort between some of his nuclear physics group and a number of low-temperature physicists under the general direction of the late Sir Francis Simon showed that it was possible to attain a substantial degree of alignment of the nuclei of radioactive cobalt-60. This field of research, in which he had first become interested in 1937, soon developed into a powerful tool for studying problems in both nuclear and solid-state physics and is being actively pursued to this day in many laboratories. In 1950 he was made a fellow of St. Antony's College in whose foundation he was deeply interested, and in 1954 the University of Oxford conferred the title of professor on him. He returned to Paris in 1956 to become professeur associé at the Ecole Normale Supérieure and a director of the Orsay laboratory, where a high-energy electron accelerator was to be installed. He retired from active nuclear physics in 1963.

Halban will be remembered by his friends and colleagues, and not least by his research students, for his boundless enthusiasm, his originality and his continuous stream of ideas. He was critical of his own work and he demanded a high standard from others, but he tempered this with great understanding, generosity, warmth and loyalty. He was a dynamic character and a great physicist.

M. A. GRACE

### Prof. A. Voisin

AN internationally recognized figure, Prof. André Voisin died in Cuba on December 22, 1964, at the age of sixty-one, while engaged on a lecturing programme connected with the reorganization of the agriculture of that country.

The study of grassland and the grazing animal, by virtue of its complexity, has given rise over the years to many prophets: men of zeal, evangelistic by nature, and



all in some degree dogmatic. Undoubtedly one of these was Voisin. He came of Norman farming stock and trained as a biochemist in Paris and at Heidelberg. It was as a practical dairy farmer in his native Normandy, however, producing milk from grass, that he gained the experience synthesized in his philosophy of 'rational' or 'timed' grazing.

Voisin burst upon the British farming scene in 1957 with the publication of possibly his best book, *Grass Productivity*. Its translation into English was followed by translation into Russian, German, Japanese and Hungarian. Here he propounded his philosophy of grassland management, the 'rational' system, basically a rotational grazing system strictly governed by defoliation frequency in relation to rate of herbage growth. The success of this work stimulated further publications—*Better Grassland Sward*, and *Soil, Grass and Cancer*, and finally, *Grass Tetany*.

Throughout his writings Voisin stressed that the grass crop must not be considered in isolation. The sward, which in turn depends on the soil, should always be related to the needs of the grazing animal, its health, well-being and production. Sward management can favour the grazing animal or the sward at any point in the grazing season without much difficulty. The results, however, may not be ultimately satisfactory. Voisin's great contribution was the evolution of a simple grazing system, soundly based, which sought and obtained the best, at one and the same time, from both sward and animal within the limits of the potential available. His reasoning quickly found favour with progressive dairy farmers, who applied his ideas with advantage. Present-day research work in plant breeding and herbage variety testing, through the use of new techniques, particularly *in vitro* studies of digestibility, is directed at taking into account the elusive 'quality' factor in fodder: in other words, the ultimate value of the feed to the animal in terms of net output. Voisin realized at an early stage that production of dry matter *per se* was not the whole answer. All his writing emphasized consideration of the needs of the animal in order to obtain optimum results.

It was unfortunate that most of his thinking centred on the permanent pasture as the most suitable medium for the dairy cow. His philosophy, moreover, hinged on only moderate use of fertilizers. Under conditions in

Britain, however, the permanent sward, because of its normally small legume contribution, is dependent on heavy fertilizer input for high herbage production, and for this reason Voisin, perhaps unknowingly, never quite completely reconciled his approach from his continental background with the fundamental economic needs of the dairy farm in Britain. These have been clear-cut over the years and have given rise to marked intensification of production determined largely by high fertilizer nitrogen application and a growing dependence on the short-term ley as a means of lengthening the growing season. In this context, Voisin's views on the 'sacrifice' of animal and plant in the cause of blind intensification of production were timely.

His brisk, forceful style and completely convincing manner, both as writer and lecturer, gained him wide popularity while provoking much controversy, to the benefit of farmer and scientific worker alike, for he gave even the most experienced grassland and animal workers cause to stop and think. From his wide knowledge of the literature, Voisin made more than a reasoned case for most of his conclusions, including those on human health arrived at in his highly controversial work, *Soil, Grass and Cancer*. His omnibus approach obviously caused him to conflict with many specialized views, but one of his great attributes was that, although acutely aware of the trees, his great concern was with the wood: the conclusions he reached were most difficult to dismiss lightly.

Above all, Voisin was a master in public relations. He aimed directly for his audience, the farming community and being a farmer himself, he knew exactly how to orientate his arguments to gain the most telling effect. His boundless energy and enthusiasm, not forgetting his humanity, are reflected throughout his work. Voisin's contribution to grassland farming practice was very large: Normandy may well be proud of her son, who found world favour in his chosen field.

Among the many honours he received abroad was an honorary degree from the University of Bonn. He was a member of the Académie d'Agriculture of his own country and also held the Croix de Guerre gained during the Second World War.

J. B. D. HERRIOTT  
CATHERINE HERRIOTT

## NEWS and VIEWS

### Chemical Crystallography in the Imperial College of Science and Technology : Prof. D. Rogers

DR. D. ROGERS, reader in chemical crystallography in the Imperial College of Science and Technology, has been appointed to the newly established chair of chemical crystallography in the Department of Chemistry of Imperial College. Although 'born and bred' a physicist, he enjoys a national and international reputation as an X-ray crystallographer, combined with a lively interest in computers and programming. Dr. Rogers was educated at Wallington (Surrey) Grammar School and graduated in 1941 at King's College, London. After securing a Ph.D. in physics at King's College in 1944, he became assistant lecturer in the College of Technology, Manchester, where his early work on X-ray crystallography was carried out. In 1948 he moved to University College, Cardiff, where he remained as a close collaborator of Prof. A. J. C. Wilson until he joined Imperial College in 1961: he held an Imperial Chemical Industries fellowship for part of this period. Since his appointment as reader at Imperial College, he has established a thriving school of chemical crystallography. Dr. Rogers's prolific studies of crystal structures have been mainly among complex organic compounds, and substances of special biological significance have provided the main field for his recent work. Nevertheless, he maintains a keen interest in other struc-

tures, and has contributed much to the work of colleagues in inorganic chemistry, particularly in the study of metal complexes. He and his research school are substantial users of the *Atlas* computer in the University of London.

### Analytical Chemistry in the Imperial College of Science and Technology : Prof. T. S. West

DR. T. S. WEST, reader in analytical chemistry at the Imperial College of Science and Technology, has been appointed as from October 1 to the newly established chair of analytical chemistry, the first in this discipline in the University of London. Dr. West was born in Peterhead, Aberdeenshire, in 1927, and after education at Tarbat Old Public School and Tain Royal Academy, winning first place in the Scottish Education Department's 'Highlands and Islands' Bursary Competition, proceeded to the University of Aberdeen, where he graduated with first-class honours in 1949. He then gained his doctorate, under Prof. Belcher, at the University of Birmingham, obtaining also his D.Sc. there in 1962. He was lecturer in the University of Birmingham until his transfer to the Imperial College of Science and Technology in 1963. Dr. West was awarded the Meldola Medal of the Royal Institute of Chemistry in 1956, the first analytical chemist to have won this distinction. In addition to his productive research activities which have

resulted in many papers, Dr. West has been an active member of various committees of the Society of Analytical Chemistry, the International Union of Pure and Applied Chemistry, etc., and is a founder member of the editorial board of *Talanta*. Dr. West's researches have been concerned with the development of submicro methods of organic analysis, titrimetric reagents and the application of co-ordinating and chelating agents in inorganic analysis; he has also written several monographs and has been a contributor to the *Annual Reports of the Chemical Society*. In addition to his research interests, Dr. West is deeply concerned with teaching and is running a postgraduate course in analytical chemistry at the Imperial College of Science and Technology as well as supervising numerous research students.

#### Experimental Education in the University of Manchester: Prof. F. W. Warburton

DR. F. W. WARBURTON, senior lecturer in education in the University of Manchester, has been appointed to the newly-established chair of experimental education at that University. Dr. Warburton entered the University of Reading in 1928 and graduated with honours in psychology in 1931. In the following year he was awarded the Diploma in Education of the University of Reading and then took up an appointment as assistant master in the London Teaching Service. He held this post until 1940 and during this time he read for, and gained, an M.A. of the University of London. In 1940 he was appointed acting headmaster of the London County Council School, Solva, Pembrokeshire, Wales. In 1945 he took up an appointment as Fellow at the London Child Guidance Clinic and in the same year was awarded a Ph.D. (London). In the following year he was appointed research officer (with the rank of major) in the Department of Personnel Selection of the War Office. In 1948 he was made officer in charge of the research team in Far Eastern Command, Singapore and Ipoh, Malaya. In 1949 he was appointed lecturer in educational psychology in the Department of Education in the University of Manchester. During 1958-59 and also the summer of 1961 he was research associate professor in the Department of Psychology in the University of Illinois. He was promoted senior lecturer in the University of Manchester in 1960 and was visiting professor in the Department of Education in the University of Illinois during the summer of 1963. Dr. Warburton has been a Fellow of the British Psychological Society since 1948 and a member of the Council of that Society since 1960. In 1960 he was appointed a member of the Committee on the New British Intelligence Test. In 1962 he became secretary of the Society of Multivariate Experimental Psychology (European Branch) and in 1963 convener of the British Psychological Society Committee on Programming of Electronic Computers.

#### Electrical Engineering in the Queen's University of Belfast: Prof. E. H. Frost Smith

DR. E. H. FROST SMITH has been appointed to the chair of heavy electrical engineering in the Queen's University, Belfast, rendered vacant by the resignation of Prof. J. C. West on his appointment to the University of Sussex (*Nature*, 203, 815; 1964). Born in 1923, Dr. Frost Smith received his early education at the Whitgift School, Croydon, and, after the outbreak of the Second World War, at Ardyn, Aberystwyth. Going up to Peterhouse College, Cambridge, in 1941, he graduated B.A. in the Mechanical Sciences Tripos in 1943. From Cambridge, Dr. Frost Smith joined the research staff of the Royal Aircraft Establishment, Farnborough, where he worked on automatic control systems associated with the electrical equipment of aircraft. In 1947 he took up postgraduate work at University College, London, and in 1950 was awarded the degree of Ph.D. for research into the behaviour of magnetic amplifiers. Returning to industry the same year, in the research laboratories of

Elliott Brothers, Dr. Frost Smith continued his work on automatic control systems and, in 1954, he became chief engineer of the Servo Mechanisms Division of that Company. In 1956 Staveley Industries appointed Dr. Frost Smith director of research at Bedford. Here he has been responsible for a wide range of research projects, mechanical, hydraulic and electromechanical, related largely to the design and manufacture of machine tools and control systems. Dr. Frost Smith brings to his new post a keen interest in the problems of engineering teaching in universities and an exceptionally wide experience of industrial research. One of his specialist interests which he may be expected to pursue in his new department is the problem of developing the low-inertia high-torque electric motor for use on control systems.

#### Sociology in the University of Kent at Canterbury: Prof. A. P. Stirling

DR. A. P. STIRLING, who has recently been appointed to the chair of sociology in the University of Kent at Canterbury, is forty-four years old. After a period at the University of Oxford, interrupted by service in the Royal Navy as Lieutenant, R.N.V.R., during 1941-45, he gained a first-class honours degree in Litt.Hum. in 1947. From 1948 until 1951 he was the holder of a senior treasury studentship for Turkish cultural studies under the Scarborough Scheme. After training in social anthropology at Oxford he carried out comparative field research on two Turkish villages. For this work he obtained his D.Phil. degree in 1951. In 1952 Dr. Stirling joined the London School of Economics and Political Science as an assistant lecturer in anthropology, becoming a lecturer in 1954. During his period at the School he made very significant contributions as a teacher, as a research worker, and as an organizer of research plans. He also served with distinction as the managing editor of the *London School of Economics Monographs on Social Anthropology*. Dr. Stirling has published a number of articles on aspects of Turkish rural society and also a book entitled *Turkish Village*, which is a substantial, meticulously documented description of two Turkish rural communities in their general social, economic and political setting.

#### Scientific Director of the High Latitude Space Environment Monitoring Station, Anchorage, Alaska:

Dr. H. J. A. Chivers

DR. H. J. A. CHIVERS has been appointed the first scientific director of the Central Radio Propagation Laboratory's High Latitude Space Environment Monitoring Station, Anchorage, Alaska. In his new position, Dr. Chivers will direct the scientific activities of the Station as it reorganizes its activities in the field of space environment monitoring and forecasting. This change is being made to allow the Anchorage facility to concentrate on monitoring and reporting solar and geophysical events of importance to users of high-frequency radio propagation and to other activities affected by solar-associated disturbances in the space environment of the Earth. Dr. Chivers, who is a graduate of the University of Manchester, has been a senior scientist with the Space Environment Task Group, and has also served as chief of the High Latitude Ionospheric Physics Section, Upper Atmosphere and Space Physics Division, for two years. His research experience includes observation of radio star scintillations at the Jodrell Bank Experimental Station, University of Manchester, and radio observation of artificial satellites by means of ground-based stations for ionospheric research, geomagnetic conjugate point studies, and D region absorption at the National Bureau of Standards Boulder Laboratories. Dr. Chivers is a member of the United States Commission III of the International Scientific Radio Union, the American Geophysical Union, and the Polar Cap Panel of the Committee on Space Research.

### Admissions to Universities and Colleges of Advanced Technology

IN written answers in the House of Commons on February 18, the Secretary of State for Education and Science, Mr. A. Crosland, stated that about 19,275 students in science and technology were admitted to universities and colleges of advanced technology in October 1964, or 1,375 more than in October 1963, but about 2,000 fewer than had been planned. Recurrent Government grants to universities and colleges during the financial years 1961-62 to 1964-65 were: 1961-62, £57.9 million; 1962-63, £56.8 million; 1963-64, £69.4 million; and 1964-65, £86 million. The corresponding capital grants were: £26.2 million, £32.8 million; £40.7 million, and £57.5 million, respectively.

### Underground Transmission Lines

IN reply to questions in the House of Commons on February 16 regarding underground transmission lines, the Parliamentary Secretary to the Ministry of Power, Mr. J. Morris, said that out of a total expenditure on research and development of about £7 million a year by the Central Electricity Generating Board and the Area Boards, roughly £1 million was spent on transmission and distribution, of which between 10 and 20 per cent was spent on placing cables underground. Much research was carried out by manufacturers and supported by the Boards through their purchases of cables. The Central Electricity Generating Board had placed, or was placing, contracts of about £21 million for 275-kV and 400-kV cables of a pioneering type, with an exceptionally large cross-section and provision of water-cooling. These contracts reflected much indirect support for the cable manufacturers' research and development. The Central Electricity Generating Board was giving much thought to the possibility of underground cables, but for a 400-kV line the cost was about £1 million a mile—between 18 and 20 times that of taking it above ground. At March 31, 1964, 7.6 per cent of circuit miles of the Central Generating Board were laid underground and 40.9 per cent of those of the Area Boards of more than 650 V. In 1962-63 the Central Electricity Board incurred 47.5 per cent of its capital expenditure on transmission cable or underground cables, and 42.6 per cent in 1963-64; for the Area Boards the corresponding figures are 82.2 per cent and 83.6 per cent.

### Water Barrages

IN reply to questions in the House of Commons on February 16 about the supply of power from water barrages, the Parliamentary Secretary to the Ministry of Power, Mr. J. Morris, said that while the Central Electricity Generating Board had noted the work being done by Mr. E. M. Wilson in the Department of Civil Engineering at the University of Sheffield on a barrage for the Solway Firth and the plan for a barrage across Morecambe Bay, it had estimated that in the most promising schemes so far proposed in Britain, the cost of generating electricity by tidal barrage would not be competitive. In reply to further questions on February 18, the Parliamentary Under-Secretary of State for Economic Affairs, Mr. W. Rodgers, said that proposals for barrage schemes in the Solway Firth, Morecambe Bay and the Dee Estuary were already being examined in the context of regional economic planning and it was hoped to make a statement shortly. It was recognized that this was not just a matter of the generation of electricity but that roads, the conservation of water and many other factors had to be taken into account. It was most important to consider all these schemes together and not the merit of any one of them in isolation.

### Carnegie Institution of Washington

THE *Report of the President* of the Carnegie Institution of Washington for 1963-64 gives new information concern-

ing the most distant visible objects in the Universe; new estimates of the age of the Earth and the size of the Earth's core; progress in the understanding of how organs of the body are formed and how living cells differentiate (Pp. 73 + xi + 5 plates. Reprinted from *Carnegie Institution of Washington Year Book 63* for the year 1963-64, Washington, D.C.: Carnegie Institution of Washington, 1964). The age of the Earth is now given as 4.7 billion years, and the examination of samples of material from St. Paul's Rocks, a group of islands located on the mid-Atlantic ridge, gave an age of 4.5 billion years; this would make them the oldest known rocks on the surface of the Earth.

### Association for Education in France

*French Science News* (No. 2; April-June 1964) record the foundation of the Association for Education in France, to promote co-operation between university and industry within the scope of the science faculties of the Universities of Paris, Grenoble and Nancy. The board of directors consists of an equal number of university professors and industrial representatives. Premises are under construction at the Orsay Faculty. The first objective will be to train engineers to a high degree of scientific culture; for the two years following their qualification they will be given special tuition which will include the new branches of science best adapted to their future industrial career. The second aim is to organize full-time refresher courses, lasting from a week to a fortnight, for practising engineers; these courses are to provide either a general or specialized training.

### L'Association des Physiologistes

L'Association des Physiologistes held the first of its biannual meetings of 1965 at Marseilles during February 10-13. About 200 members attended; the four-day meeting was divided into two days of demonstration followed by two days of short papers and discussions. More formal lectures will be included in the programme for the principal meeting of the Association, to be held at the end of June at Louvain, Belgium. The Association was founded in 1926 and owed its inception particularly to Profs. André Mayer and Emile Terroine of Strasbourg. The early membership was drawn from France, Belgium and Switzerland. The words 'de la langue Française' were added to the Association's title soon after its formation, but with the extension of its membership in recent years to countries where French is not the native tongue the original title has been resumed. The proceedings of each meeting are recorded in the *Journal de Physiologie* published biannually in Paris.

### Nuclear Structural Engineering

AN apparently widespread feeling of dissatisfaction among engineers that existing nuclear engineering literature does not yet provide an appropriate outlet for material in the field of nuclear structural engineering has led to the establishment of the new international journal *Nuclear Structural Engineering*. It is devoted to the civil mechanical and chemical structural engineering problems of nuclear power plants, of radiation facilities and of radioactive waste. Particular aspects include heat transfer, stress analysis, the design, fabrication and testing of reactor components and materials, reactor layout and safety considerations, and radiation protection. The editor, Dr. T. A. Jaeger, is a lecturer in nuclear structural engineering at the Technical University, Berlin, and he is assisted by 26 section editors from sixteen countries including Japan and the U.S.S.R., but not China. The journal is to appear bi-monthly. The first number (January 1965) contains 12 articles, two notes and two book reviews, together with bibliographical notes concerning the section editors, and consists of 139 pages. Each volume, costing £9, will have about 500 pages. It is published in Amsterdam by the North-Holland



ublishing Company. Contributions may be written in English, French or German, but the abstract must always be in English. The text and diagrams are clear and well printed and the standard of the articles conforms to that of the better scientific journals. However, one article from the Franklin Institute is an abbreviated version of a chapter of a book shortly to be published, and the units used in several of the articles reflect whether the authors are engineers or scientists.

#### Bibliography of Dictionaries

A COMPREHENSIVE bibliography, processed by punched-card, machine-sorting, and photo-offset techniques, in which are listed more than 2,800 bilingual and polyglot dictionaries, glossaries and encyclopaedias published during 1952-63, has been compiled by T. W. Marton of the National Bureau of Standards, U.S. Department of Commerce (Miscellaneous Publication No. 58. Washington, D.C.: Government Printing Office, 1964. 1.25 dollars). The entries are subdivided into 49 subject classes covering the physical sciences, engineering and technology, and are listed in alphabetical order by language. Within each language group the entries are arranged alphabetically by author or title, and consist of the names of the individual or corporate authors, the original title of the work, an abbreviated translation of foreign titles, the imprint, the Library of Congress card number, and the approximate number of terms contained in the work. Forty-seven foreign languages are represented in the bibliography. Detailed indexes, by author, language and subject, complete the publication, which by its large coverage will be of great value to scientists and engineers, abstractors, technical information officers, librarians and translators.

#### Directory of Information Resources

A DIRECTORY has been published by the National Referral Center for Science and Technology at the U.S. Library of Congress. The book, of 356 pages, is entitled *Directory of Information Resources in the United States: Physical Sciences, Biological Sciences, Engineering* (available from Superintendent of Documents, Government Printing Office, Washington, D.C. 20402, 2.25 dollars). The volume contains narrative descriptions of the subject specialization, information services, and publications of some 1,100 organizations and institutions throughout the United States. Professional societies, academic research groups, industrial firms, Government offices and technical libraries are included. The National Referral Center, supported by funds from the National Science Foundation, is now preparing a similar volume in the social sciences, based on data gathered in a special survey by the Bureau of Applied Social Research at Columbia University. Plans are being made for specialized listings to meet specific requirements in selected areas.

#### Dual Effect of Boron and Manganese on Growth of Raspberries

DR. C. L. WHITTLES, Campbell House, Mauchline, Ayrshire, Scotland, has written to the Editor emphasizing the necessity of estimating the effects of micro-elements on plant growth more comprehensively than is frequently done. In a long-term experiment with raspberries (Burnet Holme Seedling) on ground well supplied with nitrogen, phosphorus and potassium, he failed to obtain any enhancement of vigour from applications of boron alone, even though leaves from treated plants had ten times the boron content of controls. Treatment with manganese borate (1 lb. per 20-yd. row, giving 20 p.p.m. boron and 15 p.p.m. manganese in the soil), however, gave spectacular increase of growth and fruitfulness. The variety Burnet Holme is apt to decline in vigour, even with adequate soil fertility, showing increasing amounts of 'die-back' and virus-like symptoms in the process. Dual application of boron and manganese arrested the decline and rapidly

restored the vigour, eliminating also the die-back and the virus-like symptoms. Apical buds of untreated plants, moreover, became dormant in August (a condition recognized by tea planters and called 'banjhi'). Application of manganese borate broke this dormancy, and within 72 h after treatment the shoot had begun to extend. It is possible, indeed likely, that response to manganese borate will vary on different soils, but the necessity for integrated investigation of micro-nutrients of plants is an important consideration arising from this work.

#### The Institute of Physics and the Physical Society: Appointments and Awards for 1965

THE Council of the Institute and Society has made the following appointments and awards for 1965; the presentations will be made in London at the annual dinner of the Institute and Society on May 4: *Guthrie Lecturer*, Dr. J. B. Adams, director of the Culham Laboratory of the U.K. Atomic Energy Authority; *Thomas Young Medal and Prize*, to Prof. A. Maréchal of the University of Paris and the Institut d'Optique, Paris, for his distinguished work in many fields of optics; *Duddell Medal and Prize*, to Dr. H. A. Gebbie of the National Physical Laboratory, for his pioneering work in interference spectroscopy in the infra-red; *Charles Chree Medal and Prize*, to Prof. B. J. Mason of the Imperial College of Science and Technology, for his work in atmospheric physics; *Charles Vernon Boys Prize*, to Dr. A. Howie and Dr. M. J. Whelan of the University of Cambridge, for their studies of lattice defects in crystals by electron microscopy.

#### Travelling Research Fellowships to Denmark and Sweden, 1965-1966

APPLICATIONS are invited for the Carlsberg-Wellcome and Wellcome-Swedish travelling research fellowships, 1965-66. The object of these is to encourage working visits, on an exchange basis, between Danish and British, and Swedish and British, investigators in any branches of the natural sciences which have a bearing on human and animal medicine. One of each fellowship is awarded annually to a graduate from the United Kingdom for a year's work in the exchange country, and one Swedish and one Danish candidate annually may work in the United Kingdom. The stipend may range from £1,000 to £2,000 per annum; travelling and some incidental expenses are provided in addition. The Carlsberg-Wellcome fellowships are arranged with the Carlsberg Foundation in Copenhagen; the Wellcome-Swedish fellowships are provided jointly by the Medical Research Council of Sweden and the Wellcome Trust. Candidates in the United Kingdom can obtain further information from the Scientific Secretary, the Wellcome Trust, 52 Queen Anne Street, London, W.1. Completed applications must be submitted by April 9, 1965.

#### Physics Exhibition

THE Physics Exhibition, 1965, will be held in the Manchester College of Science and Technology during April 5-8. The Exhibition endeavours to show recent advances in physics and its applications and in methods of teaching physics. Emphasis is placed on new scientific instruments and apparatus and their possible uses for improving productivity. During the Exhibition, the following lectures will be given: physical instruments in biophysical and biological research (Dr. A. Elliott, King's College, London); instrumentation problems in computer control systems (J. L. W. Churchill, A.E.I. Automation, Ltd.); instrumentation in process and product control in the textile industry (Dr. D. W. Hill, Cotton, Silk and Man-made Fibres Research Association). Further information and tickets can be obtained from the Secretary, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

### John Logie Baird Travelling Scholarship

THE Television Society invites applications for the third award of the John Logie Baird Travelling Scholarship. This Scholarship, of value up to £200, is open to post-graduate students (in United Kingdom educational establishments) who are concerned with television engineering or an allied technology. It is expected that the award will be made to someone between 21 and 30 years of age, and the successful applicant will be known as the Baird Scholar. The scholarship is intended to assist the successful applicant in undertaking a period of investigation abroad of approximately 6-8 weeks. During this period some aspect of television, or an allied technology, will be investigated. Further information can be obtained from the Hon. Secretary of the Television Society, 166 Shaftesbury Avenue, London, W.C.2.

### Fishery Research Training Grants

THE Development Commissioners, in association with the Ministry of Agriculture, Fisheries and Food and the Department of Agriculture and Fisheries for Scotland, will award postgraduate training grants in fishery research, tenable from October 1. The grants are intended to enable the selected candidates to undergo a specified course of training to fit them for the investigation of problems in marine or freshwater science. Candidates must be British subjects and should be honours graduates in science from a British university, or possess equivalent qualifications. Further information and forms of application can be obtained from the Secretary, Development Commission, 3 Dean's Yard, Westminster, London, S.W.1.

### University News:

THE following lectureships have been announced: Dr. A. A. Rutherford (agricultural biometrics); Dr. A. McDowell Mercer (pure mathematics).

#### Belfast

#### London

THE following appointments have been announced: *Professorships*, Dr. K. W. S. Sing (chemistry, tenable at Brunel College). *Readerships*, Dr. D. M. Matthews (chemical pathology, tenable at Westminster Medical School); Dr. J. E. Ffowes Williams (applied mathematics, tenable at the Imperial College of Science and Technology). The following titles have been conferred: *Professorships*, Dr. M. E. Fisher (physics, at King's College); Dr. J. B. E. Baker (pharmacology, at Charing Cross Hospital Medical School); Dr. J. S. Weiner (environmental physiology, at the London School of Hygiene and Tropical Medicine). *Readerships*, Dr. J. S. L. Leach (physical metallurgy, at the Imperial College of Science and Technology); Dr. R. D. Milne (aeronautical engineering, at Queen Mary College); Dr. N. McA. Sayers (electrical engineering applied to medicine, at the Imperial College of Science and Technology).

### Royal Holloway College

IN October 1965 the Royal Holloway College will admit men as undergraduates for the first time, thereby instituting a process of expansion which is expected to more than double its size by 1967. An illustrated brochure issued by the College outlines the scope of its faculties of arts and science and sketches the proposed developments in botany, chemistry, physics and zoology (Pp. 24. Englefield Green: Royal Holloway College, 1964). By October 1967 the expansion is planned to create a mainly residential college of 1,000 students with men and women in equal numbers.

#### Swansea

DR. F. T. BARWELL, director of electrical research, British Railways Board, has been appointed professor of mechanical engineering in succession to Prof. R. H. Macmillan.

### Announcements

DR. M. TALWANI, a senior scientist at the Lamo Geological Observatory, Columbia University, has been selected by the Indian Geophysical Union as the first recipient of its newly created Krishnan Medal, for outstanding work in the field of marine geophysics.

THE Organization of the Felt Industry in Europe invites young people in the employ of members of the Organization, and of firms in the paper-making and allied industries and students in universities, colleges and other educational institutions, to submit technical or scientific papers for consideration for the annual awards of the Organization. Further information can be obtained from the General Secretariat, OFE, Raamweg 13, The Hague.

H. J. HEINZ COMPANY, LTD., and the National Vegetable Research Station Association have each agreed to finance a second postgraduate scholarship, tenable at the National Vegetable Research Station as from October 1. The purpose of the scholarships is to enable graduate students to pursue further studies and obtain training in one of the following subjects: chemistry; plant physiology; irrigation; plant pathology; ecology and control of weeds; biometry. Further information can be obtained from the Secretary, Vegetable Research Station, Wellesbourne, Warwick. The latest date for receipt of applications is April 30.

A. DOUGLAS CO., LTD., is to act as sole British importer of the multi-head linear dividing machine manufactured by V. E. B. Feinmess, of Dresden. This machine is capable of dividing 24 specimens simultaneously. The gradations may be applied to work-pieces over a wide range of materials, such as steel, aluminium, brass, plastics and glass. A wide range of literature covers a variety of dividing machines, including circular and linear machines of high precision, also universal and multi-head machines of the production type, etching pantograph and number engraving pantograph. Further information can be obtained from A. Douglas Co., Ltd., Lincoln Road, Cressex Industrial Estate, High Wycombe, Bucks.

AN ordinary meeting of the North of England Section of the Society for Analytical Chemistry will be held in Durham on March 12. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

THE tenth annual general meeting of the Midlands Section of the Society for Analytical Chemistry will be held in the University of Birmingham on March 11. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

THE Palynological Society of India was inaugurated on January 5 by Prof. T. S. Mahabale (Poona University) during the 51-52 session of the Indian Science Congress. The following have been elected to the Executive Council of the Society: *President*, Prof. K. N. Kaul; *Vice Presidents*, Prof. A. R. Rao and Prof. A. K. Ghosh; *General Secretary-cum-Treasurer*, Dr. P. K. K. Nai; *Councillors*, Dr. K. Biswas, Prof. U. N. Chatterjee, Dr. G. B. Deodkar, Prof. T. S. Mahabale, Dr. D. D. Panigrahy, Prof. V. Puri, Dr. Bhoj-Raj, Dr. J. Sen, Mr. B. D. Sharma, Dr. D. N. Shivepuri, and Dr. M. S. Swaminathan. Further information can be obtained from Dr. P. K. K. Nai, National Botanic Gardens, Lucknow.

CORRIGENDUM. In Fig. 1 of the article entitled "A Antifouling and Anti-corrosion System", by B. Wise (*Nature*, 203, 1132; 1964), a discrepancy occurs in the "scale drawing". The true boundary between the "hydrophobic layer" and the "rest" was omitted; to correct this a line needs to be added parallel to and 8 mm to the left of the line labelled "rest".



## THE SCIENCE AND TECHNOLOGY BILL

WHEN the Science and Technology Bill was considered in Committee in the House of Commons on January 20, several amendments were proposed to the clause relating to the Research Councils. However, these were withdrawn on receiving satisfactory explanations from Mr. M. Stewart, then Secretary of State for Education and Science. Mr. D. Price had raised the question of the functions of these Councils and the choice of priorities in the light of the final report of the Advisory Council on Scientific Policy. Mr. Stewart agreed as to the importance of priorities in research and said that besides their responsibility for the execution of research the Research Councils were responsible for the choice of priorities within their fields. The new Council on Scientific Policy was responsible for advising the Government on the choice of priorities in the allocation of funds between the various fields represented by the Research Councils and elsewhere. Mr. Price raised a further question regarding the Natural Environment Research Council, and while he and other speakers welcomed the establishment of this Council, there was some concern as to whether its responsibilities were wide enough in regard to water. Some natural resources are the responsibility of other Ministries: gas and coal (Ministry of Power), sand and gravel (Ministry of Public Buildings and Works), etc. Mr. Stewart did not entirely dispel these fears, and admitted that soil surveys fell within the scope of the Agricultural Research Council. Oceanographic research would come within the sphere of the Natural Resources Research Council, which would take over existing interests in the National Institute of Oceanography.

A further amendment advocated the establishment of a Work Study Research Council. However, while agreeing as to the importance of this subject, Mr. Stewart deprecated any such decision pending the report of the Heyworth Committee on the Social Sciences, which might well point to the creation of a research council for the social sciences. The question of space research and a Space Research Council was also raised by several members in this interesting and well-informed debate, but both Mr. J. Hogg and Mr. Stewart, supported by other members, resisted the argument. While agreeing that space research was an essential human effort at the present time, they

maintained that the size of the British effort in that field was a matter for the Council on Scientific Policy and the Government and not something to be written into the Bill.

Reference was also made to the position of the National Research Development Corporation. While the Corporation welcomed, in its annual report for 1963-64 (published a few days after the debate), the statement made by the previous Government in July that it had been decided to seek powers to extend the scope and scale of its work so that it would be able to contribute more effectively to industrial innovation and development, Mr. Stewart did not refer to the legislation then promised for this purpose, including provision for a modification and extension of the financial and other conditions under which the Corporation operates.

Sir Edward Appleton's opening address, "The Relationship between Science and Administration", in the Scottish Office Course on Science, given in Edinburgh on January 8, provides an interesting gloss on the debate. After reviewing the development of the relations between science and Government in Britain from the formation of the Royal Society down to the establishment of the National Physical Laboratory in 1900, and the foundation of the Department of Scientific and Industrial Research with its Advisory Council, Sir Edward referred to the Science and Technology Bill. He referred also to the Haldane Report on the machinery of government and particularly its doctrine that research and enquiry should be carried out under a Minister without specific departmental duties, although in close touch with Departments concerned with the activities under investigation. Sir Edward commented that thirty years ago potential user departments were often not well qualified to appreciate when scientific advice could be helpful, nor to estimate its value when given. It would seem that even to-day he has misgivings about the Road Research Laboratory being transferred to the Ministry of Transport. He is also uneasy as to the implications of appointing personal scientific advisers to a Minister, believing that this device contributed to difficulties during the War. It is not clear whether or not this criticism relates to the growing practice of appointing chief scientific officers of high status in Ministries concerned with scientific and technical matters.

## NORTH SEA LICENCES FOR THE GAS COUNCIL-AMOCO GROUP

THE Gas Council-Amoco Group comprises the Gas Council, Amoco U.K. Petroleum Ltd. (a subsidiary of American International Oil Company), Amerada Exploration Ltd. (a subsidiary of Amerada Petroleum Corporation), and Texas Eastern (U.K.) Ltd., all British corporations, and was formed early in 1963. According to a report issued on behalf of the Group (*P.R.* 6685, September 1964, The Gas Council, 1, Grosvenor Place, London, S.W.1), five licences to drill for oil and natural gas in the North Sea have been granted to the Group, which has already completed extensive seismic survey work within its now designated area of 3,600 square miles of sea; this area is divided into 36 blocks, with loci scattered over an area ranging from Peterhead in the north of Scotland down to offshore of the Norfolk coast.

Production licences cost £6,250 per block for an initial period of six years, with an option for a further 40 years "... on no more than half the area awarded to the Group at annual sums rising from £10,000 to £72,500 per block".

It is anticipated that drilling operations will begin in 1965. In this connexion it has recently been reported in the national Press that the Group has placed an order with John Brown and Co., Ltd., of Glasgow, worth £2.5 million, for a drilling platform, to operate in the North Sea area. The published particulars of this particular platform are impressive. It will stand on latticed steel legs 387 ft. high and will be built to accommodate 50 operatives; it will include a helicopter deck to facilitate communications with on-shore bases; it is designed to withstand winds of 115 m.p.h. and waves of 64 ft. These factors put this project in the class of super drilling platforms, one of the largest and most expensive structures so far conceived for offshore drilling for oil and gas. According to the report from the Group, it is considered unlikely that the rig, when in operation, will be able to drill more than two or three wells a year, because it is anticipated that completion depth could, at any one site, range to the 10,000 ft. mark, or even deeper.



A majority of the world's great oil and gas fields have been proved only after drilling several dry holes and expending large amounts of money; even a single field may require several holes to be drilled before it is proved a commercial proposition. The hazards of offshore drilling, where there is known continuity of seaward extension of land-based oilfields, are often formidable; in the case of the North Sea, the hazards, and consequently the risk of failure, are greatly increased because of lack of

such continuity. The North Sea, at least in so far as British licences at present extend, is a relatively unknown environment so far as oil and gas potentialities are concerned; to this extent optimism for the outcome, not only of the Gas Council-Amoco Group's enterprise, but equally for the pioneer efforts of other major oil interests now actively engaged in the search, must necessarily be tempered with caution and restraint; this is implicit in the report herein considered.

## ELECTRON MICROSCOPY OF GRAPHITIC CRYSTALLITES IN *Meta-ANTHRACITE*

By J. T. McCARTNEY and Dr. S. ERGUN

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**E**LECTRON microscopy of coals and coal components has been greatly facilitated by development of a technique for cutting ultra-thin sections (500–2000 Å) of these materials by microtomy with a diamond knife<sup>1–5</sup>. Granular structures as small as 100 Å and pores of the order of 20–50 Å have been reportedly observed in some of these investigations. Results of recent investigations of various coal components have been described elsewhere<sup>6</sup>.

Of special interest among these were observations of an apparent vitrain band in a *meta-anthracite* from Leoben, Austria. Fig. 1 shows a typical electron micrograph of an ultra-thin section of this material. Although these sections appeared to be continuous, the micrograph reveals a jumble of thin platelets interspersed with many holes. Many of the platelets are nearly parallel to the plane of the micrograph, and their oval or polygonal outlines can be traced. Some have a roughly hexagonal shape. Both X-ray and electron diffraction patterns of this material have shown most of the characteristic sharp lines of graphite<sup>7,8</sup>. The latter pattern is shown in Fig. 2. The rings, in order from the centre, are identifiable with the following reflexions of graphite: (002), (100–101 doublet), (004), (103) (weak), (110), (112), (201), (114), (121), (300), and (220). Estimations of crystallite size from measurements of line broadening in the X-ray patterns have yielded a value of 800 Å for this coal.

Measurements of diameters of about a dozen clearly defined platelets in electron micrographs resulted in an average diameter of about 800 Å. It is evident that the platelets are crystallites of graphite that have formed and grown from aromatic layer clusters during metamorphosis of this *meta-anthracite*.

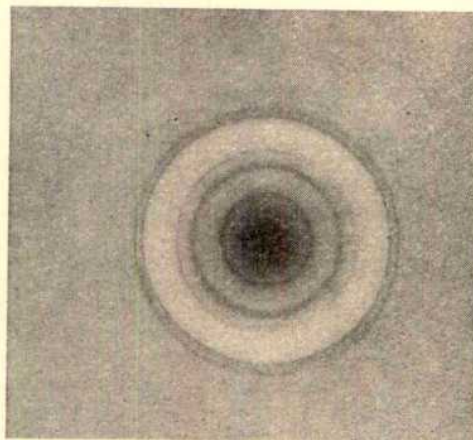


Fig. 2. Electron diffraction pattern of the section of Fig. 1

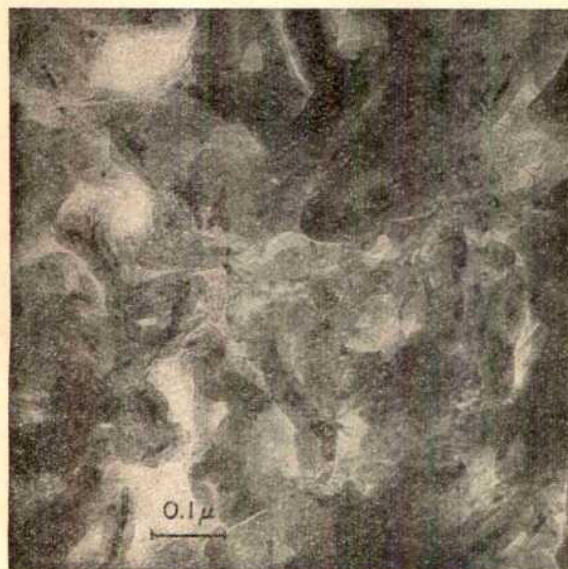


Fig. 1. Electron micrograph of an ultra-thin section of an apparent vitrain band in a *meta-anthracite* from Leoben, Austria. ( $\times 93,750$ ). (Unoriented section)

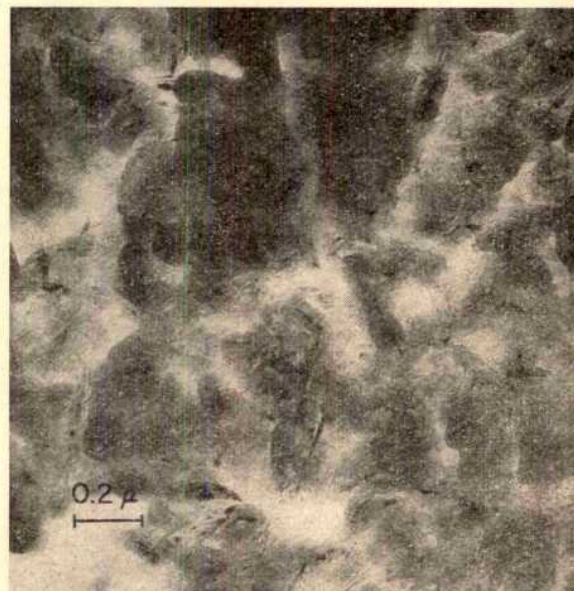


Fig. 3. Electron micrograph of a section of Leoben *meta-anthracite* cut parallel to the bedding. ( $\times 45,000$ )



To investigate the probable orientation of the crystallites with respect to the bedding planes, particles from the *meta*-anthracite vitrain band were embedded so that ultra-thin sections could be cut parallel and perpendicular to the bedding. Fig. 3 is a micrograph of a parallel section. Although the crystallite structures are not so prominent here, a general orientation of rounded platelets parallel to the film plane is evident. This is substantiated by an electron diffraction pattern of this section, shown in Fig. 4. The (002) ring is very weak, not nearly so strong as in Fig. 2, and the (004) reflexion is not detectable. Such decreased intensity of the (00 $l$ ) reflexions would be expected when the incident electron beam is more or less perpendicular to the diffracting planes.

Fig. 5 shows a section cut perpendicular to the bedding. An impression of layers of crystallites roughly perpendicular to the section is not difficult to conceive. Fig. 6, which is a diffraction pattern of this section, substantiates this conception. The rings show definite orientation effects; the accentuated arcs of the (002) and (004) rings are nearly parallel to the layer lines of Fig. 5, while those of the (100) and (110) rings are roughly perpendicular to these lines (Figs. 5 and 6 are shown in the same relative

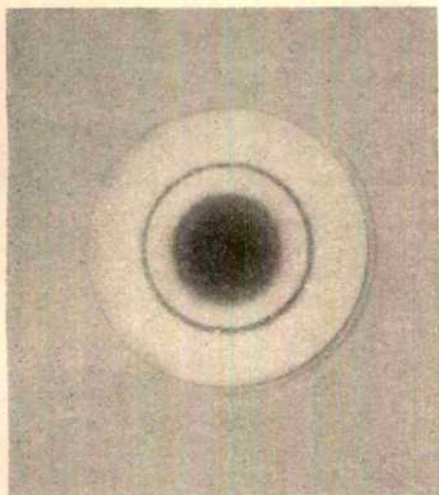


Fig. 4. Electron diffraction pattern of the section of Fig. 3

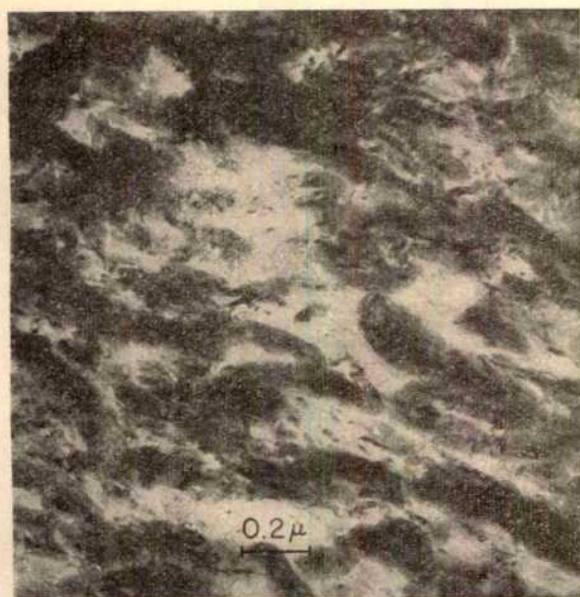


Fig. 5. Electron micrograph of a section of Leoben *meta*-anthracite cut perpendicular to the bedding. ( $\times 45,000$ )

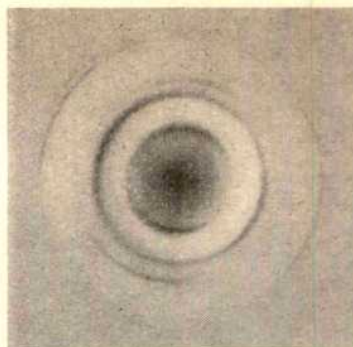


Fig. 6. Electron diffraction pattern of the section of Fig. 5

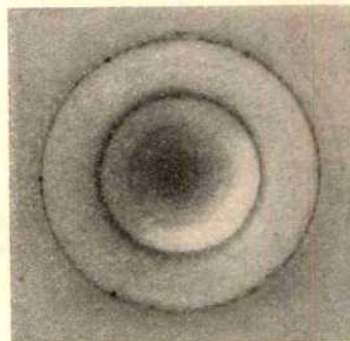


Fig. 7. Electron diffraction pattern of the section of Fig. 8

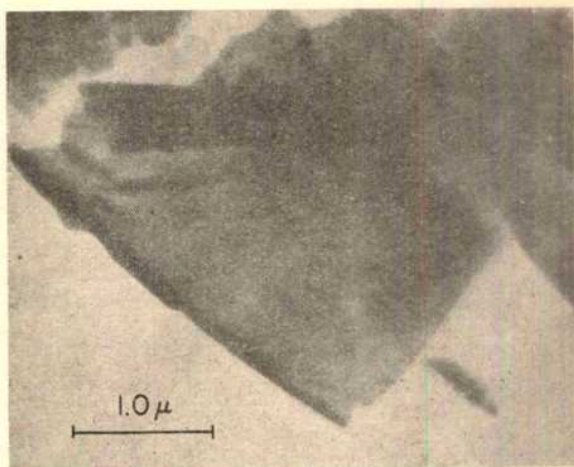


Fig. 8. Electron micrograph showing a relatively large single crystal of graphite in the Leoben *meta*-anthracite. ( $\times 18,750$ )

orientation). Since the (002) and (004) reflexions are due to interlayer spacings and the (100) and (110) are the result of in-plane atom distances, the oriented patterns are consistent with the foregoing interpretation.

During traversal of the sections while viewing the diffraction patterns, spot patterns characteristic of single crystals were often seen. These were largely attributable to mineral matter. However, in some instances, accentuated spots were seen on the graphite rings. Such a pattern is shown in Fig. 7. Six regularly spaced spots, in addition to other random ones, can be seen on both the (100) and (110) rings, with the spots on the two rings at alternate  $30^\circ$  angles. Observations of the area contributing to this pattern disclosed the large crystallite shown in Fig. 8. This crystallite, of the order of  $3\mu$  in diameter, is much larger than the average throughout this material, but a few of this size have been observed. The other, much

smaller crystallites contribute to the random spots and the nearly continuous ring.

The foregoing material presents direct evidence of the development of three-dimensional graphite crystallinity in *meta*-anthracite and the ordering of the crystallites in layer planes parallel to the bedding.

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<sup>3</sup> McCartney, J. T., Walline, R. E., and Ergun, Sabri, *Preparation of Ultra thin Sections of Coal*, U.S. Bur. Mines Rep. Inves., 5885 (1961).

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<sup>5</sup> Brown, H. B., and Taylor, G. H., *Nature*, 193, 1146 (1962).

<sup>6</sup> McCartney, J. T., O'Donnell, H. J., and Ergun, S., *Proc. First Amer. Cong. Coal Science* (in the press).

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<sup>8</sup> Ergun, Sabri, and McCartney, J. T., *Science*, 134, 1620 (1961).

## THERMAL ANOMALIES ON THE TOTALLY ECLIPSED MOON OF DECEMBER 19, 1964

By J. M. SAARI and R. W. SHORTHILL

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RECENT infra-red measurements on small portions of the lunar disk made during eclipses<sup>1,2</sup> and during the lunar night<sup>3,4</sup> have revealed prominent thermal anomalies on ray craters, and lesser anomalies in other areas. A survey of the entire lunar disk is needed to determine the thermal homogeneity of the surface. While such a survey is possible for the dark side of the Moon, the low temperatures there require a slow scanning rate and observations over many nights. During a total lunar eclipse, however, the surface cools for only a short period, and its substantially higher temperature allows rapid scanning during totality.

In 1962 we developed a rapid scan system for mapping the Moon thermally and photometrically under illumination, using the 60-in. telescope at Mount Wilson<sup>4</sup>. The scan system was modified for use on the 74-in. Kottamia telescope of the Helwan Observatory in Egypt for observations during the total lunar eclipse of December 19, 1964. Using a mercury-doped germanium detector cooled to liquid neon temperature, we were able to scan the entire disk in 16 min at 10" arc resolution with approximately 200 successive traverses. Four scans were made on the full Moon before the eclipse began, four during the first penumbral phase, three during totality, and one during the second penumbral phase. These data were recorded on magnetic tape and are now being reduced on a 7094 computer.

A great amount of detail was revealed by chart recordings made during the experiment and is the basis for this preliminary report. As expected, prominent anomalies were found on the major ray craters Tycho, Copernicus, Aristarchus, Aristoteles, Proclus, Theophilus, Langrenus and Stevinus. For example, Fig. 1 is a tracing of the infra-red signal obtained on a traverse through Tycho, an outstanding thermal anomaly on the eclipsed Moon. Preliminary calculations indicate that the maximum temperature on Tycho is 226° K and the environ temperature 178° K, a difference of 48° K. On this traverse three peaks in the signal over Tycho may correspond to the rims and the central peak.

Perhaps the most surprising result of the experiment was the discovery of hundreds of localized thermal anomalies or 'hot spots' on the surface of the Moon besides those associated with the major ray craters. An example in Fig. 1 is the 'spike' in the signal near Tycho, tentatively identified with Heinsius A. Other examples of hot spots are seen in Fig. 2, where seven spikes in the signal correspond to locations in Mare Tranquillitatis and Mare Foecunditatis and on the edges of Mare Imbrium and Mare Serenitatis. The largest spike on the chart recordings, comparable to the signal from Tycho, was observed from the crater Dawes.

The positions of 300 of the more conspicuous hot spots are plotted in Fig. 3, where the boundaries between the seas and continental areas are indicated. The distribution of these anomalies is not uniform on the disk; for

example, a great number are found in Mare Tranquillitatis and relatively few in the continental area between Tycho and Theophilus. Many hot spots can be identified with the small ray craters which appear as white spots on the full Moon; these youthful features probably are thermally anomalous, as are the major ray craters, by reason of their denser and/or rougher surfaces. Some hot spots, however, are not associated with small ray craters; accurate positioning will be the first step in seeking an interpretation for these anomalies.

Another important result is the discovery of thermal enhancements over extended regions of the seas as shown in Fig. 1. Some of these extended enhancements are plotted in Fig. 3 as lines along the traverse direction. All of Mare Humorum appears higher in temperature than its environs, while only parts of Oceanus Procellarum, Mare Imbrium, and Mare Frigoris are elevated. Extended enhancements are also found in Mare Tranquillitatis and Mare Foecunditatis as shown in Fig. 2.

The significance of these results will become clearer after contour maps are plotted and cooling curves for specific areas of interest are constructed. The observed thermal anomalies will be plotted on maps of the lunar disk and compared with surface features. The inter-

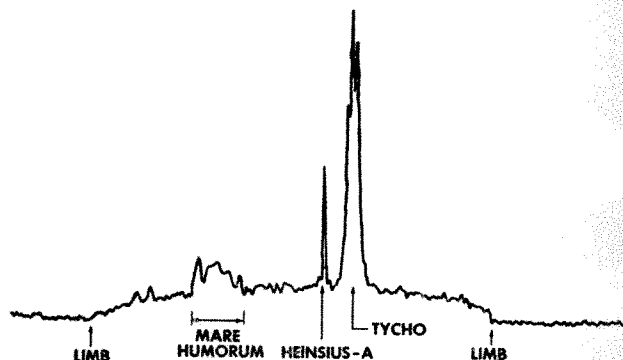


Fig. 1. Tracing of the infra-red signal obtained on traverse number 81 during the third scan in totality of the December 19, 1964, total lunar eclipse. The scan began at 2h 55-0m U.T. and ended at 3h 10-4m U.T.

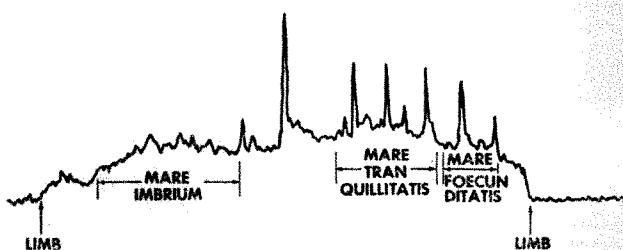


Fig. 2. Tracing of the infra-red signal on traverse 134 during the third scan in totality



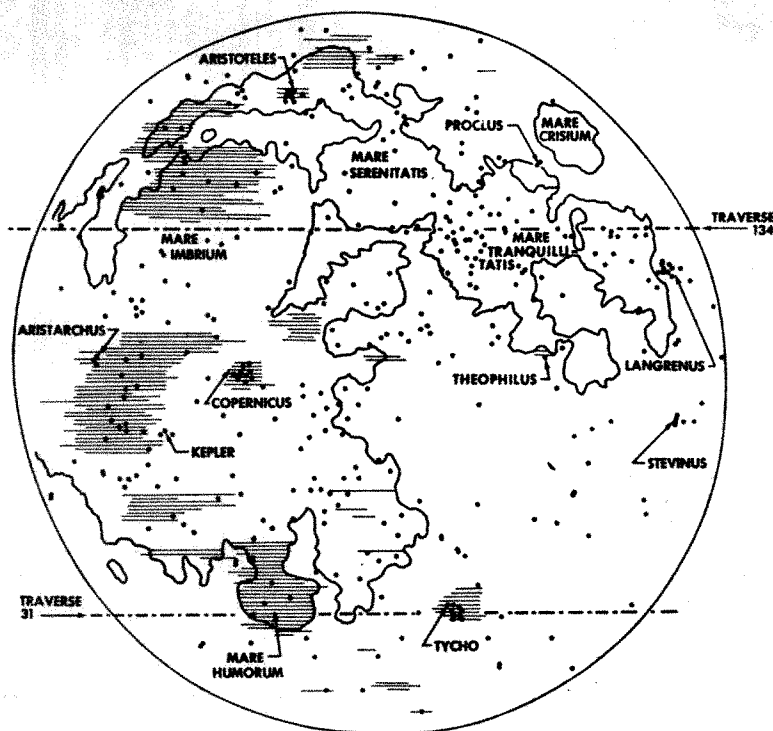


Fig. 3. Positions of 'spikes' in the infra-red signal (indicated by dots) obtained during the third scan in totality. Some of the extended thermal enhancements are indicated by lines in the traverse direction (see text). Traverses 31 and 134 are indicated by dot-dash lines

pretation of the results can then be made with respect to theoretical cooling curves and experimental data such as albedo, colour contrasts, stratigraphy, radar and infra-red measurements through a lunation.

We thank Prof. A. H. Samaha, director of the Helwan Observatory, for generous allowance of observing time on the 74-in. reflector at Kottamia; Dr. M. K. Aly, associate director, for collaboration at the telescope; and G. K. Bruce for assistance in carrying out the measurements.

Transport of the equipment was made possible through the co-operation of Dr. J. W. Salisbury, chief of Lunar-Planetary Research, U.S. Air Force Cambridge Research Laboratories, under contract AF19(628)-4371.

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<sup>2</sup> Sinton, W. M., *Lowell Obs. Bull.*, 5, 25 (1960).

<sup>3</sup> Murray, B. C., and Wildey, R. L., *Astrophys. J.*, 139, No. 2, 734 (1964).

<sup>4</sup> Shorthill, R. W., and Saari, J. M., paper presented at Conf. Geological Problems in Lunar Research sponsored by the New York Academy of Sciences, New York, May 16-19, 1964 (in the press).

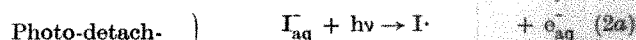
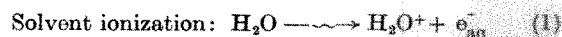
## SOME ELEMENTARY PROCESSES IN RADIATION- AND PHOTO-CHEMISTRY REVEALED BY ELECTRON SPIN RESONANCE

By DR. P. B. AYSCOUGH, R. G. COLLINS and PROF. F. S. DANTON, F.R.S.

Department of Physical Chemistry, University of Leeds

**E**LECTRONS may be generated at a uniform rate in liquids either by ionization of solvent molecules caused by the close passage of fast charged particles, for example, by irradiation with  $\alpha$ -,  $\beta$ - or  $\gamma$ -rays, or by photo-detachment from reducing solutes. Typical primary processes in aqueous systems are (1) and (2) below. In sufficiently polar media the electron may become solvated and persist long enough to establish its equilibrium ion-atmosphere so that when it reacts with a negatively or positively charged species the reaction is accelerated or decelerated by increases of ionic strength according to the Brønsted-Bjerrum equation<sup>1</sup>. The products of reaction of an electron with a solute having an even number of electrons must include a free radical the nature of which is usually inferred from the accompanying stable products, for example, nitrogen in reaction (3). Direct spectroscopic observation of such reactive species is only possible in fluid systems if their concentrations can be greatly increased by the techniques of pulse radiolysis<sup>2</sup> or flash photolysis<sup>3</sup> and provided they possess distinct, strong absorption bands in an accessible region. In this way it

has also been shown that many of the bimolecular reactions are diffusion controlled ( $k \approx 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ) and might therefore be expected to occur even at low temperatures. If a rigid medium is used the solute and the free radical product of its reaction with an electron will be immobilized so that the optical and electron spin resonance spectrum of the latter can be obtained. For the electron to react with the immobile solute the electron must be able to move from its point of origin through the medium. This is possible because as it is first formed the electron has energy considerably in excess of thermal levels.

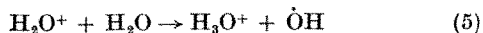




In radiation chemistry this energy is typically about 10 eV and the electron can move distances about 10–100 Å before it is thermalized: in photochemistry the energy is about 1 eV and the distance is presumed to be shorter. If there is a solute lying in the path of the electron, reaction may occur. If no solute is present the electron may either continue its travel until it encounters a positive ion resulting from the primary act, or it may reach a region in the solid where the molecules are so oriented as to form a potential well which can bind a thermal electron. We shall refer to such an immobile electron in a solid as a 'trapped' electron denoted by  $e_t^-$  to distinguish it from the mobile solvated electron formed in a liquid and denoted, in the case of aqueous systems, by  $e_{aq}^-$ . Clearly the density of traps will be lowest in crystalline solids and higher in glasses and crystals containing anion vacancies. If a solute is present in the glass in concentration comparable with the trap density it will compete with the traps for capture of the electron. This competition is not disadvantageous to the investigation of solute-electron reactions because in all cases the trapped electron can be remobilized by absorption of light according to reaction (4). The electron,  $e_m^-$ , may either be retrapped at another site or be captured by a solute so that illumination causes the physical traps to be depleted by transfer of electrons to the solute molecules. Moreover, since a rise in temperature increases the possibility of rotation of molecules of the matrix, movement of electrons and free radicals through the matrix can be thermally induced and much can be learnt about this movement from an examination of the effect of step-wise temperature increases on the optical and electron spin resonance spectra of irradiated glasses.



This article describes the electron spin resonance spectra of various odd electron species produced by  $\gamma$ -rays or light and trapped in various glassy matrices at 77° K. It should be noted that always in radiation chemical, and sometimes in photochemical, generation of the electron another free-radical species must be formed. In radiation chemistry the molecular ion produced in the primary ionization generally reacts very rapidly with a neighbouring molecule in a proton-transfer reaction, for example, reaction (5) in the case of aqueous systems. To the extent that in photochemical systems the other species formed in the photo-detachment process is often relatively stable, these systems are often simpler to interpret than the radiation-chemical ones.



**Aqueous alkaline glasses.**  $\gamma$ -Irradiated alkaline glasses (> 1 N alkali) have been investigated extensively by Schulte-Frohlinde *et al.*<sup>4</sup> and by Erbov, Pikaev, Glazunov and Spitsyn<sup>5</sup>. In an 8 N NaOH glass  $e_t^-$  has an intense optical spectrum ( $\lambda_{max} = 5860$  Å,  $G_{e_{max}} = 3.7 \times 10^4$  M<sup>-1</sup> cm<sup>-1</sup>) and an electron spin resonance signal consisting of a single narrow line at  $g = 2.002-3$  (Fig. 1). A broad electron spin resonance peak at  $g = 2.04$  has been attributed to  $O_t^-$ . Unlike earlier workers, we find that when the irradiated glass is warmed to 150° K for a few minutes or is illuminated with visible light, the initial spectrum, attributed to  $e_t^-$ ,  $O_t^-$  and  $\dot{O}H$ , changes in such a manner as to indicate that the  $O_t^-$  and  $e_t^-$  disappear together and that only  $\dot{O}H$  remains (Fig. 1 and ref. 6). Thus the suggestion<sup>4</sup> that reaction (6) occurs on warming is not supported, although this reaction may be responsible for the  $O_t^-$  produced during the  $\gamma$ -irradiation. On the other hand, a reaction leading to the conversion of  $O_t^-$  to  $\dot{O}H$  (equation (7)) would explain our observations. Attempts to obtain the spectrum of  $\dot{O}H$  in an alkaline system by photolysing  $H_2O_2$  in deaerated 8 N NaOH

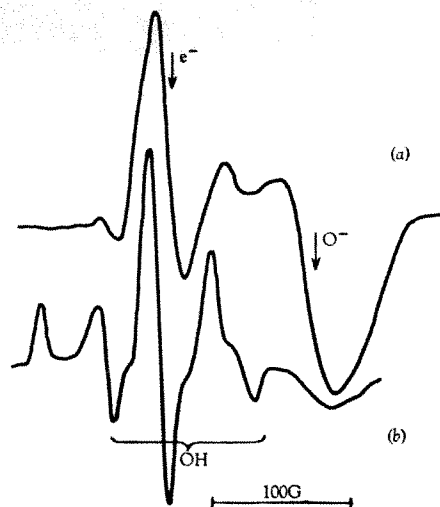
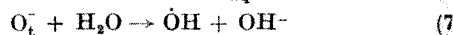
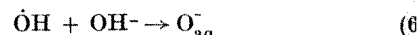
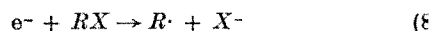


Fig. 1. Electron spin resonance spectra of  $\gamma$ -irradiated 8 N NaOH glass at 77° K. (a) Immediately after irradiation; (b) after illumination with visible light

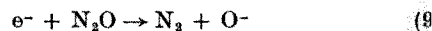
have not been successful, the only species formed being  $O_2^-$  or  $\dot{H}O_2$  (ref. 6) which appears before photolysis and which is presumed to be an intermediate in the rapid chain decomposition of  $H_2O_2$  which is observed.



Electron scavengers, added before irradiation, all reduce the  $e_t^-$  signal and replace it partially or completely by that of a neutral radical  $R\cdot$  formed in the dissociative electron capture process (8) where  $RX = CH_3Cl$ ,  $C_2H_5Cl$ ,  $ClCH_2COOH$ ,  $FCH_2COOH$ ,  $CH_2OHCOOH$ ,  $CH_3CN$ . The behaviour of a glass containing approximately  $3 \times 10^{-2}$  M  $CH_3Cl$  is typical<sup>10</sup>. The initial spectrum clearly indicates the presence of  $e_t^-$  and methyl radicals, the latter increasing very markedly on warming to 130° K or illuminating with visible light at 77° K (Figs. 2 and 3).



The effect of adding nitrous oxide (at 5 atmosphere pressure in order to increase solubility) is to increase the  $O_t^-/e_t^-$  ratio because of the occurrence of reaction (9).  $N_2O^-$  is not observed. On photobleaching, some of the mobile electrons react with  $N_2O$  so that the  $O_t^-$  increase slightly and some react with  $\dot{O}H$ , the signal from which is thereby diminished. Conversely, the addition of an  $\dot{O}H$  scavenger such as  $10^{-2}$  M glycerol before irradiation decreases the relative yield of  $\dot{O}H$ , as expected, and after photobleaching the residual  $\dot{O}H$  is an order of magnitude less than that observed in the pure samples.



The  $e_t^-$  peak and blue coloration are also seen when 8 N NaOH containing  $10^{-4}$  M  $K_4Fe(CN)_6$  is irradiated with light of wave-length 2537 Å. In this case no  $O_t^-$  and no  $\dot{O}H$  is seen (Fig. 3a). The addition of electron scavenger to these glasses reduces the  $e_t^-$  peak and replaces it by that of the appropriate radical (reaction (8)) confirming the identity of the reducing species and the reality of reaction (2c) in the system (Fig. 3b). Photo-ejection of an electron from  $I^-$ ,  $OH^-$  and  $N_3^-$ , which occurs readily in fluid media, has not been observed in alkaline glasses using light of wave-length 2537 Å or 2230 Å, possibly due to the marked blue shift of electron detachment spectra caused by a 200 deg. temperature drop<sup>7</sup>.

**Aqueous acid glasses.** In  $\gamma$ -irradiated glasses of 6 N  $H_2SO_4$  or in ultra-violet-irradiated glasses containin

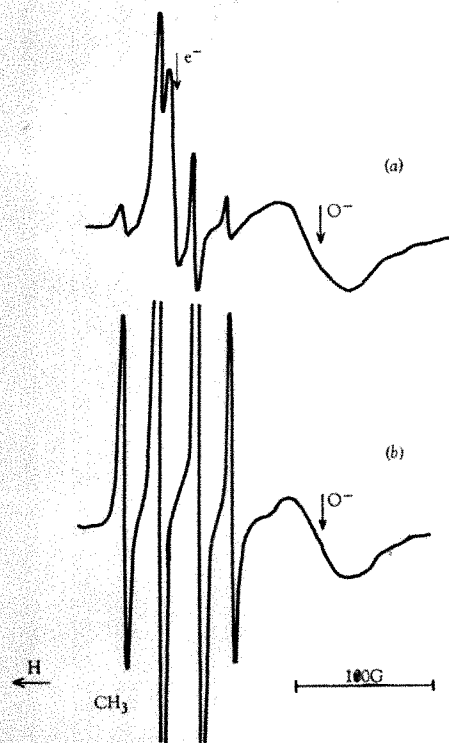


Fig. 2. Electron spin resonance spectra of  $\gamma$ -irradiated 8 N NaOH glass containing  $3 \times 10^{-4}$  M  $\text{CH}_2\text{Cl}$  at  $77^\circ \text{K}$ . (a) Immediately after irradiation; (b) after warming to  $150^\circ \text{K}$  for 5 min

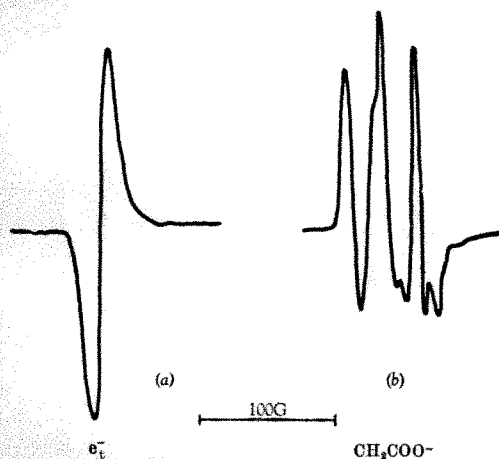
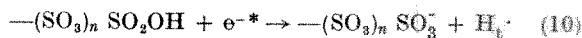


Fig. 3. (a) Electron spin resonance spectrum of NaOH glass containing  $10^{-4}$  M  $\text{K}_2\text{Fe}(\text{CN})_6$ , after illumination with light of wave-length  $2537 \text{ Å}$ ; (b) as (3a) above, with addition of  $\text{CH}_2\text{ClCOOH}$  before irradiation

$0.4 \text{ M Fe}^{2+}$ , no blue colour develops and no electron spin resonance signal attributable to  $e^-$  is observed. Instead there is a strong signal from trapped hydrogen atoms<sup>8,9</sup> which are formed by reaction of the electron with OH groups on the  $\text{SO}_2$  polymer chains according to (10). In addition, there is a large asymmetric peak at  $g = 2$  which appears to be associated with the acid residue and is probably formed by reaction of  $\text{H}_2\text{O}^+$  with  $-(\text{SO}_3)_n\text{O}_2\text{OH}$  in a manner analogous to reaction (5) (Fig. 4a). The paramagnetic centre probably has the structure  $-\text{SO}_2-\dot{\text{O}}$ . If nitrous oxide is added before irradiation the concentration of H atoms is reduced to an extent which is closely related to the amount of additive. The addition of  $\text{ClCH}_2\text{COOH}$  results in the formation of  $\text{H}_2\text{COOH}$ , identified by its electron spin resonance spectrum<sup>10</sup>, similar to that shown in Fig. 3b. This evidence, together with the kinetic evidence presented elsewhere<sup>10</sup>, suggests that  $e^-$  is a precursor of H in these systems, and

that both  $\text{N}_2\text{O}$  and  $\text{ClCH}_2\text{COOH}$  can compete successfully with  $\text{H}_2\text{O}^+$  and  $-\text{SO}_2\text{OH}$  for the mobile electrons at concentrations of  $10^{-3} \text{ M}$  or less.



However, these systems are very much more complicated than the alkaline glasses as may be adduced from their behaviour on warming. The hydrogen atoms disappear by a second-order process at a measurable rate at  $85^\circ \text{K}$  and very rapidly at  $95^\circ \text{K}$ , while little change occurs in the central peak, attributed to  $-\text{SO}_2-\dot{\text{O}}$  (ref. 9). However, above  $100^\circ \text{K}$  this peak increases very markedly in size, reaching a maximum height (when measured at  $77^\circ \text{K}$ ) five or six times that observed initially. This maximum occurs after warming to  $120^\circ$ – $125^\circ \text{K}$ ; further warming causes a diminution until the signal disappears after warming to about  $150^\circ \text{K}$ . During these changes the signal becomes almost symmetrical and develops small shoulders. Furthermore, in samples containing nitrous oxide, additional fine structure centred at  $g = 2$  is observed (Fig. 4b). This appears at about  $110^\circ \text{K}$  and disappears at  $130^\circ \text{K}$ . Finally, ultra-violet irradiation at  $77^\circ \text{K}$  of samples which have been warmed above  $100^\circ \text{K}$  causes a reduction in size of the central peak which may be restored by warming to the maximum temperature reached during the previous warm-up.

These observations, while not fully understood, suggest the presence of another form of electron trap from which the electron may be ejected by relaxation of the matrix. This trap might be a point at which the electron could interact with oxygen atoms from two or more  $-(\text{SO}_2-\text{O})_n$  chains, the resulting anisotropy rendering the absorption line too broad for detection. Mobility might then be restored to the electron by thermal relaxation, leaving an  $-\text{SO}_2-\dot{\text{O}}$  radical. The temporary appearance of hyperfine structure in samples containing nitrous oxide must be similarly associated with the formation of  $\text{N}_2\text{O}^-$  by a species not observed initially at  $77^\circ \text{K}$ . ( $\gamma$ -Irradiation of pure  $\text{N}_2\text{O}$  gives a weak triplet having line separation about 6 gauss and line-width about 2 gauss, very similar to that of nitrogen atoms trapped in solid nitrogen at  $4^\circ \text{K}$ <sup>11</sup>). More detailed quantitative investigations are needed before this problem can be resolved.

*Non-aqueous glasses* (methanol,  $\alpha$ -methyl tetrahydrofuran and olefines). The behaviour of these non-aqueous glasses is in many ways analogous to that of the alkaline aqueous glasses. For example,  $\gamma$ -irradiation of a methanol glass at  $77^\circ \text{K}$  produced electron spin resonance and optical spectra which indicate the presence of trapped

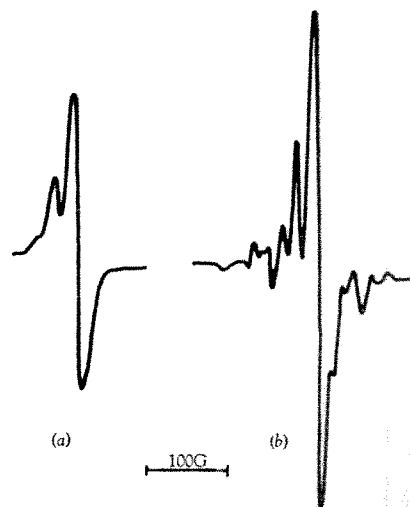
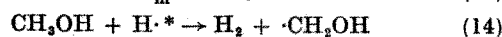
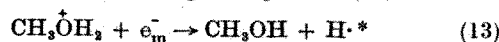


Fig. 4. Electron spin resonance spectrum of  $\gamma$ -irradiated 6 N  $\text{H}_2\text{SO}_4$  containing  $\text{N}_2\text{O}$  at 1 atm. pressure. (a) Immediately after irradiation at  $77^\circ \text{K}$ ; (b) after warming to about  $130^\circ \text{K}$

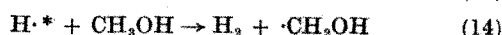
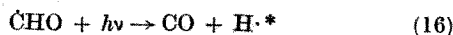
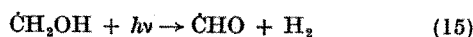
electrons and  $\dot{\text{C}}\text{H}_2\text{OH}$  radicals<sup>12,13</sup>. The sequence of reactions is summarized in equations (11) and (12):



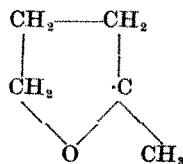
The trapped electrons have an optical absorption band with  $\lambda_{\text{max}} = 5330 \text{ \AA}$  to be compared with  $\lambda_{\text{max}} = 5400 \text{ \AA}$  for  $e_t^-$  in alkaline methanolic glass and  $\lambda_{\text{max}} = 6500 \text{ \AA}$  for the solvated electron in methanol<sup>14,15</sup>. Similarly,  $\cdot\text{CH}_2\text{OH}$  has an absorption maximum at a much shorter wavelength in the glass at  $77^\circ \text{K}$  than in the liquid at room temperature<sup>15</sup>. The electron spin resonance spectrum of  $e_t^-$  is much broader than that observed in the alkaline glasses and may have hyperfine structure. Unfortunately, the insolubility of  $\text{K}_4\text{Fe}(\text{CN})_6$  precluded the use of reaction (2c) to obtain the spectrum of  $e_t^-$  in the absence of  $\dot{\text{C}}\text{H}_2\text{OH}$  radicals, so this interpretation is tentative. However, hyperfine structure has been observed in electrons trapped in neutral aqueous glasses prepared by the reaction of sodium with ice at  $77^\circ \text{K}$  (ref. 16). Some confirmation of this interpretation is provided by the observation that illumination of the  $\gamma$ -irradiated methanol glass with green light approximately doubles the size of the triplet of  $\dot{\text{C}}\text{H}_2\text{OH}$  and reduces that of an underlying peak but the total peak area remains the same. This effect is attributed<sup>15</sup> to photo-detachment of  $e^-$  in reaction (4) followed ultimately by reaction (13) in which the hydrogen atom is 'hot' and therefore reacts immediately with a neighbouring methanol molecule according to equation (14).



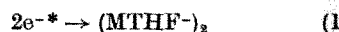
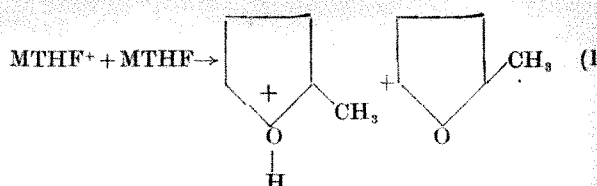
This effect is quite different from that of irradiation with ultra-violet light which causes decomposition of  $\dot{\text{C}}\text{H}_2\text{OH}$  (ref. 17) and of  $\dot{\text{C}}\text{HO}$  so that steady-state concentrations of  $\dot{\text{C}}\text{H}_2\text{OH}$  and  $\dot{\text{C}}\text{HO}$  are ultimately attained through the cycle of the following reactions<sup>17</sup>:



$\gamma$ -Irradiation of glasses made by rapidly cooling  $\alpha$ -methyltetrahydrofuran (MTHF) is known from Hamill's work<sup>18</sup> to result in the formation of  $e_t^-$  and the electron spin resonance spectrum shown in Fig. 5a has also been found<sup>19,20</sup>. The central peak, about 3 gauss wide, is believed to be associated with trapped electrons, since it shows the same thermal and photochemical relaxation pattern as the broad optical absorption based with  $\lambda_{\text{max}} = 12,500 \text{ \AA}$ . Both are destroyed by illumination with red light. The outer structure and the absorption at  $2500 \text{ \AA}$ , which are unaffected by illumination with red light<sup>15</sup>, may be assigned to the radical



in which two  $\beta$ -protons have hyperfine splittings of about 35 and 20 gauss, respectively, and the methyl protons about 20 gauss. The areas of these two features are comparable when observed under sufficiently low microwave power, and it is believed that the reactions involved are:



The addition of small quantities of naphthalene or carbon tetrachloride to MTHF before irradiation causes diminution of the  $e_t^-$  peak, and the appearance of feature corresponding to the attachment of  $e^-$  to the additive. In the case of naphthalene a structureless peak about 3 gauss wide is observed (the naphthalene monoanion). When carbon tetrachloride is added, an additional asymmetric peak at  $g = 2.02$  is seen (Fig. 5b). This probably indicates the presence of  $\text{CCl}_3$  formed by dissociation of  $\text{CCl}_4$ . The addition of nitrous oxide also reduces  $e_t^-$  but no additional spectrum is seen (Fig. 5c).

Olefines such as 2-methyl-butene-2 and hexene-2 which form transparent glasses at  $77^\circ \text{K}$ , also develop a weak blue coloration when  $\gamma$ -irradiated in the absence of light. It has been suggested<sup>21</sup> that the visible absorption in these systems indicates the presence of cationic species and that an absorption band in the infra-red corresponds to trapped electrons. We find that the electron spin resonance spectra of these olefines when examined in the absence of light consist of a narrow single peak about three gauss wide superimposed on the broad hyperfine spectrum of trapped alkyl radicals<sup>22</sup>. The singlet peak is very weak in 2-methyl-butene-2 but quite marked in hexene-2. When illuminated with visible light the blue colour disappears almost immediately, but the electron spin resonance singlet persists for about 5 min. Thus the photochemical relaxation of the optical absorption and the electron spin resonance signal follow different courses in contradistinction to the behaviour of the MTHF glasses. Although it is very likely that the electron spin singlet is caused by  $e_t^-$ , there is as yet no proof of this. These conclusions are in agreement with our earlier observations<sup>23</sup> on thermal annealing of *n*-hexadecene-1 in which it was suggested that the decrease in concentration of the trapped dimeric radical-cation was caused by the progressive release of a mobile species, presumably electrons, from traps of different energies.

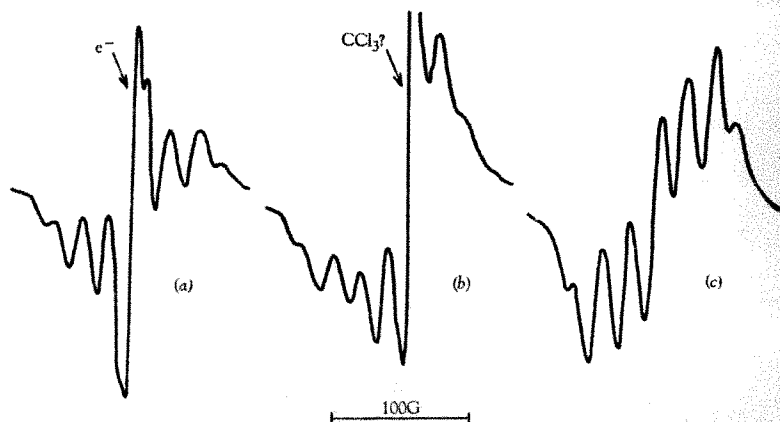


Fig. 5. Electron spin resonance spectra of  $\gamma$ -irradiated  $\alpha$ -methyltetrahydrofuran at  $77^\circ \text{K}$ . (a) Immediately after irradiation; (b) as above, with addition of  $\text{CCl}_4$  before irradiation; (c) as above, with addition of  $\text{N}_2\text{O}$  before irradiation.



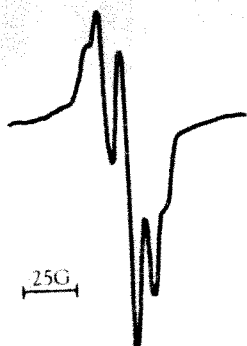


Fig. 6. Low-field hydrogen atom peak in  $\gamma$ -irradiated 6 N  $\text{H}_2\text{SO}_4$  at 77° K

**Matrix effects.** While the foregoing electron spin resonance data do not confirm the mechanisms already outlined here, they provide powerful support for the conclusions based on detailed analyses of the kinetics of the decompositions. It is significant that the basic reactions are very similar despite the widely different matrices. However, the differences in the spectra associated with the trapped electron need some comment. There has already been much speculation on the nature of the electron traps in alkaline glasses<sup>24,25</sup>, and there is increasing support for the view that the electron is trapped in an anion vacancy surrounded by water molecules, rather than in an expanded orbital of the cations as has been suggested for electrons in liquid ammonia. The line width (about 15 gauss in 8 N NaOH) is reduced to about 6 gauss on replacing  $\text{H}_2\text{O}$  by  $\text{D}_2\text{O}$ , so that some proton interaction is implied. The observation of proton interaction in neutral ice where the individual line width is only 2–3 gauss<sup>16</sup> and our observations on methanolic glasses support this view. The absence of such interaction in ethereal systems (for example, MTHF) and the long relaxation time probably indicate that the vacancy occupied by the electron lies between two MTHF rings and is too distant from the protons to give either an observable interaction or rapid relaxation. A further observation of some relevance here is that in some samples of 6 N  $\text{H}_2\text{SO}_4$  the hydrogen atom peaks appear as quintets or septets with peak separation of 7 gauss and line width about 2 gauss (see Fig. 6), almost identical with the values quoted for the  $e_{\text{aq}}^-$  peak in ice. One is tempted, therefore, to suggest that the H atom traps have a very similar conformation, interacting weakly with four or six neighbouring protons. This

behaviour is not very reproducible, the H atom peaks sometimes appearing with satellites as reported by Livingston<sup>26</sup>. In glasses containing  $\text{Fe}^{2+}$  (and, of course,  $\text{Fe}^{3+}$ ) any such structure is obscured by the greater line width (7–8 gauss) presumably caused by the presence of a relatively high concentration of paramagnetic ions in the matrix.

We thank G. A. Salmon, C. G. Gopinathan, T. J. Kemp and J. Russell, of the University of Leeds, and J. Teply, of the Nuclear Research Institute of the Czechoslovak Academy of Sciences, for permission to quote some of their results prior to publication. We also thank the Rockefeller Foundation, the Department of Scientific and Industrial Research and the International Atomic Energy Agency for financial assistance in the form of capital grants and maintenance allowances.

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## A COMMERCIAL SPECTROMETER MODIFIED FOR X-RAY SPECTROSCOPY OF THE LIGHT ELEMENTS

By PROF. RALPH W. G. WYCKOFF, For.Mem.R.S., and FRANKLIN D. DAVIDSON

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CONVENTIONAL X-ray spectroscopy has been very successful in the analysis of elements heavier than silicon and has given results of limited accuracy and sensitivity for aluminium and magnesium. It has failed for lighter elements because of the low efficiency of the fluorescent excitation of their characteristic X-rays and because their very long wave-lengths could not be analysed in spectrometers built to deal with the harder radiations from heavier elements. Recent developments in ways to excite very soft X-rays and in techniques for their measurement now make it relatively easy to analyse for the lighter elements through boron [ $\lambda(K\alpha) = 67 \text{ \AA}$ ]. We describe here a simple modification of a commercial X-ray spectrometer which permits this extension.

The problem of low X-ray production by light elements can be met<sup>1,2</sup> by eliminating the window between the tube target and a 'fluorescing' sample; this will increase by as much as a thousand times the output of characteristic X-rays. Single crystals are not available with spacings great enough to reflect the X-rays from elements lighter than oxygen, but stacked monomolecular films of lead stearate or other fatty acid salts will act as very efficient gratings with  $2d$  spacings at least as large as 125 Å. The counter tubes ordinarily used will not record the X-rays in question because of absorption in their windows, but they are satisfactory when equipped with stretched polypropylene or even with thin collodion windows.



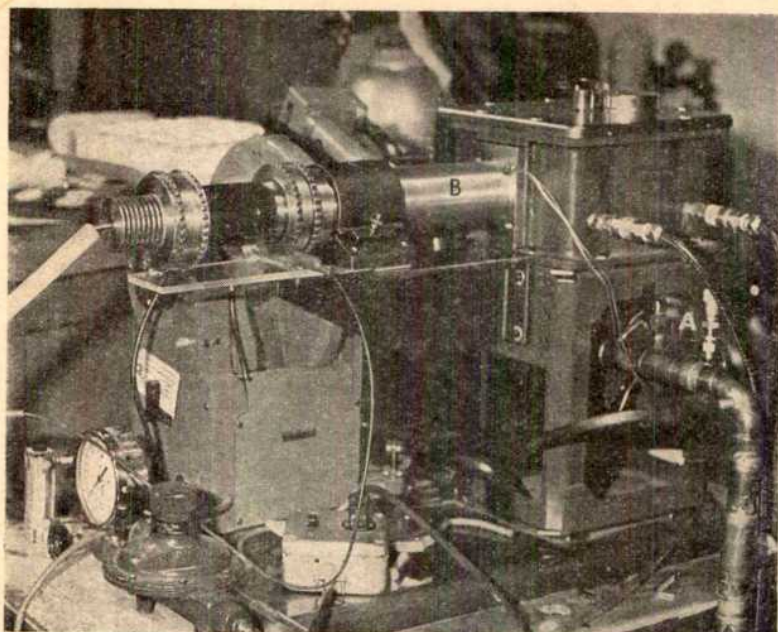


Fig. 1. Photograph of the Philips vacuum spectrometer arranged for soft X-ray measurements. The gas type X-ray tube is at B and the vacuum leak at A.

After having carried out extensive measurements with a spectrometer especially arranged for the examination of long X-rays, we have applied the X-ray source, grating and detector developed for this instrument to the commercially available Philips vacuum spectrometer to see how it would perform in this extension of X-ray spectroscopy. The gas type X-ray tube (B in Fig. 1), mounted in this Philips spectrometer, is an obvious modification of that recently described<sup>1</sup>; it is built round a casting for a standard FA-60 X-ray tube and fits as a direct replacement for such a tube. The target, which is exchangeable, is water cooled through the same leads which cool an FA-60. The power consumption of the gas tube for this work is so small (about 25 W) that no other water cooling is required. The sample is placed in one of the usual specimen holders from which the mylar has been removed and there is no window between it and the anode of the tube. For work with soft X-rays it is important that low voltages be used to minimize background due to white radiation. The standard, unmodified Philips power supply, operated at its lowest value between 10 and 15 kV, can be used for the analysis of elements heavier than fluorine, but for this element and those still lighter better results are obtained if the tube is operated in the region 5–10 kV. In view of the small power needed—in none of our experiments with this instrument has it exceeded 100 W—an inexpensive power supply has been adequate. The tube operates at a vacuum of about 20  $\mu$  and no difficulty has been experienced in creating a better vacuum than this throughout the apparatus with the Welch No. 1402 oil pump which routinely evacuates the spectrometer. For quantitative measurements of intensity it is obviously essential to have a constant X-ray output. This is readily obtainable by introducing a controlled air leak into the pumping system. It can be done with such a precision needle valve as the Whitey 22RS4-316 (purchased from Whitey Research Tool Co., 5525 Marshall Street, Oakland, 8, California), connected directly to the atmosphere, but we find it somewhat better to connect the valve to a buffer tank (an old butane bottle, for example) which is partially evacuated every day or two.

As an analysing 'crystal' a 130-layer<sup>3</sup> lead stearate (LS) grating is commonly used. Such a 'grating' is not difficult to prepare with a modified Langmuir trough. Analyses with greatly increased sensitivity can now be

made for silicon and aluminium with an ethylenediamine ditartrate (EDDT) crystal and for lighter elements with mica or potassium acid phthalate (KAP) crystals. If the stearate grating is to be used to measure the heavier of these light elements, this should be done in a higher order to avoid the high background at low angles of reflexion. An idea of the relative efficiencies of the three crystals and the stearate grating can be gained from the fact that the intensities of the first order  $K\alpha$  lines of aluminium are in the ratio 100 (EDDT): 27 (mica): 70 (KAP): 200 (LS). Intensities of the several orders of a spectral line from a typical LS grating are in the approximate ratio 100:19:12:3:2 for its first five orders. As we make them, such a grating is built up on a 1 in.  $\times$  3 in. microscope slide which can very simply replace the second crystal in the double crystal holder of the Philips spectrometer.

The flow proportional counter of the commercial instrument can be used as detector for the long wave-lengths with only a change of window. This window is, however, so large that it is customary to coat its inner surface with aluminium or carbon for stable operation. Such a coating increases unnecessarily the opacity of the stretched polypropylene; it can be avoided by facing the new window on the counter side with aluminium foil cut to cover all but an area of about 7 mm  $\times$  25 mm of the Soller slit. The P-10 (argon 90 per cent-methane 10 per cent) counter gas conventionally used in X-ray spectroscopy will record long X-rays; and the higher counter voltages required, up to about 1,900 V, are within the range of the circuit panel customarily used with this instrument.

Fig. 2 shows typical spectra obtained with the Philips spectrometer as modified above; backgrounds are low

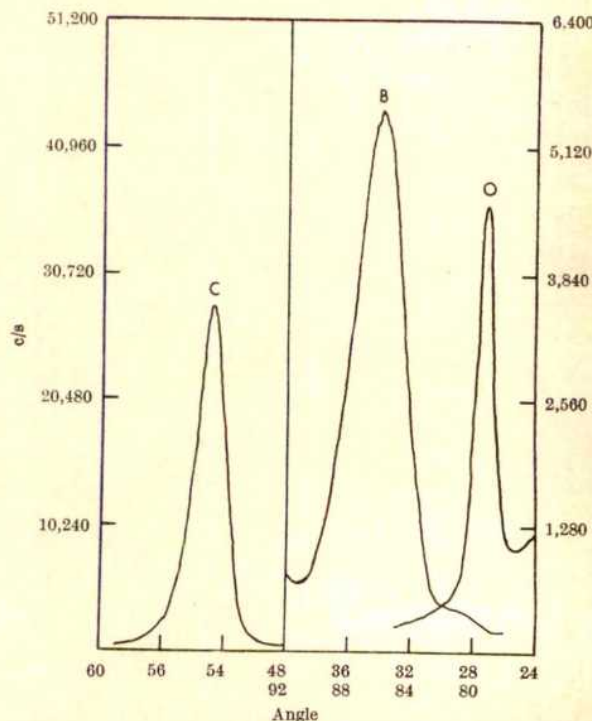


Fig. 2. The  $K\alpha$  lines of boron, carbon and oxygen as recorded with the spectrometer of Fig. 1. All spectra were taken with 25 W through the tube. For carbon the sample was compressed graphite. The spectra for boron and oxygen at the right were obtained from pressed boric acid



Table 1. OBSERVED PEAK HEIGHTS (IN C/S/W) OF LS SPECTRA OF LIGHT ELEMENTS

Element	Spectral order	
	First	Second
Aluminium	—	400
Magnesium	—	394
Sodium (NaCl)	—	(292)
Fluorine (LiF)	(840)	(203)
Oxygen	(196)	(28)
Nitrogen	(13)	—
Carbon	1,100	—
Boron	(480)	—

and peak-to-background ratios satisfactory. Table 1 gives the observed efficiencies of  $K\alpha$  lines (in c/s/W of energy through the X-ray tube) measured for several elements. Numbers in parentheses are observed values adjusted for 100 per cent content of the element but not for absorption. They have no absolute significance and depend on many factors including a tube voltage which will ordinarily be chosen to give the optimal peak-to-

background ratio for the elements being analysed. The intensities shown in the figure can easily be increased by raising the power through the tube, but they are sufficient to permit accurate analyses for small amounts of all the light elements except nitrogen. The low efficiency for nitrogen recorded in the table is due to the extreme absorption of its characteristic X-rays by the carbon of the counter window; this is a situation that will always prevail unless a suitable non-carbonaceous window material is found.

This work was supported by National Aeronautics and Space Administration grant *NsG-120*, National Science Foundation grant *GB 1322* and National Institutes of Health grant *DE 01919*.

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## SIZE EFFECT OF OXYGEN ANION ON PARTITION COEFFICIENTS

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MATSUI and I have attempted to evaluate the partition coefficients of elements for the process of crystallization of silicate melt, mainly basic<sup>1-5</sup>. It was the analysis<sup>4</sup> of abundance variation of lanthanides that stimulated us to undertake this series of investigations.

Hence, the partition coefficients were first obtained for lanthanides<sup>4,5</sup>. These values resulted from the interpretation that the partition coefficients of lanthanides, arranged in order of atomic number, formed an arithmetical progression.

In this connexion, furthermore, it was found<sup>6</sup> that the reciprocal of ionic radius rather than the ionic radius itself, of lanthanides, changed linearly with atomic number. (It must be added here that this finding is derived from a set of ionic radii given by Templeton and Dauben<sup>7</sup> for lanthanides. So far as the relative magnitude is concerned, their values for lanthanides are much more precise than those presented by Goldschmidt<sup>8</sup>, apart from the absolute scale.) Fig. 1 shows the relation between the partition coefficient obtained by me and the reciprocal of ionic radius. The correlating line in this diagram is intriguing in two respects<sup>6</sup>: the positions of the intersections of partition coefficients at 1 and 0. The correlating broken lines based on Goldschmidt's scale intersect the level of unity at  $1.195 \sim 1.23$  ( $= 1/0.84 \sim 1/0.81$ )  $\text{\AA}^{-1}$ . It seems noteworthy that the ionic radius,  $0.81 \sim 0.84$   $\text{\AA}$ , corresponding to the concerned intersection, is almost equal to the ionic radius of magnesium or ferrous iron. (According to Goldschmidt<sup>8</sup>, the ionic radii of magnesium and iron (II) are  $0.78$  and  $0.83$   $\text{\AA}$ , respectively.) The fact that the partition coefficient for a hypothetical lanthanide with the ionic radius similar to magnesium or ferrous iron is presumed to be 1 can be regarded as very reasonable, inasmuch as the species of leading metallic cations in ultrabasic and basic rocks are magnesium and ferrous iron.

On the other hand, the broken lines in Fig. 1 intersect the level of partition coefficient 0 at  $0.73 \sim 0.765$  ( $1/1.37 \sim 1/1.31$ )  $\text{\AA}^{-1}$ . Although it is difficult to present a unique interpretation of this result on limiting size of ionic radius, it is of interest to note that the ionic radius of oxygen anion usually adopted ranges from  $1.32$  to  $1.40$   $\text{\AA}$  (ref. 9). It may be justifiable to infer that the cation having the ionic radius larger than that of oxygen anion is prohibited

from finding its primary way into the closely packed structure of solid oxide. (This interpretation was applied to the estimation of partition coefficients of lanthanides<sup>10</sup> for calcium-controlled process.)

The extension and general application of these findings to other elements form a very interesting problem. Actually, such an investigation<sup>6</sup> was made on the basis of crustal abundances presented by Ahrens and Taylor<sup>11</sup>, and of chondritic abundances of elements. Although the

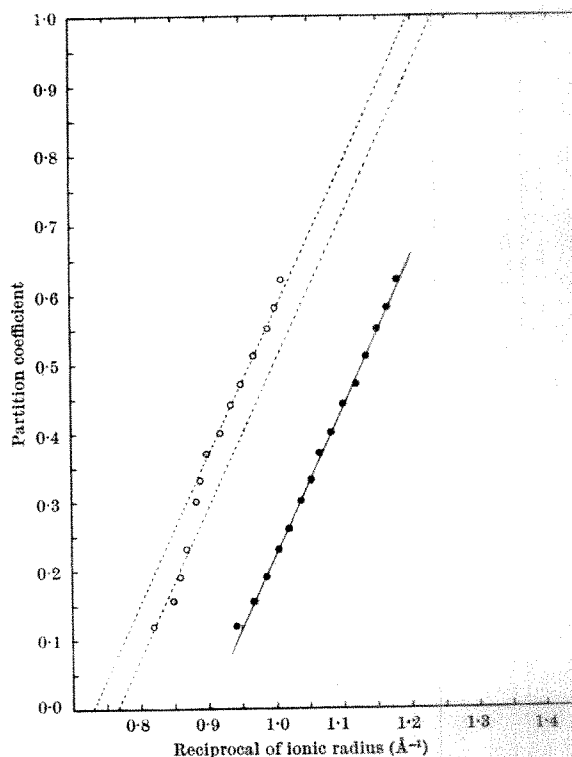


Fig. 1. Correlation between partition coefficients of lanthanides and reciprocals of their ionic radii<sup>6</sup>. Open circles are based on ionic radii by Goldschmidt<sup>8</sup>, while solid circles on ionic radii are from Templeton and Dauben<sup>7</sup>.



sequence of such an investigation appeared to conform to the regularity expected from lanthanides, some uncertainty remained, for it is doubtful whether Ahrens-Taylor's abundances are representative of crustal abundances of elements and whether they correspond to the same liquid fraction value.

Therefore, it is desirable to direct attention to the average abundances for basic rocks, though it is also more or less uncertain whether they correspond to the same liquid fraction value. However, the basic rocks are preferable in that most of them can be considered as liquid-type material and their genetic nature and chemical composition are very simple compared with acidic rocks. For this reason, I dealt with the average abundances for basic rocks<sup>12</sup>. Since the liquid fraction value for average basic rock<sup>3</sup> has been estimated to be about 1/80, it is possible to evaluate the partition coefficient<sup>6</sup> from the average abundance for basic rocks, if the relevant abundance for initial material is known. For some elements such as lanthanides<sup>4,5</sup>, uranium and thorium<sup>3</sup>, it was shown that the chondritic abundances can be regarded as representing the initial concentrations for the Earth. As regards alkali metals, however, it has been questioned<sup>13</sup> whether their chondritic abundances can represent their abundances in the original oxide material of the Earth. In fact, if the partition coefficients of alkali metals are calculated, taking their chondritic abundance to be representative of the Earth's initial abundance of alkali metals, values much smaller than expected are obtained. Consequently, so long as reference is made to chondrites, it appears to be impossible to extend the limiting effect by oxygen anion to alkali metals.

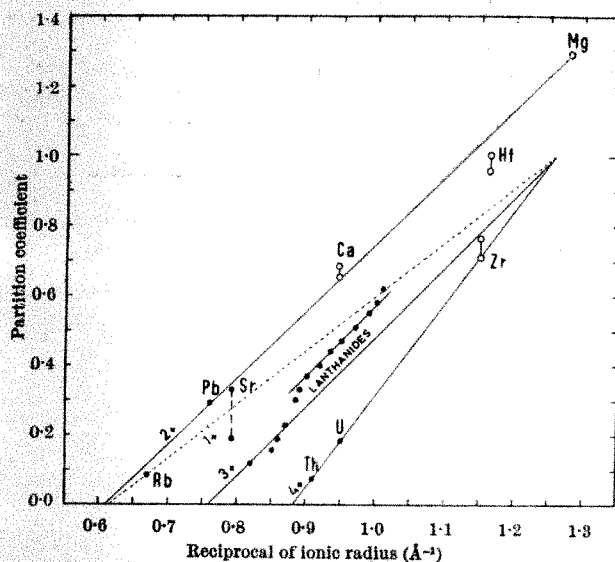


Fig. 2. Dependence of partition coefficient on the reciprocal of the ionic radius and on valency. Partition coefficients for rubidium and strontium are from ref. 1, that for lead from ref. 2, those for thorium and uranium from ref. 3, and those for lanthanides from refs. 4 and 5; for open circles, see text. (Correlating lines may not converge but tend to run in parallel. However, it makes a slight difference to the size limit, because the partition coefficients have been obtained for large ions)

Meanwhile, I have succeeded<sup>1</sup> recently in evaluating the partition coefficient of rubidium exclusively from the abundance variation in terrestrial materials (basic rocks) without any reliance on meteorites. As a result, it has been shown that the value for the abundance of rubidium in chondrite cannot be used as an initial rubidium abundance which is pertinent to the genesis of our basic rocks. Moreover, in contrast to the result of preliminary inspection based on the chondritic data, it has been also indicated that the partition coefficient of rubidium is not aberrant but regular in relation to the bearing on ionic radius.

The resulting partition coefficients have been plotted in Fig. 2 together with those for other elements. (In this diagram, the partition coefficients derived from, or endorsed by, terrestrial abundance variations are shown in solid circles, while those calculated merely from a comparison<sup>6</sup> of the average basic rock with the chondrite are expressed by open circles.) As to bivalent elements, it is a debatable problem whether the major elements magnesium and calcium can be treated in the same way as many of the minor elements. Nevertheless, it has been shown<sup>12</sup> that the major bivalent elements can in general be treated in the same way as minor bivalent elements. In any case, it may be necessary to deal separately with a group of bivalent elements, probably inclusive of minor elements, for the leading metallic cations in ultrabasic or basic rocks belong to this group.

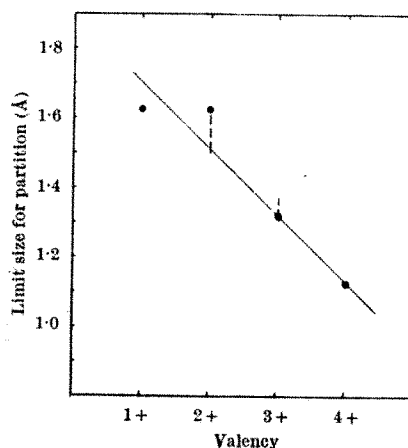


Fig. 3. Dependence of size limit for partition on valency (cf. Fig. 2)

The limiting value of ionic radius corresponding to partition coefficient 0 (cf. Fig. 2) is shown as a function of valency in Fig. 3. (As to the partition coefficient of a univalent element, there is only one value, that is, that for rubidium. Accordingly there exists some uncertainty about the size limit for univalent series, but the value shown in Fig. 3 could be regarded as fairly reliable.) It is seen that the size limits for partition are comparable with that of the oxygen anion. In addition, it seems to be significant that the size limit for partition of elements with increasingly larger electric positive charge becomes smaller. As a possibility, this can be interpreted as suggesting that the oxygen anion behaves as a smaller sphere towards the cation with higher electric charge. (It should be noted that Pauling<sup>14</sup> gave the value of 1.76 Å to oxygen for a univalent crystal and 1.40 Å to oxygen for a polyvalent crystal.) That is, Fig. 3 can be interpreted as reflecting the coulomb effect. However, the possibility cannot be ruled out that compressibility may be connected to a lesser extent with this phenomenon.

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## DISTRIBUTION PATTERN OF AMYLASE ACTIVITY IN SERUM PROTEINS

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**A**MYLASE activity of serum has been reported by several workers to be associated either with albumin or with globulin or with both. McGeachin and Lewis<sup>1</sup> reported that in normal human serum amylase activity was chiefly associated with the albumin fraction of a serum, a conclusion based on electrophoretic investigations. McGeachin and Potter<sup>2</sup> have suggested that this was not the case for rat serum, and the largest fraction of the serum amylase recovered from the electrophoretic pattern was associated with  $\beta$ -globulin and a lesser amount with  $\gamma$ -globulin. It was further suggested that the distribution pattern of serum amylase may vary in different species.

The amount of serum amylase activity varies widely in different species and the activity is not related to the carbohydrate content of the normal diet of a particular species. For example, rabbits have a lower serum amylase content than dogs. Evidence is presented here that the distribution pattern of amylase activity in the serum proteins of man, dog, rat and guinea-pig is the same and that amylase activity is associated with the globulin fraction.

The blood samples from guinea-pigs and rats were withdrawn directly from the heart under mild ether anaesthesia. The samples from men and dogs were obtained by vein-puncture. Serum was obtained from fresh blood samples and the serum proteins were fractionated by the method of Reinhold<sup>3</sup>, where the precipitation of globulin is effected by a solution of a mixture of sodium sulphate and sodium sulphite (27.8 per cent). This method was originally recommended for routine estimation of albumin and globulin fractions.

Serum and sulphate-sulphite solution were mixed in the ratio of 1:15 in a centrifuge tube; 7.5 ml. of ether per ml. serum was added and shaken. Usually 2 ml. of serum was taken in each case. It was centrifuged just long enough for a firm globulin layer to form. The clear solution below the globulin layer was carefully removed quantitatively and contained all the albumin fraction. The globulin precipitate was washed twice with sulphate-sulphite mixture and the washings were added to the albumin fraction. The globulin precipitate was dissolved in saline after removal of ether. The albumin and globulin fractions were dialysed separately against running saline at 4° C for 36–48 h to remove all the sodium sulphate and sodium sulphite. The whole serum was also similarly dialysed. The contents of the dialysing bags were then adjusted to a known volume with saline and amylase activity was measured.

Serum amylase activity was determined in terms of the amount of reducing sugar formed when serum was incubated with starch at 38° C. The amount of the solution equivalent to 0.05 to 0.1 ml. of the original serum was added to a buffered starch substrate (0.1 per cent starch in saline with 12.5 mM potassium phosphate buffer, pH 6.9) containing 1 mg of soluble starch (Fisher Scientific Co.), and incubated for 15 or 30 min. A control was similarly prepared without starch. At the end of the incubation period, enzyme activity was stopped by addition of 1 ml. of 0.1 N sodium hydroxide and 5 ml. of 0.45 per cent zinc sulphate solution. The solutions were kept in boiling water for 4 min and filtered through a cotton plug. The precipitate was washed with two 3-ml.

Table 1. SERUM AMYLASE ACTIVITY

Amylase activity is expressed in terms of mg of glucose equivalent released by 100 ml. of serum in 30 min. Numbers in parentheses represent the number of samples tested in each species

	Whole serum	Amylase units per 100 ml. of serum Dialysed serum	Albumin fraction	Globulin fraction	Total
Man (5)	50	38	2	41	43
Guinea-pig (8)	421	417	4	364	368
Rat (4)	637	618	8	547	555
Dog (6)	793	759	14	729	743

portions of hot water. The reducing sugar was estimated in the filtrate by Hagedorn and Jensen's method.

The difference between test and control values provides a measurement of the amylase activity which was calculated as mg of glucose equivalent released by 100 ml. of serum in 30 min. Two blank experiments were simultaneously run on each day of estimation. The first blank was used to determine the reducing power of 1 mg starch and the value was subtracted from the test values. Another blank was carried out without starch and the value was subtracted from all control experiments.

After dialysis, some of the samples of the serum protein fractions were electrophoresed on paper in barbitol buffer at pH 8.6 and ionic strength of 0.05. The amount of solution of albumin or globulin equivalent to 0.02 ml. of the original serum was subjected to electrophoresis for 16–18 h, along with the samples of dialysed and undialysed serum. The strips were dyed and scanned in the usual way.

The mean values found for human and for guinea-pig, rat and dog sera are summarized in Table 1. The results show that amylase activity was always associated with the globulin fraction of the serum proteins and that activity was slightly less on dialysis.

Fig. 1, which denotes one of the typical electrophoretic patterns of the human serum protein fractions and those

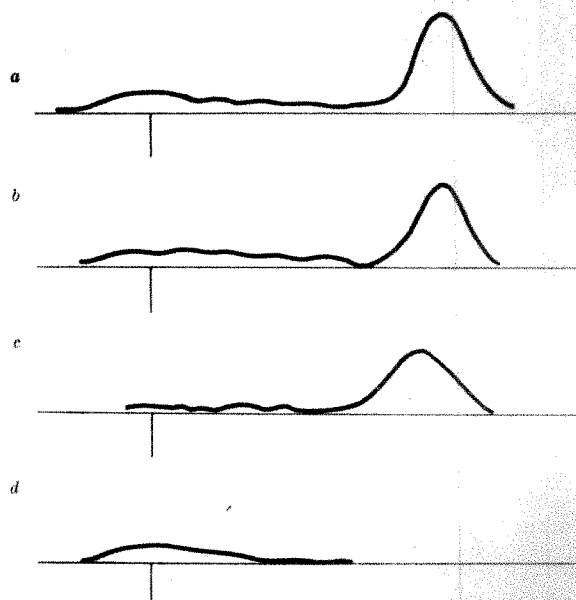


Fig. 1. Electrophoretic pattern of human serum: a, whole serum; b, dialysed serum; c, albumin fraction; d, globulin fraction

and undialysed serum, indicates that the fraction was free from globulin and vice versa, though further separation of the different fractions of globulins was not clear, probably due to dilution and ageing of the serum samples.

These results suggest that enzyme activity is always associated with globulin fraction and does not differ from one species to another, at least among the species tested. The results are therefore not in agreement with those of McGeachin and his co-workers<sup>1,2</sup>, but they do agree with those of Baker and Pellegrino<sup>4</sup>, who showed that amylase activity was principally in the globulin fraction. Both groups of workers have based their separation of the fractions on electrophoresis. It has been pointed out that the measurement of paper electrophoresis is uncertain because of protein-protein and protein-paper indications<sup>3</sup>.

These observations, apart from showing that enzyme activity is always associated with the globulin fraction, also indicate that the separation of the fractions of the serum proteins by the Reinhold method is complete.

McGeachin and Lewis<sup>1</sup> indicated the presence of an inhibitor of amylase in human serum which was undialysable; they found that the sum of the amylase activities of the electrophoretically separated fractions of normal serum was greater than the amylase activity of the whole serum. In this investigation the presence of inhibitor was not indicated at all when the serum proteins were fractionated into albumin and globulin fractions. The total amylase activity of the two fractions was, however, slightly lower than the amylase activity of the whole serum, dialysed or undialysed, except in the case of human serum, when the total activity of the albumin and globulin fractions (Table 1) was higher than that of the activity of dialysed serum. This suggests that if a protein inhibitor is present, then this protein is associated with the globulin fraction, as nearly all the amylase activity is associated with this fraction.

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## RUMEN MICROBIAL POLYNUCLEOTIDE SYNTHESIS AND ITS POSSIBLE ROLE IN RUMINANT NITROGEN UTILIZATION

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IT now appears that a major proportion of the nitrogen absorbed from the ruminant's digestive tract is represented by molecular combinations arising from the metabolic action of rumen micro-organisms on dietary forms of nitrogen. The principal products of this microbial metabolism are ammonia and amino-acids derived from the subsequent intestinal digestion of rumen microbial protein. This report presents evidence that microbial polynucleotide nitrogen represents an appreciable proportion of rumen microbial nitrogen and suggests that the conversion of dietary nitrogen to polynucleotides, and their subsequent digestion and metabolism, may explain several phenomena related to ruminant nitrogen utilization.

Rumen-cannulated sheep were fed diets essentially devoid of ribonucleic (RNA) and deoxyribonucleic (DNA) acids and protein (by the analytical methods employed) and supplemented at a level of 10 per cent with one of the nitrogen sources listed in Table 1. These sources were employed because, due to their solubility in 70–80 per cent ethanol, they could be separated from microbial protein and nucleic acids. Samples of rumen ingesta were obtained shortly after the morning feed, and placed in 39° C anaerobic incubation tubes. 10 ml. sub-samples were removed immediately and at intervals up to 12 h. Methods of analysis were based on a modification<sup>1</sup> of the Schmidt and Thannhauser<sup>2</sup> procedure by which RNA and DNA phosphorus were determined. RNA and DNA nitrogen values were calculated assuming a phosphorus to nitrogen ratio of 8:15.3 (ref. 3).

Microbial nitrogen per 10 ml. ingesta increased with incubation time during all experiments, as indicated by the ranges recorded in Table 1, and was highly significantly, positively correlated with RNA ( $r = 0.72$ ) and total polynucleotide ( $r = 0.80$ ) nitrogen. Therefore, for brevity, the nitrogen contribution of these polynucleotides is expressed in Table 1 as a mean percentage of microbial nitrogen. RNA-N comprised from 10.4 to 14.8; DNA-N, 2.2–4.1, and total polynucleotide nitrogen, 13.8–18.4 per cent of the microbial nitrogen.

An indication of the magnitude of conversion of dietary nitrogen to microbial nitrogen and polynucleotide nitrogen can be obtained from the proportion of the total ingesta nitrogen represented by each. Since each represented an increasing proportion of the total ingesta nitrogen with increasing incubation time, the range and mean of microbial nitrogen and polynucleotide nitrogen expressed as a percentage of total ingesta nitrogen is presented in Table 1. Microbial nitrogen represented 41.1–51.6 per cent of the total ingesta nitrogen in Exp. C involving zein-values which agree well with the *in vivo* conversion of zein nitrogen to microbial nitrogen (at least 40 per cent) reported by McDonald<sup>4</sup>. Microbial nitrogen represented a similar proportion of the total ingesta nitrogen in Exp. G and H to that reported for the *in vivo* conversion of wheat hay nitrogen to microbial nitrogen (62–82 per cent<sup>5</sup>).

Microbial polynucleotide nitrogen represented from 5.0 to 7.6 per cent of the ingesta nitrogen from the sheep fed zein and 8.4–13.3 per cent of the ingesta nitrogen from sheep fed urea and amino-acids. A private communication from Dr. I. W. McDonald indicated that (based on analysis for adenine and guanine and assuming that adenine and guanine represented two-thirds of the total nucleic acid nitrogen) nucleic acid nitrogen represented over 10 per cent of the microbial nitrogen leaving the rumen of sheep fed casein<sup>6</sup>. The *in vitro* data presented here for Exps. G and H agree well with McDonald's *in vivo* results. The lower value for zein, as compared with urea and amino-acids, is consistent with the demonstrated resistance of zein to intra-ruminal degradation<sup>4</sup>. These data suggest that a significant proportion (from 5.0 to 13.3 per cent) of the ruminant's dietary nitrogen is converted to rumen microbial polynucleotide nitrogen and, since microbial nucleosidal and nucleotidal nitrogen was not determined, possibly a larger proportion is converted to microbial purine and pyrimidine nitrogen. This is consistent with an extensive conversion of dietary nitrogen to rumen microbial nitrogen and the relatively large concentration of nucleic acids in various species of bacteria<sup>7</sup>. It also serves to explain several reported observations concerning ruminant nitrogen utilization and excretion.

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Table 1. DISTRIBUTION OF NITROGEN IN RUMEN INGESTA

Experiment	A	B	C	D	E	F	G	H
Nitrogen source	Urea*	Urea*	Zein†	Urea‡ + Zein	Urea§ + M.I.	Urea§ + M.I.	Urea¶ + A.A.	Urea¶ + A.A.
Number of sub-samples analysed	15	13	16	16	16	16	9	9
Range of microbial nitrogen, mg/10 ml. ingesta	22.3-28.2	22.8-26.0	16.0-20.4	26.0-30.5	23.6-27.4	32.6-38.2	18.9-24.3	20.8-25.9
RNA-N, mean % of microbial N	14.7 ± 0.2	11.1 ± 0.1	10.8 ± 0.4	14.5 ± 0.2	14.8 ± 0.2	10.4 ± 0.2	13.8 ± 0.2	14.5 ± 0.3
DNA-N, mean % of microbial N	2.6 ± 0.1	4.1 ± 0.1	3.4 ± 0.3	3.6 ± 0.1	3.1 ± 0.03	2.9 ± 0.03	2.2 ± 0.2	3.9 ± 0.3
Total polynucleotide N, mean % of microbial N	17.3 ± 0.2	15.2 ± 0.2	14.0 ± 0.3	18.2 ± 0.2	17.9 ± 0.2	13.3 ± 0.2	16.0 ± 0.2	18.4 ± 0.3
Mean of total ingesta N, mg/10 ml. ingesta	—	—	39.9	—	—	—	32.2	34.6
Range and mean of microbial N, % of total ingesta nitrogen	—	—	41.1-51.6	—	—	—	56.9-70.0	60.8-72.5
Range and mean of polynucleotide N, % of total ingesta nitrogen	—	—	46.5	—	—	—	64.3	67.5
	—	—	5.0-7.6	—	—	—	8.4-11.8	10.5-13.3
	—	—	6.8	—	—	—	10.4	11.9

\* Urea, 37.7; starch, 62.3. † Zein, 100. ‡ Zein, 50; urea, 17.7; starch, 32.3. § Urea, 31.4; methionine hydroxy analogue, 5.0; isoleucine, 18.0; starch, 45.6. ¶ Urea, 21.0; methionine hydroxy analogue, 5.0; DL-tryptophan, 3.0; L-lysine monohydrochloride, 6.0; isoleucine, 18; DL-valine, 10; and starch, 37.0. ±, Standard error of mean.

Blaxter<sup>8</sup> has summarized data indicating that ruminants, as compared with monogastric animals of similar body-weight, excrete a greater (three- to six-fold) daily quantity of purine nitrogen. Whereas daily purine excretion remains essentially constant for the fed and fasted monogastric<sup>9</sup> and suckling calf<sup>10</sup>, it decreases by approximately 50 per cent in the mature ruminant<sup>11</sup> on fasting—thereby indicating an exogenous source of purine excretion by the fed ruminant at least equal to that of endogenous origin. Blaxter and Martin<sup>12</sup> reported experiments in which the intraruminal infusion of casein, free of purine and pyrimidine bases, increased allantoin and hippuric acid excretion when compared with abomasal infusion of identical quantities of casein. These observations are consistent with an extensive conversion of dietary nitrogen to rumen microbial polynucleotides and their subsequent digestion in and absorption from the small intestines, thereby providing a large exogenous source of innutritious purines which are excreted.

Such an intra-ruminal conversion of dietary nitrogen to innutritious purine nitrogen may also partially explain the more efficient utilization of the readily degraded casein for nitrogen balance<sup>13</sup> and weight gains when it is either heat-treated to resist microbial degradation or administered into the alimentary canal posterior to the rumen. Several investigators have determined the biological value (biological value as conventionally used here represents the proportion of nitrogen absorbed from the digestive tract which is utilized for maintenance and growth purposes, that is, retained) of rumen microbial preparations when fed to monogastric animals and found it to be quite high (80 (ref. 14)). Considering the appreciable content of unnutritious purine nitrogen likely to have been present in such microbial preparations, it appears that the biological value of rumen microbial true protein must have been considerably higher than that of total microbial nitrogen. Such a diluting effect by unnutritious purine nitrogen formed from dietary nitrogen offers an explanation for the lower biological value of such proteins as whole egg when fed to the ruminant. Whole egg protein has a biological value close to 100 when fed to growing rats. Several investigators<sup>15,16</sup> have

determined the biological value of whole egg protein to be 80-86.7 for the growing lamb.

There is little doubt that the extensive protein anabolic potential of rumen micro-organisms is indeed a beneficial process to the ruminant's net nitrogen utilization under its usual dietary regime. Such anabolic capabilities permit the ruminant to utilize a wide variety of dietary nitrogenous compounds with an efficiency which varies within a relatively small range<sup>15-17</sup>. It also permits a "protein regeneration cycle"<sup>18</sup> for blood urea<sup>18</sup> and ammonia resorbed into the rumen. It has been the intent of the foregoing discussion to point out that part of the cost of this beneficial rumen microbial protein anabolism is a concurrent conversion of potentially nutritious dietary nitrogen to rumen microbial purine nitrogen which, on subsequent digestion and absorption, is innutritious to the ruminant's tissues and is the origin of the comparatively higher purine excretion of ruminants as compared with monogastric animals.

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## STOCHASTIC CONCEPTS IN CLINICAL RED CELL SURVIVAL STUDIES

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**R**ADIOACTIVELY labelled red blood cells have to-day become an important tool in the investigation of cell survival in circulating blood. Two basically different methods of labelling can be recognized: (1)

labelling of circulating red blood cells; (2) labelling of red blood cell precursors. The following is essentially confined to (1).

For labelling according to (1),  $^{51}\text{CrO}_4^{3-}$  and  $\text{DF}^{32}\text{P}$  (di-isopropylfluorophosphonate) are to-day the most frequently used substances. The data one generally gets

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from such experiments consist of the number of labelled circulating red blood cells as a function of time, that is, the survival curve. However, while much work is frequently devoted to the experimental technique, the theoretical analysis of the data is comparatively confined and primitive. Commonly the only form of 'calculation' performed consists of the determination of the 'half-life'. This parameter, usually denoted by  $t_1$ , has hitherto not been given any generally physico-biological significance and its commonly accepted clinical significance is merely phenomenological and is due to the parameter having been presented together with clinical data for such a long time.

In the literature, the half-life is often calculated from semilogarithmic plots of survival curves; this gives the impression that the parameter originates from an analogy with certain physicochemical processes, for example, radioactive decay. This analogy is misleading and the use of the term 't-fifty' (with the corresponding notation  $t_{50}$ ) would perhaps prevent some confusion and would also be consistent with the unquestionable significance of the parameter: the time after which 50 per cent of the members of the population originally present have died (cf.  $LD_{50}$ ). But the physico-biological significance of the 'half-life' is, of course, still obscure.

In this respect the concept of 'life span' is preferable: knowledge of the life-span distribution over the population of circulating red blood cells would doubtless give some insight into what could be called the 'physiological state' of the system considered. A number of papers discuss cell survival curves on the basis of such a concept<sup>1-3</sup> (for further references, see the review by Berlin *et al.*<sup>4</sup>), but, as explicitly stated by Dornhorst<sup>3</sup>, it is almost impossible to determine the life-span distribution from a survival curve alone. Some knowledge in addition to the survival curve is thus necessary and, since this knowledge must be physiological in character, the question arises whether the conception of life-span has a physico-biological significance that will provide an efficient basis for intuitive reasoning in physiological terms.

From this point of view the 'death probability of a cell' is a more satisfactory concept: considered as a function of the cell age, it is more directly connected with the destruction mechanism than is the life-span distribution. Now, as shown elsewhere<sup>5</sup>, what has commonly been assumed *ad hoc* about the life-span (for example, that it shows a Gaussian distribution) is not consistent with what intuitively seems natural when the death-probability function is considered. So far, the theoretical work indicates that the interpretation of survival data should be performed on the basis of the death-probability function rather than of the life-span distribution. The final choice between the two entities, however, depends to a certain extent on which one is most easily calculated from the results available.

As already stated, the life-span distribution cannot be determined from the survival curve only, and the same is true also for the death probability as a function of the age of the cell. However, a knowledge of the mean value of the corresponding variables even without the complete distribution should be valuable, and in a note published in 1946, Mills<sup>6</sup> has claimed that 'mean life-span' can, in fact, be determined from the initial slope of the survival curve: a statement which implicitly follows from the works by Callender *et al.*<sup>1</sup> and Dornhorst<sup>3</sup>. These authors, however, all include the general assumption of steady-state, and under that assumption the mean life-span is just the inverse of the 'mean death probability'<sup>5</sup>; consequently, under steady-state conditions, the two mean values are equally easy to determine.

But to assume steady state puts a strong restriction on to the system, and the assumption can scarcely be realistic in clinical work. The question then is whether the initial slope of a survival curve has the same significance also under non-steady-state conditions, and, as recent work

has shown (Bergner, in preparation), a definite answer does exist: if the cells are irreversibly labelled independently of their age, the initial slope of a survival curve always determines the mean death probability (the value that this parameter has at the time when the cells are labelled). Or, in more technical terms, if the labelling is proper (according to the preceding sentence) and the number of labelled cells is plotted against time, the initial slope of the curve, when divided by the initial number of labelled cells, is equal to the mean death probability at the time of labelling, independently of whether or not the system is stationary.

In the light of this recent result, let us consider the following equation, derived by Uhlhorn (unpublished work), which gives the general relation between the mean life-span and the mean death probability:

$$\bar{l}(t) = \frac{1}{P(t)} \left( 1 - \frac{d[n(t)\bar{a}(t)]/dt}{n(t)} \right)$$

where  $\bar{l}(t)$  is the mean life-span at the time  $t$ ,  $P(t)$  the mean death probability at the time  $t$ ,  $n(t)$  the total number of circulating red blood cells at the time  $t$ , and  $\bar{a}(t)$  the mean age at the time  $t$  of the circulating red blood cells. In this equation the time derivative becomes different from zero when the system is not stationary, and the relationship might then not only be complicated but also quite undetermined; at present it does not seem possible to determine the derivative experimentally. Consequently, the equation indicates that, under non-steady-state conditions, the mean life-span is not so easily calculated by existing theoretical tools as is the mean death probability, the latter being determinable under quite general conditions.

The theoretical analysis thus supports the suggestion by Garby<sup>7</sup> that the initial slope is a fundamental parameter in clinical practice, not only for theoretical reasons but also as a consequence of the simple fact that in clinical work short-term investigations are preferable to long-term ones. However, many difficulties are associated with initial slope measurements. At present DFP appears to be the only labelling substance that might satisfy the basic requirements, but still more investigations must be carried out to evaluate the details of the labelling mechanism in normal and non-normal systems. Moreover, certain mathematical difficulties are associated with the estimation of initial slopes in general; some of those problems have been investigated in other contexts<sup>8</sup> and the results there might also be applicable here.

By making use of the idea that the red blood cells possess a definite death probability, which is a function of the age of the cell only, it has thus been possible to ascribe a general and, as it seems, practically useful significance to the initial slope of cell survival curves. For a more detailed interpretation of survival curves, however, the form of this death-probability function must be known to a certain extent; this is made necessary by the foregoing fact that the death-probability function cannot be estimated from the survival curve alone. Some knowledge about the death-probability function does already exist, mainly on the basis of precursor labelling (for example, <sup>14</sup>C-glycine)<sup>4</sup>, but more information is needed, especially about certain disease states. Cohort methods, according to the principles recently introduced by Cline *et al.*<sup>9</sup>, might prove useful here.

But, in order to make possible an efficient use of old and new experimental techniques and data, a more sophisticated approach is necessary rather than that creating such a relatively primitive concept as  $t_1$ . The degree of sophistication necessary might be illustrated as follows. Distinction has here been made between the life-span distribution function and the death-probability function; the former gives the probability that a newborn cell will die at a certain age, while the latter, referring



to a randomly chosen cell, gives the probability that the cell will die within the time unit, under the condition of the cell's age. The distinction is evident and significant to those who are familiar with stochastic methodology, but it might be difficult for others to grasp. However, the distinction is fundamental physiologically, showing that the death probability is a more basic concept than is the life-span distribution. To mention but two motives for the last statement: in a non-stationary system the life-span distribution can only occasionally be determined even when the destruction mechanism is known, whereas this very mechanism immediately defines the death-probability function, which also can be directly observed by cohort methods. The second motive has a more direct clinical significance: together with an estimate of the

total number of circulating cells, the mean death probability always determines the total destruction rate (the total number of cells dying per unit of time) while this rate can be determined from the mean life-span only when the system is stationary.

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## REVERSIBLE CHANGES IN SHAPE OF RED CELLS IN ELECTRICAL FIELDS

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IN the course of our investigations on the mechanical properties of the membrane of the human red cell by use of the micromanipulator on single cells<sup>1-3</sup>, we inserted polarizable stainless-steel microelectrodes into a hanging drop of isotonic saline containing the cells. When a voltage difference of approximately 2 V or less was applied to the electrodes, some remarkable shape-changes were seen that were completely reversible. Before, the cells were of the normal biconcave disk shape, but when the voltage was applied shape-changes occurred near the anode which were completely different from those near the cathode. Higher voltages lead to the liberation of gas near the electrodes which disturbed the field optically.

Those cells that were near the cathode assumed the shape of crenated spheres (Fig. 1), while those near the anode remained smooth in outline but cup-shaped when seen edge on, with a region of high optical density (a 'refractile body') in the 'dimple' region. The transformation took only a few seconds in the case of those cells which were close to the electrodes, and longer for those farther away. Reversal of the voltage gradient reversed the transformation of individual cells. For lower voltages, the time required for the transformation was greater. On removal of the voltage gradient the cells return to their normal biconcave shape. The crenated and cup-shaped cells could be seen clearly in the area of current flow, while cells outside this showed very little or no change. Some cells, usually those showing micro-crenation, did not change on reversing the current. Fig. 2 shows a magnified picture of a group of cells between the electrodes, in which the changes in individual cells could be seen when the polarity of that electrode was reversed.

One observation made in these experiments seemed to us to be of importance. If a single cell undergoing crenation was observed, the emergence of a particular 'bump' or 'pimple' of crenation could be seen, which disappeared when the voltage was removed; this occurred repeatedly at the same place on the membrane of that cell. Furchgott<sup>4</sup> and Ponder<sup>5</sup> also made this observation. This would strongly suggest that crenation does not represent a random sort of folding of the membrane, but

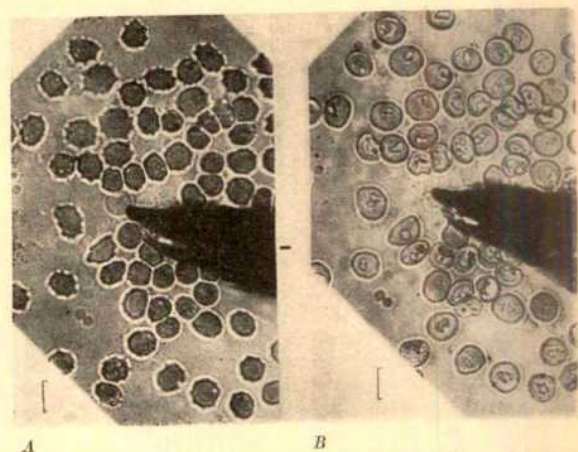


Fig. 1. Shape changes in red cells close to the same electrode when it changes from cathode (A) to anode (B). The voltage difference was about 2 V. The scale indicates 10  $\mu$ . With no voltage, most of the cells in the field returned to normal biconcave shape.

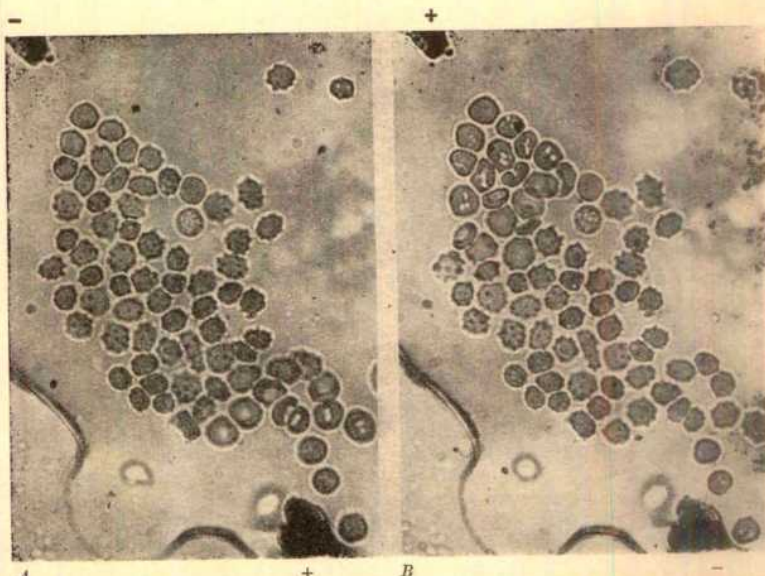


Fig. 2. The field between the two electrodes, showing changes in individual cells when the polarity was reversed. Cells outside the main potency of the current, or in the centre of the field, change very little.



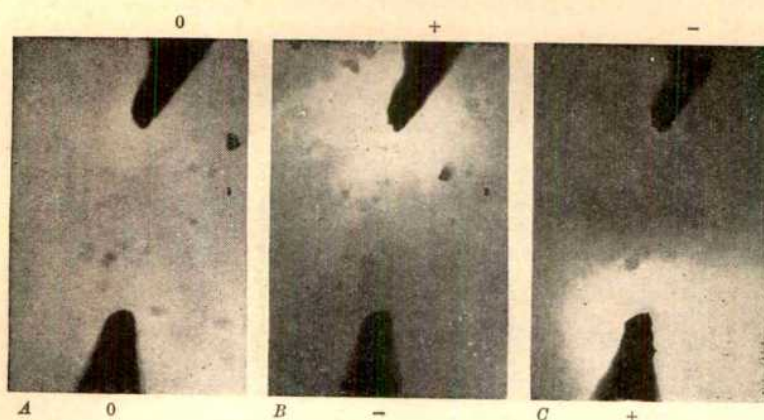


Fig. 3. Demonstrating the violent change in pH occurring between the electrodes when the voltage is applied. This, rather than electrical forces, may be the primary factor

that a definite mosaic of discontinuities exists over the membrane of the normal cell.

It is not at all likely that the effects are primarily due to the electrostatic field. There is a considerable density of current which produces a marked local change of pH, with a very high pH near the cathode, and a very low pH near the anode. This was demonstrated by adding a mixture of two indicators, bromthymol blue and phenocresol purple (yellow below pH 6, purple above pH 8), to the hanging drop. The areas of pH change could easily be observed by the colour changes, but were less easily photographed (Fig. 3). Changes of pH produced without any applied electrical field also lead to crenation at high pH, and smooth cup-shaped cells at low pH. This was demonstrated by spraying the cells with isotonic saline which had been made very alkaline with sodium hydroxide, or made very acid with hydrochloric acid. These changes in shape were also demonstrated by Furchgott<sup>4</sup>, but little was made of the fact that the shape changes were completely different at the 'two' levels of pH. In the case of the low pH the unique region of concavity (dimple) was preserved (as the cup region), but at the high pH the dimple region was lost and the crenated cells were spherically symmetrical.

It was questionable whether the presence of the normal concentrations of haemoglobin was required for this phenomenon. In order to answer this, we investigated the phenomenon in red cell ghosts. Since phase-contrast microscopy is required in this case, we have not yet succeeded in carrying out the experiments with electrical

fields, and an apparatus will have to be specially designed to avoid optical disturbance. However, the changes in high and low pH (which are apparently identical with those in the electrical field for normal cells) have been investigated in ghosts. Ghost cells became extremely thin at high pH rather than crenated like normal cells. At low pH, ghost cells showed no changes comparable with those of normal cells (they are not cup-shaped, with region of high optical density). We concluded that the cup-shape phenomenon is dependent on the presence of haemoglobin, and that crenation is considerably modified in the absence of high concentration of haemoglobin.

Whatever the mechanism and interpretation of this phenomenon, the use of the electrodes provides a convenient way of producing perfectly reversible changes in the shape of the red cell. Such an investigation may help to elucidate the difficult problem of forces responsible for the normal biconcave shape of the red cell. Biophysical analysis of this has led us to search for an external force, such as a mutual attraction between the membranes in the dimple region.

From these and other observations of the process of crenation, we are forced to accept the view that the membrane of the red cell cannot be uniform in its own plane, but has a mosaic pattern of charges, or of active groups, fairly regularly spaced over the surface, at intervals of 0.5–1.0  $\mu$ . Possibly the forces of interaction between these centres of charge may underlie the force of attraction between the membranes, which we suggest is responsible for the biconcave shape and for the positive hydrostatic pressure within the cell<sup>1</sup>. The same reasoning may hold for the obvious attraction of the membrane of one red cell for that of another which shows itself in the tendency to form rouleaux. Experiments with red cells treated with the enzyme neuraminidase, which removes the surface charges, are being pursued to supply further evidence on this view.

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## BIOCHEMICAL DEFECT CAUSING CONGENITAL GOITRE IN SHEEP

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**C**ONGENITAL goitre in man, which is associated with defects in the biosynthesis of thyroid hormone, has recently aroused much interest, and a number of these cases have been described<sup>1</sup>. In three of the congenital disorders a definite biochemical lesion has been demonstrated in the goitrous gland<sup>2-4</sup>, but the other categories of congenital defects are heterogeneous groups.

The recent widespread occurrence of goitre in South Australian Merino lambs born in areas with no previous history of thyroid abnormality has led to an investigation of the underlying causes. The data presented in this article are restricted to a biochemical investigation of the goitre

tissue, and do not deal with results for the parallel work on field occurrence, pathology and genetic origin of the goitre.

The goitres were obtained from 1–3-year-old Merino rams and ewes. The sheep were held in partially roofed pens and fed on hay chaff and oats. Goitrous tissue excised under aseptic surgical conditions was immediately sliced into strips and chilled in ice. The goitres varied in weight from 13 g to more than 100 g for the two lobes. The general appearance of the goitre on surgical exposure was of a firm elastic structure of deep red colour with an extremely good blood supply. In some cases the lobes were fused into a single mass, but in the majority the two lobes were of normal location and shape. Normal sheep thyroid glands were obtained immediately after

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slaughter at the abattoirs, and placed in ice. Until it was required for analysis the thyroid tissue was stored at  $-15^{\circ}\text{C}$ .

The iodinated amino-acid content of normal and goitrous thyroid tissue was determined after enzymatic hydrolysis of homogenates, followed by ion exchange column separation of amino-acids<sup>6</sup>. The column eluate was bulked and evaporated to dryness in a rotary film evaporator, and the solid residue re-dissolved in 2 N ammonium hydroxide 1 part: ethanol 3 parts. Portions of the re-dissolved amino-acid extract were chromatographed on Whatman No. 1 paper using a solvent of butanol 2 parts: 2 N ammonium hydroxide 1 part: ethanol 1 part. The chromatograms were cut up and the iodinated amino-acid content determined<sup>6</sup>. The results of this analysis are shown in Table 1.

Table 1. IODINATED AMINO-ACIDS LIBERATED BY ENZYMATIC HYDROLYSIS FROM HOMOGENATES OF GOITROUS AND NORMAL THYROID GLANDS OF SHEEP ( $\mu\text{g/g}$  FRESH WT)

Sample	Moniodotyrosine	Diiodotyrosine	Thyroxine
Normal	60	215	58
Goitre Y87	15.1	23.2	0.4
Goitre Y135	2.8	23.7	2.5
Goitre Y190		7.1	0.2
Goitre WL133	9.7	10.0	0.0
Goitre L1	5.8	32.9	2.0

\* Total moniodotyrosine and diiodotyrosine.

Although the iodinated amino-acid content of the goitrous thyroids varies considerably, it is clear that they contain about one-tenth that of the normal. Diiodotyrosine was in the largest proportion, as in the normal gland.

The protein patterns of normal and abnormal thyroid tissue were examined<sup>7</sup> in a double sector cell run on a Spinco model E analytical centrifuge, and the mobility of the protein components measured. Extracts of the goitrous thyroids gave a characteristic ultracentrifuge pattern (Fig. 1a), with one peak of  $S_{20w}$  3.2–7.6, and a very small peak of  $S_{20w}$  50.4 and 55.9 in two out of 12 glands. Normal thyroid extracts showed three consistent peaks in the ultracentrifuge (Fig. 1b) at  $S_{20w}$  3.3–5.0,  $S_{20w}$  18.5–19.1 and  $S_{20w}$  27.6–27.9. The thyroglobulin peak at  $S_{20w}$  18.5–19.1 in the normal thyroid extract contains 72.7 per cent of the total protein, as measured from the peak area corrected for radial dilution. It is clear that there is no detectable thyroglobulin in the goitrous glands of untreated sheep.

The effects of thyroxine therapy on the accumulation of thyroglobulin in the goitres was investigated in three sheep injected intramuscularly with 0.1, 1.0 and 2.0 mg of thyroxine daily for 40 days. The two higher doses of thyroxine were approximately three and six times the daily requirement. The protein-bound iodide level of the serum of the animal given 1 mg/day increased from 3.9  $\mu\text{g}/100\text{ ml.}$  to 17.7  $\mu\text{g}/100\text{ ml.}$  in 17 days. Thyroglobulin was not detected in these treated goitres by the ultracentrifuge method (Fig. 1c). It therefore appears likely that the synthesis of thyroglobulin was impaired in these

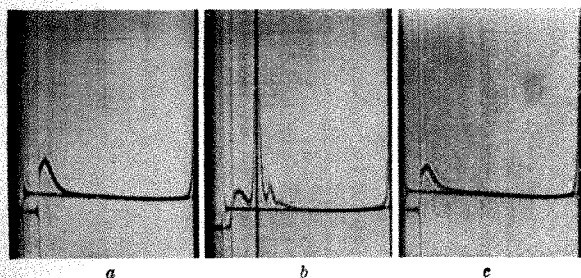


Fig. 1. Ultracentrifuge patterns obtained from 0.9 per cent sodium chloride extracts of thyroid tissue. a, Goitrous thyroid extract, 0.84 per cent protein; 28 min at 37,000 r.p.m. Spinco E, double-sector cell. b, Normal thyroid extract 0.99 per cent protein; 28 min at 36,960 r.p.m. Spinco E, double-sector cell. c, Goitrous thyroid extract from sheep given 1 mg thyroxine/day for 40 days, 0.96 per cent protein; 29 min at 35,000 r.p.m., Spinco E, double-sector cell.

goitres, rather than rapid proteolysis preventing the appearance of any thyroglobulin.

The synthesis of thyroglobulin in normal and goitrous thyroid glands was examined by following the incorporation of  $^{14}\text{C}$ -proline and  $^{14}\text{C}$ -leucine into thyroid tissue slices. Fresh thyroid tissue chilled in ice was chopped to 0.5 mm slices on a Mickle (Gomshall, Surrey) tissue chopper and 0.2-g portions were quickly transferred to 1-ml. portions of chilled Tyrode's solution (pH 7.4). Duplicate portions contained 20  $\mu\text{g}$  cycloheximide ('Actidione', Upjohn Corp.), which is an inhibitor of protein synthesis<sup>8</sup>. The tissues were incubated with either 0.5  $\mu\text{C}$ . or 0.25  $\mu\text{C}$ .  $^{14}\text{C}$ -proline or  $^{14}\text{C}$ -leucine (Schwarz BioResearch Inc.) 100 mc./mmole. Duplicate portions of normal and goitrous slices were immediately frozen in dry ice-acetone mixture. The remaining portions were incubated at  $37.5^{\circ}\text{C}$  in a water bath for up to 3 h and at the end of incubation the material was frozen and stored at  $-15^{\circ}\text{C}$ .

The incubated slices were thoroughly homogenized with 3 M 'Ballotini' beads using a motor-driven 'Teflon' pestle, while partially frozen. Following the addition of 2-ml. proline solution (1 mg/ml.), cellular debris and larger particles were sedimented at 20,000g in a Spinco model L preparative ultracentrifuge.

The supernatant material was fractionated with ammonium sulphate as shown in the individual experiments. The protein fractions were redissolved in water, and precipitated with trichloroacetic acid at 5 per cent w/v. The resulting precipitate was resuspended in water and centrifuged at 2,000g. The precipitate was resuspended in 1 ml. of water and 0.5 ml. of it was transferred to 10 ml. 'Diotol' liquid scintillator fluid<sup>9</sup>. After thorough dispersion of the protein and 24-h storage at room temperature in the dark, the radioactivity was determined on an Ekco liquid scintillation counter and scaler (models N664A, N610A).

The incorporation of 0.5  $\mu\text{C}$ .  $^{14}\text{C}$ -proline into the protein fraction precipitated by 50 per cent saturated ammonium sulphate is shown in Fig. 2. The incorporation of the tracer (1.8 per cent), which was linear over 3 h in both normal and goitre preparations, was largely inhibited by 'Actidione'.

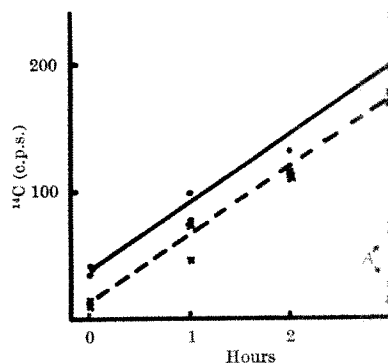


Fig. 2. Incorporation of  $^{14}\text{C}$ -proline into soluble proteins of normal (●) and goitrous (×) thyroid slices. 0.2 g of 0.5 mm thick slices incubated with 0.5  $\mu\text{C}$ .  $^{14}\text{C}$ -proline at  $37.5^{\circ}\text{C}$  for up to 3 h. Duplicate samples incubated with 20  $\mu\text{g}$  'Actidione' (cycloheximide) (Δ). Radioactivity measured in 50 per cent saturated ammonium sulphate precipitate from 20,000g supernatant of a slice homogenate.

Further experiments were carried out in which 0.25 and 0.5  $\mu\text{C}$ .  $^{14}\text{C}$ -proline and  $^{14}\text{C}$ -leucine were incubated with the thyroid slices. The supernatant material was again fractionated with ammonium sulphate. Precipitates were obtained at 0–35 and 35–45 per cent saturation, and the fraction soluble at 45 per cent saturation retained. All fractions were finally precipitated with trichloroacetic acid to 5 per cent. The results of one of these experiments is shown in Fig. 3.

The incorporation of  $^{14}\text{C}$ -amino-acids into the 35–45 per cent ammonium sulphate fraction, which is largely thyroglobulin, is greatly reduced in the goitrous slices.

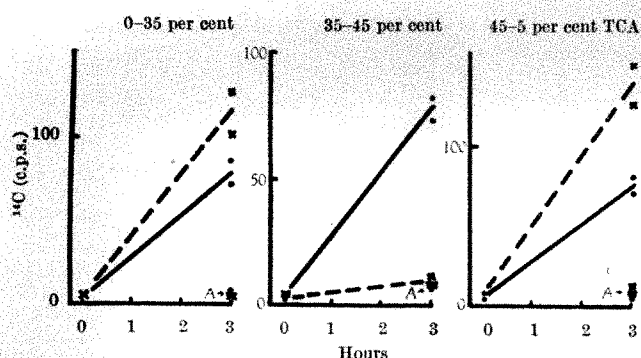


Fig. 3. Incorporation of  $^{14}\text{C}$ -leucine into soluble proteins of normal (●) and goitrous (x) thyroid slices. 0.2 g of 0.5 mm thick slices incubated with 0.25  $\mu\text{C}$   $^{14}\text{C}$ -leucine at  $37.5^\circ\text{C}$  for 3 h. Duplicate samples incubated with 20  $\mu\text{g}$  'Actidione' (cycloheximide) (A). Radioactivity measured in 20,000g supernatant fractions of a slice homogenate precipitating at 0-35 per cent saturated ammonium sulphate, 35-45 per cent saturated ammonium sulphate (thyroglobulin fraction) and 45 per cent saturated ammonium sulphate-5 per cent trichloroacetic acid.

This is in marked contrast to the incorporation of  $^{14}\text{C}$ -leucine into the other fractions which is higher in the goitrous than in the normal slices. Thyroxine therapy at 1 mg/day for 40 days to the goitrous animal repressed the incorporation of  $^{14}\text{C}$ -proline to approximately normal levels in the 0-35 per cent ammonium sulphate and 45 per cent ammonium sulphate-5 per cent trichloroacetic acid fractions (Fig. 4).

Recent investigations of thyroglobulin synthesis in pieces of normal thyroid tissue<sup>10</sup> have shown that the  $^{14}\text{C}$ -amino-acids appear in the follicular lumen as thyroglobulin after an incubation period of 4 h. During this time it is suggested that the uniodinated protein is synthesized, four large polypeptide units are aggregated, and the protein passes out of the cell. It is likely, therefore, that the results of 3-h incubation with  $^{14}\text{C}$ -amino-acids, as described here, indicate the rate of intracellular biosynthesis of thyroglobulin, which is relatively unaffected by proteolytic reactions in the follicular lumen.

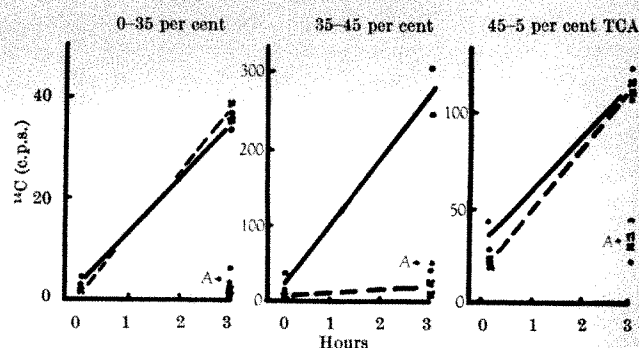


Fig. 4. Incorporation of  $^{14}\text{C}$ -proline into soluble proteins of normal (●) and goitrous (x) thyroid slices. The goitrous tissue was removed from a rat after 40 days therapy with 1 mg thyroxine/day. 0.2 g of 0.5 mm thick slices incubated with 0.5  $\mu\text{C}$   $^{14}\text{C}$ -proline at  $37.5^\circ\text{C}$  for 3 h. Otherwise as described for Fig. 3.

It is clear that the ultracentrifuge patterns and the results of  $^{14}\text{C}$ -amino-acid incorporation studies described in this article indicate a defect in the biosynthesis of thyroglobulin in the congenital goitre. This primary lesion is in turn responsible for the secondary effects of thyroid enlargement, elevated  $^{131}\text{I}$  uptake, low iodinated amino-acid content of the gland and high level of thyroid-stimulating hormone in the serum.

I thank Mr. C. Mulhearn and the South Australian Department of Agriculture for the experimental animals, and Dr. S. B. Wilson for the ultracentrifuge analysis.

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## RECOVERY FROM THE 'LETHAL' EFFECTS OF CROSS-LINKING ALKYLATION

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IT is well established that the ability of bacteria to survive to produce visible colonies after exposure to ultra-violet or ionizing radiation is markedly dependent on post-irradiation cultural conditions. In particular, incubation under otherwise sub-optimal conditions, as on minimal medium or at low temperatures, generally favours survival though the response may not be the same for different strains<sup>1</sup>. In the case of ultra-violet irradiation it is becoming clear that survival is enhanced in circumstances which permit metabolic repair of otherwise lethal primary lesions, that is the excision and replacement of DNA nucleotides which have suffered dimerization between adjacent thymine moieties<sup>2</sup>. Radiation-sensitive strains may lack the repair equipment.

That a comparable phenomenon may occur after alkylation is indicated by the work of Strauss<sup>3,4</sup> and of Strauss and Wahl<sup>5</sup>, who investigated the consequences of methylation of *Bacillus subtilis* transforming principle by the monofunctional alkylating agent methyl methane sulphonate. It is known from the work of Lawley and Brookes<sup>6</sup> that the most important site of reaction by this agent in DNA is N<sub>7</sub> of guanine with N<sub>3</sub> of adenine coming

a poor second; reaction at other sites is negligible at 'physiological' doses.

Bifunctional alkylating agents such as the di(2-chloro-ethyl) amines and sulphides (nitrogen and sulphur mustards) are enormously more nucleotoxic than their monofunctional analogues and there is now little doubt that this is due to their ability to establish covalent cross-links between the two strands of a duplex DNA<sup>7,8</sup>. The earliest published indication that the survival of bacteria exposed to these agents might also depend on post-treatment cultural conditions is to be found in a report by Harold and Ziporin<sup>9</sup>, who mention in a footnote that survival of *Escherichia coli* was higher on a minimal salts medium than on nutrient agar. (In 1955 Loveless and Miss Stock observed an apparent recovery of both ultra-violet- and nitrogen mustard-treated *E. coli* during overnight storage in phosphate buffer solution. True recovery appeared so unlikely at that time that the phenomenon was attributed to residual division and the work was not reported.) Harold and Ziporin also observed excretion of material absorbing at 260 mμ after mustard gas treatment even when the cells were suspended in



buffer solution. No such excretion occurred, however, from nitrogen mustard-treated cells. Shields<sup>10</sup> found that the survival of nitrogen mustard-treated *E. coli* K-12 may be considerably enhanced by a variety of post-treatment manipulations including the use of minimal media and what would normally be non-optimal incubation temperatures. Haynes (ref. 11 and personal communication) observed that the colony counts afforded by plating *E. coli* exposed *in perpetuo* to nitrogen mustard in phosphate buffer fell to a minimum (corresponding to the exhaustion of the reagent by hydrolysis) and thereafter rose again. This observation was not extended, but Patrick, Haynes and Uretz<sup>12</sup> have reported the recovery of nitrogen mustard-treated yeast during storage in buffer.

We have investigated the phenomenon of recovery in *E. coli* strains *B*, *B/r* and K-12 ( $\lambda$ ) treated with mustard gas (di(2-chloroethyl)sulphide, 'H'). *H* has one great advantage over a nitrogen mustard such as di(2-chloroethyl)methylamine ('HN 2') for such investigations in that it can be assumed to have reacted completely in 20 min at 37° C. The reaction of HN 2 may not be complete in under 3 h under comparable conditions<sup>13</sup> and thus one might search for evidence of recovery while inactivation continues. We also report some experiments concerned with spontaneous recovery of mustard-treated phage T 2 *in vitro*.

Cells in logarithmic phase in broth culture were centrifuged, washed and resuspended in M-9 buffer in stoppered tubes for treatment at 37° C. *H* was added as a 0.1 M solution in dry acetone and the tubes vigorously agitated during and after addition. After 2 min, sodium thiosulphate was added to give 2 per cent w/v and a further 5 min was allowed for the destruction of any available *H*. Controls were run with acetone alone. The treated cells were then diluted extensively ( $> 10^{-3}$ ) into M-9 buffer for storage at 37° C. In initial experiments care was taken that the concentration of dead cells was the same in each storage tube, where necessary by the addition of heat-killed cells, lest these should act as a source of nutrients for survivors. This was found not to be the case and the precaution was afterwards relaxed. Platings were done immediately on nutrient broth (Difco) and on minimal-salts (Davis's<sup>14</sup>) agar and at intervals thereafter up to about 24 h on broth agar. Samples were run over the surface of the agar without use of a contact spreader since the latter has been shown by Shields (personal communication) to result in a considerable reduction in the colony count afforded by cells treated

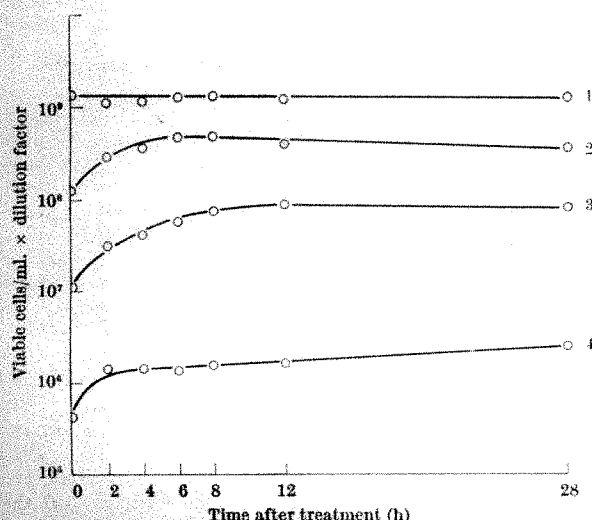


Fig. 1. Recovery pattern of *H*-treated *E. coli* *B* in phosphate buffer. 1, Control; 2,  $0.5 \times 10^{-4}$  M; 3,  $2.0 \times 10^{-4}$  M; 4,  $5.0 \times 10^{-4}$  M. All treatments for 2 min

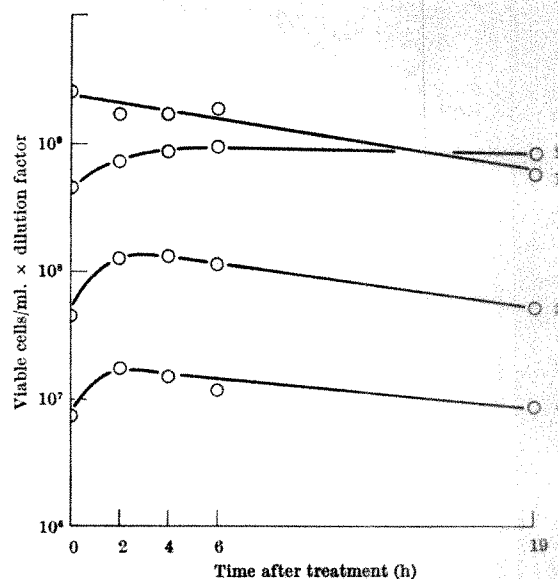


Fig. 2. Recovery pattern of *H*-treated *E. coli* *B/r* in phosphate buffer. 1, Control; 2,  $1.5 \times 10^{-4}$  M; 3,  $4.0 \times 10^{-4}$  M; 4,  $6.4 \times 10^{-4}$  M. All treatments for 2 min

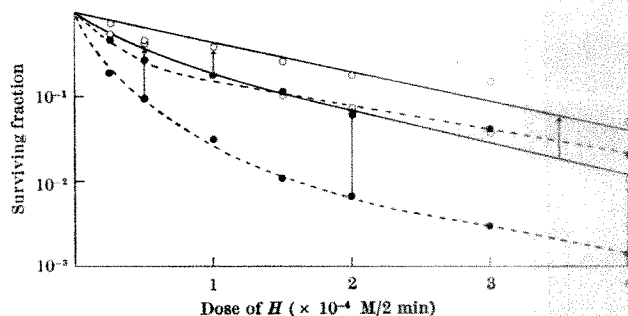


Fig. 3. Survival curves for *H*-treated *E. coli* strains *B* and *B/r* plated on nutrient broth and minimal medium. —●—, Strain *B*; lower curve, broth; upper curve, minimal. —○—, Strain *B/r*; lower curve, broth; upper curve, minimal

with alkylating agents. Plates were conveyed to the incubator immediately after spreading since keeping them at low temperature for a time enhanced the survival on broth of both *B* and *B/r*.

Untreated cells of strain *B* proved wholly stable in buffer at 37° C, showing no significant fluctuation in viable count up to 28 h. *H*-treated cells gave steadily rising viable counts which took longer to reach a maximum the more severe the treatment. Untreated *B/r* appeared to be less stable, although the counts were not wholly reliable. As with strain *B*, treated cells showed an appreciable recovery at all doses, but the erratic control prevented any conclusion regarding the positions of the maxima. Figs. 1 and 2 depict the viable counts adjusted only for the plating dilutions.

Fig. 3 presents the survival curves obtained by plating immediately after the thiosulphate 'decontamination' on broth and minimal agar. The vertical arrows indicate the recovery observed on storage in buffer; in the case of strain *B* these all refer to 12-h storage, in the case of *B/r* the maximum observed counts. A third 'recovery point' for *B/r* lies off the figure but it falls very close to the extrapolated curve for survival on minimal medium.

We have performed a few auxiliary experiments, comparable with the foregoing but using the mono-functional sulphur mustard 2-chloroethyl 2'-hydroxyethyl sulphide ('half-*H*'). Unfortunately, at the considerably higher stoichiometric doses that it is necessary

to use with monofunctional compounds to effect a degree of primary inactivation comparable with that achieved with their bifunctional analogues, the lethal effects of spontaneous depurination<sup>15</sup> must become a significant factor during post-treatment storage. Thus, as with *HN 2*, one is seeking evidence of recovery against a background of continuing inactivation but in this case one which persists longer. With a treatment leaving about 5 per cent initial survivors a three-fold increase in the viable count of strain *B* was observed during 6 h. When the initial survival was reduced to 0.1 per cent the subsequent counts remained constant. With *B/r*, which requires higher doses than *B* for equal degrees of inactivation, the counts either remained constant (10 per cent survival) or declined (0.2 per cent survival). This is the sort of result one would expect if recovery were competing with a secondary inactivation effect from which no recovery is possible.

No recovery of *T 2* monocomplexes as plaque-forming units was observed following treatment with *H* to any level of survival. *H* is an extremely good inducing agent of phage  $\lambda$  in *E. coli* strain *K-12* ( $\lambda$ ) (ref. 16). We have treated *K-12* ( $\lambda$ ) at optimal inducing doses and then followed the plaque (induced cell) and colony (viable cell) counts afforded by the treated culture during storage in buffer. The plaque counts fell rapidly, reducing to 1 per cent in 1.5 h. However, after a transient increase (up to four-fold during the first 30 min), the viable counts also fell away. Thus it remains doubtful whether a recovery from the inducing lesion has been observed.

Apart from any considerations of metabolic recovery from the effects of cross-linking alkylation, there is reason to suppose that recovery might occur spontaneously in some circumstances. Phage *T 2* retains infectivity following alkylation by *HN 2* if only one arm of each molecule of the compound is allowed to react with the phage<sup>13</sup>. Brookes and Lawley<sup>17</sup> present results suggesting that *T 2* may also suffer a modicum of base release without loss of infectivity; this suggestion is also implicit in the upward convexity of the survival curves obtained for monofunctionally alkylated *T 2* when plating follows a period of post-treatment incubation<sup>13,18</sup>. Thus a lethal covalent cross-link, established between the two strands of the DNA of a phage particle by *HN 2*, should be able to 'come undone' by base release at one end, leaving the particle only monofunctionally alkylated and carrying an apurinic site. If neither of these conditions is sufficient to inactivate, infectivity should be restored.

We have treated preparations of phage *T 2* with *HN 2* at doses estimated to be equivalent to 1, 2 and 4 phage-lethal 'hits' when sufficient time (3 h) has been allowed for full second-arm reaction (compare ref. 13). After the initial treatment, occupying 2 min, the phage was diluted extensively into *M-9* buffer containing 15 per cent v/v ethanol and a little dissolved gelatine, a medium in which, in other experiments, *T 2* had proved exceptionally stable. Duplicate controls and treatments were run and phage assays carried out at 3 h after treatment, and daily for 3 days thereafter in triplicate. The results are summarized in Table 1, which indicates the proportions of infective centres surviving *HN 2* relative to controls at these times and the mean lethal hit values indicated by these proportions at 3 h (effective end of treatment) and at 19 h ( $\equiv$  maximum of apparent recovery).

Table 1. CHANGES IN INFECTIVE TITRE OF *HN 2*-TREATED PHAGE RELATIVE TO UNTREATED PHAGE DURING POST-TREATMENT STORAGE

Dose (mg/ml/ 2 min)	Mean lethal hits at 3 h	3 h	Infective fraction				Mean lethal hits at 19 h
			19 h	45 h	69 h		
0.1	1	1.3	0.28	0.33	0.34	0.34	1.1
	2	1.2	0.29	0.33	0.31	0.32	1.1
0.2	1	2.2	0.11	0.18	0.12	0.14	1.7
	2	1.9	0.15	0.26	0.14	0.18	1.3
0.4	1	4.0	0.018	0.038	0.025	0.026	3.25
	2	3.9	0.019	0.035	0.024	0.028	3.35

Phage in the control tubes was not so stable as one had hoped, falling to nearly 1/4 of the initial titre at 69 h. However, the absolute counts for the 0.4 mg treatment were higher at 19 h than at 3 h. Moreover, very similar results were obtained with *H*-treated *T 2* whereas the surviving fractions of *T 2* given commensurate doses of ultra-violet or X-radiation behaved in the same way as controls.

**Discussion.** There seems little doubt that the recovery of bacteria from treatment by mustard gas is metabolically mediated. Our colleague Dr. D. Rosen has derived a mathematical expression to describe the spontaneous recovery expected from uncoupling of cross-links by base release as described by Brookes and Lawley, making the additional assumption that base release at both ends of such a link would be secondarily lethal. The degree of recovery observed (Table 1) falls considerably below the expectation from Rosen's equation, but it would be idle to speculate on the reasons for this at present. On the other hand, the recovery observed with bacteria is greater than could be accounted for in this way.

At this stage the most profitable supposition is that recovery occurs by excision of the damaged DNA regions followed by synthetic repair in a manner analogous to that envisaged by Setlow and Carrier<sup>2</sup> for recovery from ultra-violet lesions. However, in the interpretation of our results we are confronted with two additional difficulties. First, recovery appears to occur in the absence of an energy source and, secondly, we are concerned with a lesion involving both strands of the DNA at points set apart by but a single nucleotide.

It is to be expected that the excision of a damaged region would be accomplished exothermically and would therefore be fully able to proceed in buffer. On the other hand, it is unlikely that synthetic replacement could so proceed unless the cells, albeit washed and diluted, retain a reservoir of nutrients sufficient for this purpose. It could be that only the excision stage in fact occurs in the buffer but that on transfer to broth agar there is still a sufficient lag during which replacement can be accomplished prior to DNA replication. If this were the case it would need to be supposed that the longer lag occurring on minimal medium was sufficient to permit both stages of recovery to occur on the plate.

With regard to considerations of how the excision might come about, a number of possibilities come to mind involving either uni- or bi-lateral excision; if excision were unilateral, repair would not in theory be essential since monofunctional alkylation does not necessarily impair the replicative function. What does seem to be excluded is simultaneous bilateral excision since, although full pairing information would be retained, the DNA would surely lose its linear integrity. These considerations are best left until more experimental material is available; at the time of writing we learn privately from Drs. Brookes and Lawley that they have observed a marked loss of label from the DNA of <sup>35</sup>S mustard gas-treated *E. coli B/r* during post-treatment cultivation in full (glucose-containing) *M-9* medium.

One further consideration. We have asked ourselves whether there is any connexion between the ability to recover and the tendency to form filaments or not during cultivation in nutritive media after an exposure to mustard or radiation. Strain *B* forms long filaments in broth after biologically mild doses of *H* or *HN 2* (corresponding to an initial survival of 50 per cent or more); it does so even more markedly after full recovery in buffer. Strain *B/r* does not filament in either circumstance.

This work is preliminary in nature and indicates a number of lines for future experiment; in particular, attention should be directed to the influence of enzyme inhibition on recovery.

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## IMMUNOELECTROPHORETIC ANALYSIS OF *Ascaris suum* ANTIGENS

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MUCH is already known about the analysis of *Ascaris* antigens by agar double diffusion. Kent<sup>1</sup> has found in the aqueous extract of *Ascaris* at least four active antigens, using Ouchterlony method. Soulsby<sup>2</sup> has shown by double diffusion that each fraction is a mixture of antigenic components. Kagan *et al.*<sup>3</sup> find 4-11 bands by double diffusion agar in tube, and more recently Huntley and Moreland<sup>4</sup> found a similar number of bands. Kagan *et al.*<sup>5</sup> have shown that the number of immunodiffusion bands of each antigenic fraction varies according to the chosen physico-chemical method of fractionation.

However, immunoelectrophoretic investigation of all the components in *Ascaris* antigens are not, to our knowledge, available. This article describes an analysis of the proteins of seven antigen preparations of *Ascaris suum* as revealed by immunoelectrophoresis. It was the purpose of this work to determine the complexity of each antigen preparation, and to determine which antigenic components were the most active in serological diagnostic tests.

Besides perienteric fluid, two saline extracts of *Ascaris suum* have been studied. One antigen (Z) was obtained following the technique of Oliver-González<sup>6</sup>, and the other saline extract (ETSA) was prepared with a constant content of protein nitrogen. Four protein-polysaccharide antigens have also been examined. One (V) was obtained according to Fuller's method<sup>7</sup>, and the other three were prepared by the technique of Oliver-González<sup>6</sup> modified by Kagan<sup>8</sup>. Antigen X was deproteinized only by heat, antigen T was also subject to several physico-chemical procedures, and antigen Y was obtained by precipitation with acetone of the saline extract. Antigens prepared from proteins of host (pig serum) and intestinal contents have also been studied, to determine whether there were any antigenic similarities between the components of the *Ascaris* and those of the host.

Antisera of rabbits immunized with perienteric fluid or with pig serum, and infected orally with eggs of *Ascaris suum*, were used to develop the immunoelectrophoretic patterns. Immunoelectrophoresis was carried out according to the micromethod described by Scheidegger<sup>9</sup> with light modifications as previously described<sup>8</sup>.

The lines of precipitation produced when several antigen preparations are subject to electrophoresis and then reacted with immunoserum anti-ETSA are shown in Fig. 1. In the drawings each arc with the same number belongs to the same antigenic component. Immunoelectrophoresis of ETSA developed with anti-ETSA showed 20 different components. Fig. 2 is a photographic reproduction of this system.

Fig. 3 shows the bands obtained with the anti-perienteric fluid immunoserum. Fewer bands were found with all the antigen preparations even with homologous perienteric fluid. Apparently the perienteric fluid showed less power of stimulating antibodies than the

standardized ETSA antigen. The results of immunoelectrophoresis of antigen preparations of *Ascaris suum* developed against the serum of an experimentally infected rabbit and developed against a serum from a patient suffering from visceral larva migrans are shown in Fig. 4. ETSA antigen was that which showed more bands (six) with the anti-*Ascaris* serum of an infected rabbit. This antigen only showed 3 bands with the human diagnostic serum.

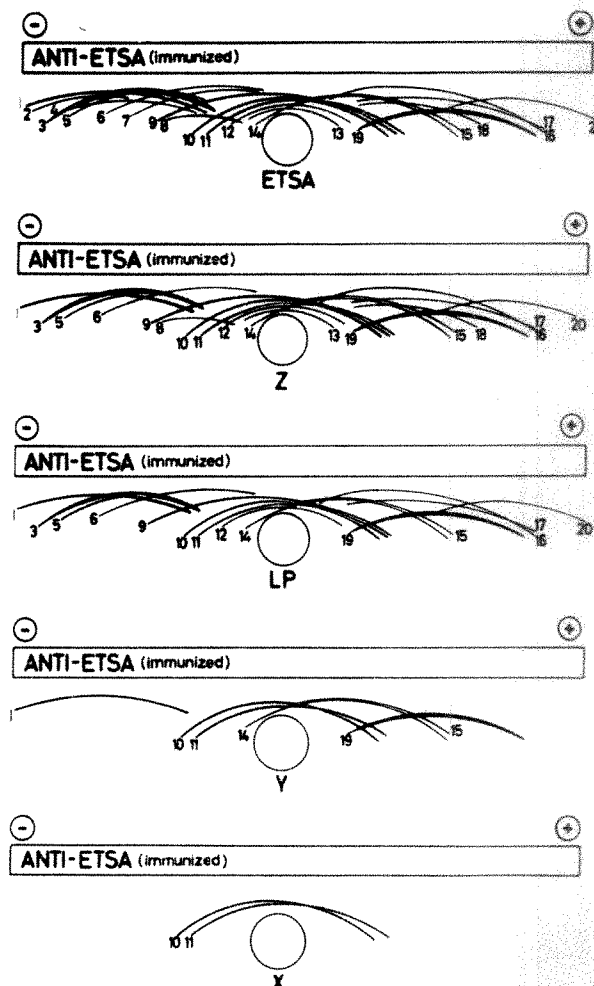


Fig. 1. Immunoelectrophoretic patterns of total saline extract (ETSA), total antigen of Oliver-González (Z), perienteric fluid (LP), and two protein-polysaccharide antigens of Oliver-González and Kagan (Y and X), developed by immunoserum anti-ETSA.



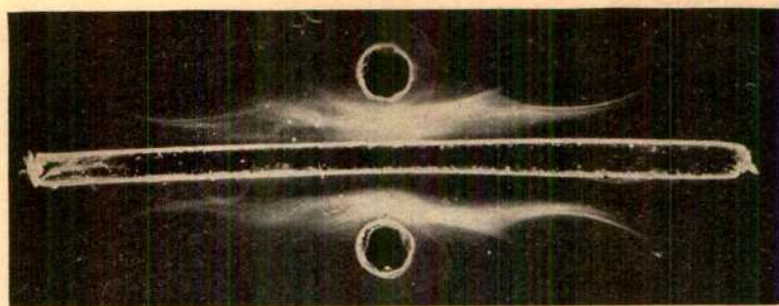


Fig. 2. Photographic illustration of immunoelectrophoretic pattern between antigen preparation ETSA and anti-ETSA immunoserum

Anti-*Ascaris* sera do not give reaction by Ouchterlony and immunoelectrophoresis with the protein components of the host (pig serum) nor with those of its intestinal content. Nor was there any reaction between the antigens of *Ascaris* with the serum anti-protein of the host. It was clear that the antigen preparations of *Ascaris* used had no protein components of the host nor of its intestinal content. In the immunoelectrophoresis of pig serum, 24 different components of the host were identified.

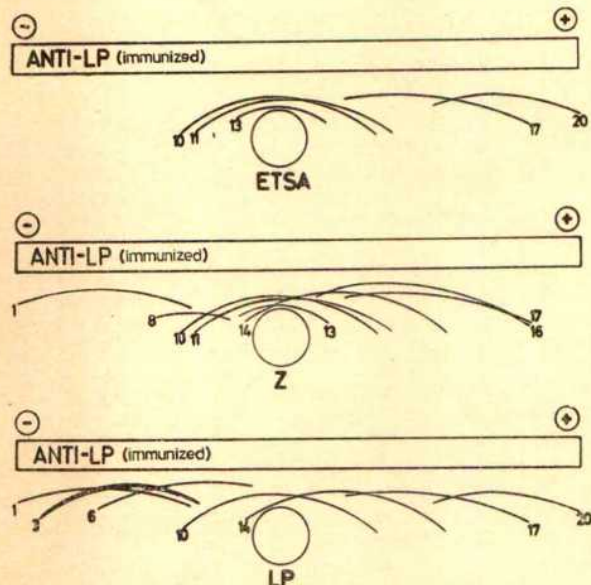


Fig. 3. Immunoelectrophoresis of antigen preparations ETSA, Z and LP developed by immunoserum anti-LP

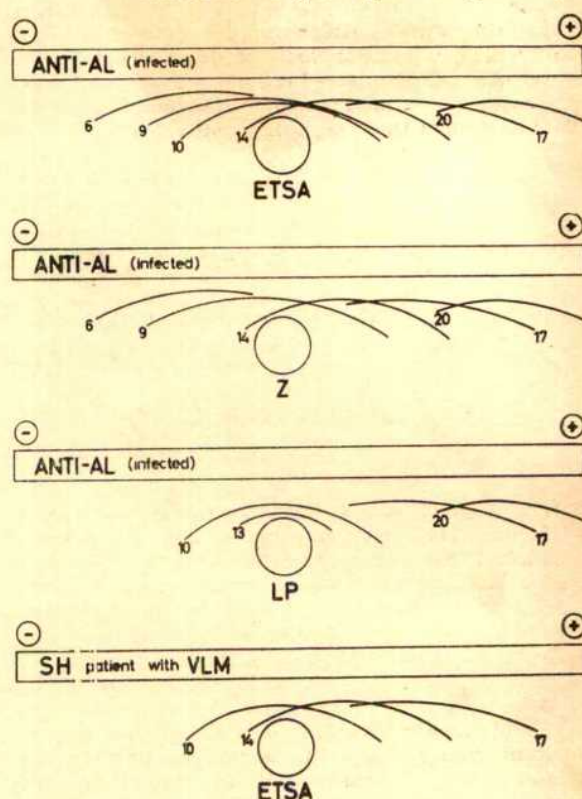


Fig. 4. Immunoelectrophoresis of antigen preparations developed against the serum of an experimentally infected rabbit (anti-AL) and developed against a serum from a patient with visceral larva migrans

Table 1. ANTIGENIC COMPONENTS IN THE IMMUNOELECTROPHORESIS OF *Ascaris* ANTIGEN PREPARATIONS AND OF ANTIBODIES TO THE ANTIGENIC COMPONENTS OF *Ascaris* FOUND IN INFECTED RABBITS AND IN A PATIENT WITH VISCERAL LARVA MIGRANS (VLM)

Band designation	Electrophoretic mobility relative to pig serum	Stain characteristics	Antigenic components					Antibodies in infected rabbits	Antibodies in patient with VLM
			ETSA	Z	LP	Y	X		
1	$\gamma$	Glycoprotein	+	+	+	+			
2	$\gamma$		+	+	+				
3	$\gamma$		+	+	+				
4	$\gamma$		+	+	+				
5	$\gamma$		+	+	+				
6	$\beta_2$		+	+	+			+	
7	$\beta_2$		+	+	+				
8	$\beta_2$		+	+	+			+	
9	$\beta_1$		+	+	+	+	+	+	+
10	$\beta_1$		+	+	+				
11	$\beta_1$		+	+	+				
12	$\beta_1$		+	+	+				
13	$\beta_1$		+	+	+			+	+
14	$\alpha_2$		+	+	+	+		+	+
15	$\alpha_2$	Glycoprotein (weak)	+	+	+				
16	$\alpha_1$		+	+	+				
17	$\alpha_1$		+	+	+			+	+
18	$\alpha_1$		+	+	+				
19	Albumin		+	+	+	+		+	
20	Albumin		+	+	+				

believe, may be the cause of this absence of reaction in the immunodiffusion techniques.

Table 1 summarizes the results obtained during antigenic analysis by immunoelectrophoresis. In it are listed the antigenic components found in *Ascaris* antigen preparations in order of appearance from the cathode to the anode side of the immunoelectrophoresis slide. In addition, the electrophoretic mobility zone relative to pig serum, the staining characteristics (when determined), the antigenic components of the several preparations and the antibodies to the antigenic components of *Ascaris* found in the serum of infected rabbits and in the human serum from a patient with visceral larva migrans are also listed.

The components for serological diagnosis are those against which the antibodies in human patients or in infected animals are produced. In case of ascariidiosis, and having in mind the results of this investigation, the selected antigen preparations would be those that present the antigenic components Nos. 6, 9, 10, 13, 14, 17 and 20, since experimentally infected rabbits, and human patients with visceral larva migrans, produced large amounts of antibodies against these antigenic components.

Thus, the protein polysaccharide antigen X deproteinized by heat is not a good antigen preparation for serological diagnosis since it lacks 6 of 7 principal antigenic components for serodiagnosis. Nor is the antigen Y good for serodiagnosis, because it lacks five of the principal antigenic components for serodiagnosical work.

On the other hand, the perienteric fluid had all but one, and both saline extracts showed all the important antigenic components. They are therefore the most thoroughly investigated antigens for a high sensitivity in the serological tests, since they are rich in important antigenic components for serodiagnosis.

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## INHIBITION OF INTERFERON SYNTHESIS AND STIMULATION OF VIRUS PLAQUE DEVELOPMENT IN MAMMALIAN CELL CULTURES AFTER ULTRA-VIOLET IRRADIATION

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THE polycyclic carcinogenic hydrocarbons 3-methylcholanthrene, benzo(a)pyrene and 7,12-dimethylbenzo(a)anthracene inhibit interferon synthesis and stimulate Sindbis virus plaque development in rat embryo cell cultures<sup>1-3</sup>. Chemically related but non-carcinogenic compounds, such as benzo(e)pyrene and pyrene, have no enhancing effect on virus plaque size<sup>3</sup>. This increase in the spread of viral infection under an agar overlay, and the decrease in interferon synthesis in cultures with fluid medium, obtained with chemical carcinogens, prompted us to look for similar effects with carcinogens of a completely different nature. The effect of ultra-violet irradiation, known to exert carcinogenic activity<sup>4-6</sup>, was therefore investigated.

Secondary cultures of rat embryo cells, grown in plastic Petri dishes in a humidified atmosphere of 5 per cent CO<sub>2</sub> in air at 36.5° C, were used. The origin of these cells, preparation of the cultures and composition of the culture medium have been previously described in detail<sup>1</sup>. Cultures were irradiated only when complete monolayers had been obtained, usually 4-5 days after seeding. All irradiations were carried out with a germicidal mercury low-pressure vapour lamp (Westinghouse Sterilamp G36T6-L) with maximal emission at 2537 Å. Cultures were irradiated at a distance where the energy received was 60 ergs mm<sup>-2</sup>/sec, as measured with a Latarjet dosimeter<sup>7</sup>. Before irradiation, the growth medium was replaced by 5 ml. of phosphate-buffered saline; the average thickness of the layer of fluid covering the cell sheet was 0.9 mm. Immediately after irradiation the phosphate-buffered saline was removed, and 2 ml. of a Sindbis virus suspension, calculated to give an average of 20 plaques per dish, was added. The virus was left to adsorb to the cells for one hour, after which the inoculum fluid was removed, and 15 ml. of a nutrient agar overlay was added to each plate. The Sindbis virus

used and the composition of the agar overlay have been described in detail<sup>1</sup>. One day before counting and measuring the plaques, 10 ml. of a second nutrient overlay of the same composition as the first, but supplemented with neutral red, was added to each culture. Table 1 presents some results of an experiment where the development of plaques in control and irradiated cultures was followed for several days. It can be seen that there was a significant increase of virus plaque size in cultures previously exposed to ultra-violet rays (see also Fig. 1). The dose of irradiation enhancing plaque size did not exert any lethal effect on the cells when fully developed (5-day-old) cultures were irradiated, as measured by cell counts and uptake of the vital dye neutral red during the days following irradiation. However, if one-day-old cultures were irradiated, a 30 per cent decrease in growth rate was observed. A striking enhancement of Sindbis virus plaque size was afterwards also obtained in cultures of African green monkey (*Cercopithecus aethiops*) kidney cells irradiated for either 4, 8 or 16 sec (see Fig. 2).

The higher susceptibility to virus infection of mammalian cells under agar after ultra-violet irradiation resembles the effect we observed with some carcinogens. This does not necessarily imply that both phenomena are due to the same mechanism, and several possibilities can be envisaged. An increased cellular permeability for viruses, due to the irradiation, is conceivable; this would be in line with a hypothesis recently advanced<sup>8</sup>. It is

Table 1. AVERAGE SINDBIS VIRUS PLAQUE DIAMETERS IN CONTROL AND ULTRA-VIOLET-IRRADIATED RAT EMBRYO CELL CULTURES, 4 DAYS AFTER INOCULATION WITH THE VIRUS

Period of irradiation	Number of plaques measured	Average diameter in mm
Controls	27	4.4 (0.44)*
2 sec.	32	5.9 (0.36)
4 sec.	26	6.8 (0.40)
8 sec.	31	6.0 (0.44)

\* The standard deviation is given after each value.

\* Fellow of the Lady Tata Memorial Trust.



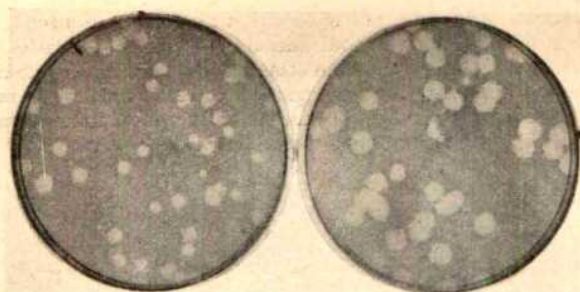


Fig. 1. Sindbis plaques in control and irradiated rat embryo cell cultures, four days after seeding. Left, control culture; right, culture irradiated during 4 seconds

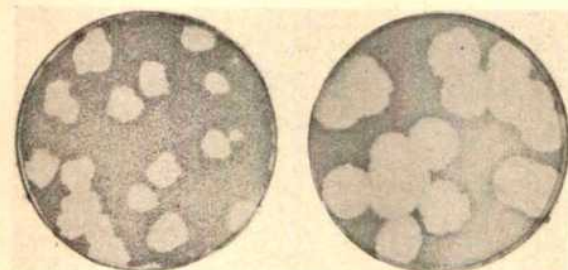


Fig. 2. Sindbis plaques in control and irradiated monkey kidney cell cultures, seven days after seeding. Left, control culture; right, culture irradiated during 16 seconds

also conceivable that the enhancing effect on viral plaque formation is due to a general depression of the rate of cellular protein synthesis, caused by an impairment of cellular DNA function. Under such conditions, the synthesis of any cellular factor(s) directly or indirectly responsible for the limitation of the spread of viral infection under the agar would be temporarily or definitively decreased; ribosomal and mitochondrial function on the contrary would be left relatively untouched, and would remain available for synthesis of viral material. One factor capable of limiting viral infection is interferon<sup>9</sup>, and it was therefore of interest to examine the effect of ultra-violet irradiation on its synthesis, especially in view of the decreased synthesis observed with chemical carcinogens.

Four secondary cultures of rat embryo cells were irradiated during 8 sec. Immediately afterwards, these cultures, together with four control cultures, were inoculated with Newcastle Disease virus, at a multiplicity of about 1 egg  $ID_{50}$  per cell. The virus was left to adsorb to the cells during 2 h, after which the excess inoculum was removed, and 20 ml. of maintenance medium was added to each culture. 44 h later all media were gathered. Portions of the media to be tested for interferon were then dialysed during 96 h against Sørensen buffer at pH 2, in order to destroy any living virus. The dialysed fluids were afterwards titrated for interferon activity, following a method previously described in detail<sup>1</sup>. The results of this titration are given in Table 2. It can be seen that irradiation of cells before addition of the virus completely inhibited interferon production, whereas a

Table 2. INTERFERON ACTIVITY OF FLUIDS DERIVED FROM IRRADIATED AND CONTROL CULTURES, AS EXPRESSED BY REDUCTION OF CHALLENGE VIRUS PLAQUES

		Dilution tested for interferon activity			
		1/50		1/500	
		Average plaque number	% Reduction of challenge plaques	Average plaque number	% Reduction of challenge plaques
Control cultures	1	0*	100†	5	91
	2	0	100	11	81
	3	0	100	12	80
	4	0	100	3	95
Irradiated cultures	1	51	12	63	0
	2	49	15	55	5
	3	58	0	72	0
	4	55	5	65	0

\* Each value represents the average plaque reading of four cultures.

† The average number of challenge plaques was 58.

considerable amount of interferon was produced in non irradiated control cultures. This interferon furthermore was completely inactive when tested in chick cells, which agrees with the species specificity described for interferon and rules out any interfering activity due to residual virus. In another experiment, not reported in detail here, an inhibition of interferon synthesis was observed in ultra-violet irradiated cultures subsequently infected with Sindbis virus. Virus yields, however, were the same in control and irradiated cultures.

The inhibition of interferon synthesis, and the increase of viral plaque size, both observed after ultra-violet irradiation, are perhaps provoked independently by the irradiation, or there might be a causal relationship between the inhibition of interferon synthesis and enhancement of plaque size.

Some indirect evidence in favour of the latter hypothesis was obtained in the following way. Use was made of a continuous line of rat cells, called 'DE40' cells, maintained in this laboratory since 1959. These cells are insusceptible to the antiviral activity of interferon, as described in detail elsewhere<sup>3</sup>. It was reasoned that if the increased plaque size observed after irradiation were due to an inhibition of interferon, there should be no increase of plaque size in 'DE40' cells, since these cells are insusceptible to interferon. In a preliminary experiment, it was established that the energy required to kill the 'DE40' cells was about the same as that needed to kill rat embryo cells. This experiment was carried out in order to have an indication of the relative susceptibility to ultra-violet irradiation of both cell systems. The influence of irradiation on plaque size was then investigated as follows. Four-day-old monolayer cultures of 'DE40' cells grown in plastic Petri dishes in an atmosphere of 5 per cent  $CO_2$  in air were irradiated during 10 or 15 sec; the energy received was 60 ergs  $mm^{-2}/sec$ . Immediately after irradiation a plaque assay with Sindbis virus was carried out, following the procedure described above. Five days later plaques were counted and measured. There was no difference either in plaque number or in plaque size between irradiated and control cultures (see Fig. 3). A second experiment carried out under similar conditions gave identical results.

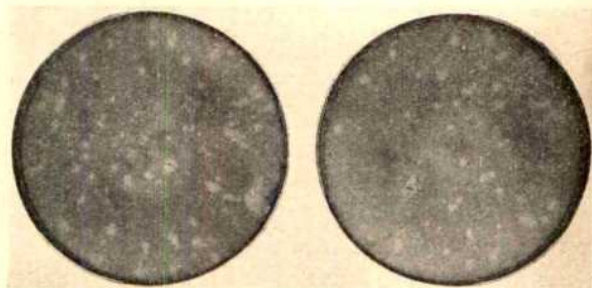


Fig. 3. Sindbis virus plaques in control and irradiated 'DE40' cell cultures, five days after seeding. Left, control culture; right, culture irradiated during 15 seconds

The inhibition of interferon synthesis and the enhancement of virus plaque size after ultra-violet irradiation closely resemble the effects obtained with carcinogenic hydrocarbons under similar conditions. This suggests the existence of a common target site at some cellular level. It is known that ultra-violet irradiation can cause alteration of DNA<sup>8,10</sup>. Therefore it is possible that the inhibition of interferon synthesis as observed in irradiated cells is due to an impairment of cellular DNA function; it has indeed been shown that interferon synthesis is coded by the genome of the cell<sup>11</sup>. The possibility that inhibition of interferon synthesis by polycyclic carcinogenic hydrocarbons is the result of an interaction with the cellular genome should be considered.

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U.S. Public Health Service, and by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

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## PREPARATION OF SYNCHRONOUS CELL CULTURES BY SEDIMENTATION

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A NUMBER of techniques for obtaining synchronous cell cultures have been worked out in recent years with the prime object of investigating the biochemical events of the cell cycle<sup>1</sup>. In most cases, the cells are subjected to a series of changes in their environment (for example, temperature, light or nutrients) which cause the culture to become synchronous. With a few cell systems, however, it has been possible to use another method which is different in principle. Cells at a particular stage of the cell cycle have been separated physically from a normal growing culture and then grown separately as a synchronous culture<sup>2-5</sup>. Separation methods tend to give a low yield, but have the merit that there is less likely to be a drastic change in cell metabolism during the process of synchronization.

We have developed another separation method which relies on differential sedimentation, and have used it to produce synchrony in fission yeast, budding yeast and *Escherichia coli*. In essence, the method consists of centrifuging cells from an exponentially growing culture through a sucrose gradient. Small cells, at the start of the cell cycle, move through the gradient more slowly than large cells, or clumps of cells, and can be separated off and grown as a synchronous culture. The main function of the sucrose gradient is to stabilize the column of liquid through which the cells sediment. It also provides a dense medium on top of which the initial cell suspension can be layered. It does not act as an equilibrium gradient since in time all the cells can be centrifuged to the bottom of the tube.

The details of the method are as follows:

(1) *Gradients*. Linear sucrose gradients are set up in straight-sided centrifuge tubes by means of a gradient machine<sup>6</sup>. The gradients should occupy 80-90 per cent of the volume of the tubes. We have used two sizes of tube—small ones (16 × 150 mm) with 15-ml. capacity and large ones (25 × 170 mm) with 80-ml. capacity. A gradient from 2 per cent sucrose (w/v) at the top to 12 per cent at the bottom is sufficient to stabilize the small tubes, but the large tubes need a 10-40 per cent gradient. It might be possible to use shallower gradients with careful régimes for starting and stopping the centrifuge or with sector tubes<sup>7,8</sup>. The sucrose was commercial sugar and in some cases was made up in a minimal medium rather than water. Once made, the gradients are stable for several days but they should be kept cool unless they are sterile.

(2) *Loading*. Cells from a culture of exponential growth are centrifuged to give a concentrated suspension. This suspension is then layered carefully on top of a gradient. We have used 0.5-1 ml. suspension for the small tubes and 2-5 ml. for the large tubes. As soon as the suspension has been put on, it should be stirred with a thin rod to mix it with the top of the sucrose until the milky layer of cells is about twice its original thickness. This tends

to produce an inverse gradient of cells at the top of the tube and hence prevents the formation of streamers of cells. We have tried loading with an inverse gradient of cells from a small gradient machine<sup>9</sup> but it does not give an improved separation. The number of cells that can be loaded will depend on the cell size. We have had good results from  $2 \times 10^{10}$  exponential phase cells of *Schizosaccharomyces pombe* in 4.7 ml. loaded on a large tube. This number of cells has a dry weight of about 0.7 g and would be produced by 670 ml. of a stationary phase culture ( $3 \times 10^7$  cells/ml.) or 4,000 ml. of an exponential culture ( $5 \times 10^6$  cells/ml.). It might be possible to increase the loading somewhat above this figure, though the cell suspension would become viscous and difficult to handle. The loading for an experiment could certainly be increased, however, by using tubes of larger diameter or, of course, by using several tubes.

(3) *Centrifuging*. The loaded gradients are centrifuged in swing-out buckets. The speed and time of centrifuging will depend on the cells, the gradient and the temperature. We used 500g for 10 min at 25° C for yeast, and 2,500g for 20 min at 12° C for *E. coli*. Cells sediment considerably faster at higher temperatures. During the centrifuging, the milky cell layer moves down the tube, broadening as it goes. It is best to stop when the layer has moved one-half to two-thirds of the way down. By this time there is often a pellet of clumped cells at the bottom. In the case of yeast, a compromise has to be reached about the temperature in the centrifuge. If it is too low, there is a risk of the temperature shock affecting the subsequent growth. If it is at the optimum growth temperature, and the gradient is heavily loaded, gas bubbles will be produced by the growing cells which will disturb the gradient.

(4) *Sampling*. After centrifuging, the top of the cell layer is removed by a syringe with a long needle (held in a clamp with a vertical screw movement) and suspended in fresh medium to produce a synchronous culture. The amount of the layer that is removed is again a matter of compromise. If the amount is large the yield is large, but the separation of small cells is less efficient and the synchrony of the resulting culture is poor. We have usually removed the top 5-20 per cent of the length of the layer. Since the top of the layer has a lower cell density than the rest of the layer, this gives a recovery of 1-5 per cent of the cells which were loaded originally. The point of the needle should be placed at the bottom of the region to be sampled because, in a density gradient, the liquid which is removed comes from above the needle point.

The cells that have been tested are given in Table 1, together with their growth media and temperatures. Two main sets of experiments were carried out using them. First, cell lengths were measured in a top sample from a gradient and in a control taken either from the rest of the cells in the gradient or from the initial suspension. The measurements were made on photographs of living

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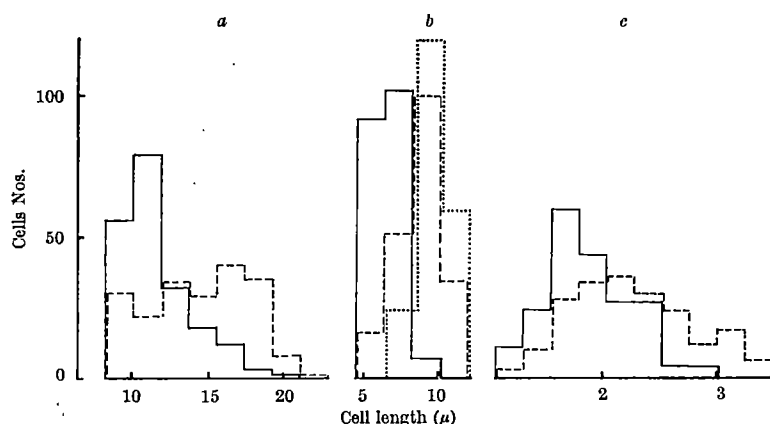


Fig. 1. Frequency histograms of cell length from 200 cells. (a) *S. pombe*, 10–40 per cent gradient in 80-ml. tube loaded with  $2 \times 10^{10}$  cells from exponential culture ( $8.6 \times 10^8$  cells/ml.). Centrifuged at 500g for 10 min at 20° C. —, Top sample (0.5 per cent of cells loaded); — — —, control. (b) *S. cerevisiae*, 10–40 per cent gradient in 15-ml. tube loaded with  $6 \times 10^8$  cells from stationary culture ( $11 \times 10^8$  cells/ml.). Centrifuged at 200g for 6 min at 20° C. —, Top sample (top 10 per cent of cell layer); . . . , bottom sample (bottom 20 per cent of cell layer); — — —, control. (c) *E. coli*, 10–40 per cent gradient in 15-ml. tube loaded with  $2 \times 10^{10}$  cells from exponential culture ( $6 \times 10^8$  cells/ml.). Centrifuged at 2,500g for 20 min at 12° C. —, Top sample (2 per cent of cells loaded); — — —, control.

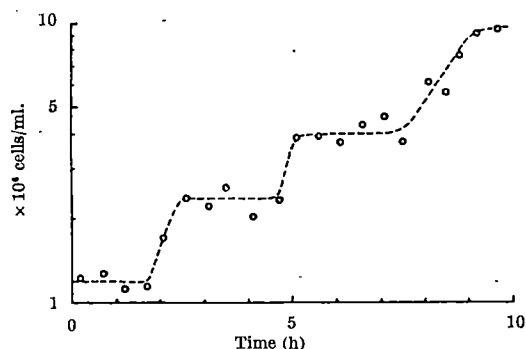


Fig. 2. Cell numbers in synchronous culture of *S. pombe*. 10–40 per cent gradient in 80-ml. tube loaded with  $3 \times 10^8$  cells from exponential culture ( $3.2 \times 10^8$  cells/ml.). Centrifuged at 500g for 10 min at 25° C. Top sample suspended in fresh medium.

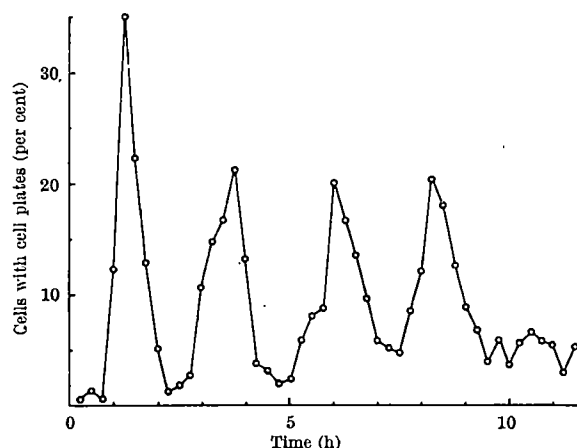


Fig. 3. Percentage of cells with cell plates in synchronous culture of *S. pombe*. 10–40 per cent gradient in 80-ml. tube loaded with  $3 \times 10^8$  cells from exponential culture ( $2 \times 10^8$  cells/ml.). Centrifuged at 500g for 9 min at 25° C. Top sample (4 per cent of cells loaded) suspended in fresh medium.

cells in the case of the yeasts and of fixed and stained cells in the case of *E. coli*. Results for each kind of cell are shown as frequency histograms in Fig. 1. There is a definite separation of the smaller cells into the top sample, but it is by no means perfect. It is possible that a better separation might be obtained with some types of cell if the top layer were centrifuged again in a second gradient, but we have tried this with *S. pombe* and did not get an improved separation. It is likely that an accurate sizing

is impossible by this method because of the variations in density among cells of the same size. The ratio of standard deviation over mean for cell density in *S. pombe* is 0.12 (ref. 9). A stationary culture (without buds) was used for *S. cerevisiae* since measurements of cell length carry less meaning in growing cells of this organism. The separation in these roughly spherical cells was somewhat better than it was for the other two rod-shaped cells, as would be expected from the physical laws of sedimentation. A histogram for the bottom layer of *S. cerevisiae* is included in Fig. 1b, and it shows a preponderance of large cells. This also occurred with the bacterium and the fission yeast, but the biological picture in the lower layers here was complicated by the presence of lumps and of pairs of young cells which had not at that time separated.

In the second set of experiments, top samples from gradients were removed and suspended in fresh media. Their progress as synchronous cultures was followed by cell counting in haemocytometers with the yeasts (using the criteria of Williamson and Scopes<sup>10</sup> for *S. cerevisiae*) and by colony counting with *E. coli*. Fig. 2 shows an experiment with *S. pombe* where there are two, perhaps three, synchronous divisions. A criticism of it as a synchronous culture is that there is not an accurate doubling of numbers in the second step at 5 h. Another sample, however, was taken from the same gradient just below the top one and also followed, though it is not reproduced here. Its steps were not quite so steep but did show accurate

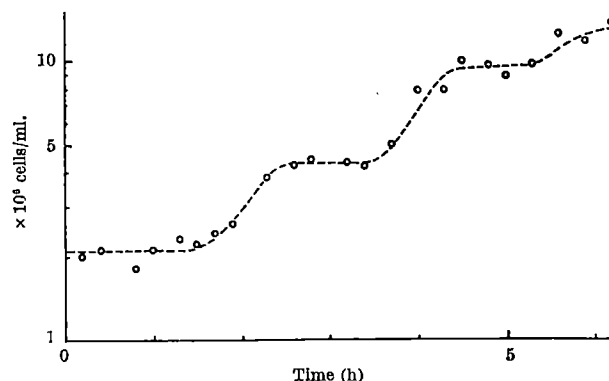


Fig. 4. Cell numbers in synchronous culture of *S. cerevisiae*. 10–40 per cent gradient in 80-ml. tube loaded with  $4 \times 10^8$  cells from exponential culture ( $2.9 \times 10^8$  cells/ml.). Centrifuged at 500g for 10 min at 25° C. Top sample (3 per cent of cells loaded) suspended in fresh medium.

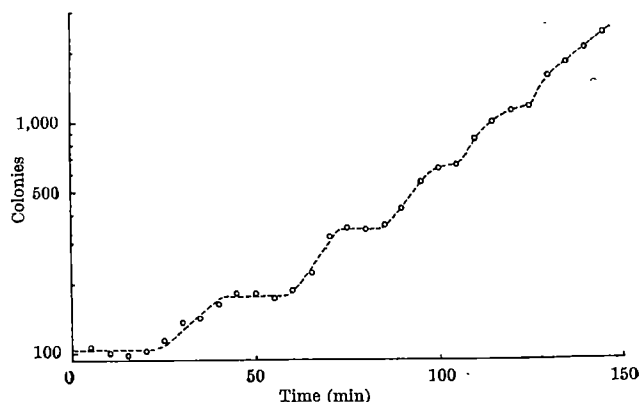


Fig. 5. Colony counts from synchronous culture of *E. coli*. 10–40 per cent gradient in 15-ml. tube loaded with  $2 \times 10^{10}$  cells from exponential culture ( $6 \times 10^8$  cells/ml.). Centrifuged at 2,500g for 20 min at 12° C. Top sample (2 per cent of cells loaded) suspended in fresh medium.

Table 1

Organism	Medium	Growth temperature (°C)
<i>Schizosaccharomyces pombe</i> (N.C.Y.C. 132)	Minimal medium 'E.M.M.' <sup>11</sup>	32
<i>Saccharomyces cerevisiae</i> (commercial bakers' yeast)	Minimal medium 'E.M.M.' <sup>11</sup>	30
<i>Escherichia coli</i> K12AF <sup>-</sup>	Double strength 'Oxoid' nutrient broth	37

doublings. The remaining cells from the gradient (about 90 per cent of the cells which were loaded) were also resuspended and followed, but showed no signs of synchrony. Another method of following synchronous cultures in *S. pombe* is to count the number of cells showing cell plates. This is equivalent to a mitotic index, and, in a normal growing culture, about 10 per cent of the cells have cell plates. Fig. 3 shows a synchronous culture followed this way. There were four synchronous divisions which lasted until the culture ran into the stationary phase after 10 h. Fig. 4 shows an experiment with *S. cerevisiae*. The steps were not quite so sharp as those with the fission yeast and there was a slight initial lag. The culture was in the stationary phase at 6 h. Fig. 5 shows an experiment with *E. coli*. There were three synchronous divisions and signs of a fourth. The growth rate appears to accelerate for the first 75 min, which might be due to cellular changes occurring during the period of 45 min between collecting the initial log phase culture and setting up the synchronous culture.

This is not a perfect method for making synchronous cultures since the separation of small cells is incomplete and the initial steps in the cell number curves are not vertical. Nor is it as good as the best of the existing methods. For example, the budding yeast synchrony in Fig. 4 is worse than that produced by the method of Williamson and Scopes<sup>10</sup>. It does, however, have many advantages. It is simple, and requires little in the way of apparatus except a centrifuge and a gradient machine. It is relatively quick compared with many other methods. The whole procedure, including collecting, but excluding the preparation of gradients, takes 20–80 min. It gives a reasonably large yield. With a loading of  $2 \times 10^{10}$  yeast

cells and a recovery of 5 per cent, it gives a 200 ml. synchronous culture. It works with exponential phase cells and so avoids the criticism that can be made of methods which start with stationary cells—that the initial period of growth represents recovery from the stationary phase. Above all, it is a method which should work with a wide variety of cells. We have tried it with three kinds of cells of different shapes and sizes, and we have little doubt that it will work with many other types. Good size separations have already been obtained with strain *L* mouse cells by Dr. D. H. Bishop and Dr. R. Sinclair in this laboratory, and work on this cell system is in progress. With cells which are affected by the osmotic pressure of sucrose, it should be possible to substitute gradients of protein, dextran or 'Ficoll'. Dextran gradients have been used by Lieblova *et al.*<sup>8</sup> to separate budding yeasts with different numbers of bud scars. Since dextran is not metabolized, these gradients are not disturbed by gas bubbles. It is more difficult, however, to make steep gradients because concentrated dextran solutions are very viscous.

We thank Dr. D. H. Bishop for his help in carrying out the experiments with *E. coli*, Prof. M. M. Swann for valuable discussions, and Mr. J. Creanor for his help. One of us (J. M. M.) was the recipient of a special research grant from the Department of Scientific and Industrial Research, and the other (W. S. V.) the holder of grant GM11480 from the National Institutes of Health, U.S. Public Health Service.

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## BEHAVIOUR OF CERTAIN MARINE ORGANISMS DURING THE SOLAR ECLIPSE OF JULY 20, 1963

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THE effect of solar eclipses on animal activity is the subject of a literature which, though not extensive, has ancient beginnings. Much of it has been summarized recently by Cloudsley-Thompson<sup>1</sup>, who concludes: "Some animals show exogenous rhythms, others are more markedly endogenous". That is, some animals engage in crepuscular activities during the eclipse, while others continue their natural mid-day ones. In this literature only the briefest attention has been directed to marine organisms.

This article reports the similar behaviour of two sorts of phylogenetically unrelated marine organisms during the solar eclipse of July 20, 1963. Observations were made of so-called 'deep scattering layers' and of the bioluminescence of certain planktonic micro-organisms.

In those cases where sound-scattering from the deep scattering layers has been examined critically, using broad-band sound sources and spectrum analysis, a gas-bubble-bearing sound scatterer is indicated<sup>2</sup>. Since mesopelagic fishes with swim-bladders are such bubble-bearers, since many of these fishes correspond generally in their vertical and geographic distributions with the deep-

scattering layers<sup>3</sup>, and since such fishes may be taken by nets towed in deep-scattering layers, it is widely accepted that such animals are indeed the agents responsible for many or most of the deep-scattering layers observable throughout the oceans of the world. Certain other gas-bubble bearers among the plankton and nekton cannot be excluded, however. For example, Barham<sup>4</sup> has made certain observations from the bathyscaphe *Trieste* off Monterey, California, suggesting that the bubble-bearing siphonophore *Nanomia bijuga* may be responsible for a deep-scattering layer there. Characteristic of most deep-scattering layers is the upward vertical migration, the extensive and rapid part of which begins about sunset, and the subsequent retreat to the depths that begins with morning twilight (see recent reviews of the deep-scattering layer 'problem' by Hersey and Backus<sup>5</sup> and by Boden<sup>6</sup>).

Similar behaviour (in that it is apparently light-controlled) has been reported by us<sup>7</sup> for certain bioluminescent micro-organisms at the sea surface, both in coastal waters and on the deep ocean. These organisms, taken to be dinoflagellates chiefly, are luminescent at night, but mainly not so during the day. The change from the one



condition to the other and back occurs quickly at sunrise and sunset.

The literature on the effects of solar eclipses on animal activity can be taken to be a special part of a now massive literature concerning circadian endogenous rhythms in a host of plants and animals (much of which is summarized by Bunning<sup>8</sup>, Cloudsley-Thompson<sup>1</sup>, and in *Biological Clocks*<sup>9</sup>). Sweeney and Hastings<sup>10</sup>, for example, show that the dinoflagellate *Gonyaulax polyhedra*, when kept in constant dim light, has a cycle of luminescence-non-luminescence close to 24 h in period. In fishes, it has been demonstrated, at least, that several species orient with respect to the Sun while allowing for its changing azimuth, thus demonstrating their perhaps endogenous ability to measure the passage of time<sup>9</sup>.

Are the vertical migrations of the deep-scattering layers and the diurnal cycle of luminescence in dinoflagellates events principally or wholly under the control of the exogenous factor of light, or are they controlled, or modified, to a large extent by endogenous rhythms? In the scattering layer case, the answer has already been suggested by Hersey and Moore<sup>11</sup>, who detected, during the daytime, the beginnings of an upward migration of a shallow (about 50 fathoms) scattering layer during the passage of "a particularly heavy rain squall". In similar circumstances we have observed a tendency for the bioluminescent activity of dinoflagellates to increase. But events such as passing rain squalls are not sufficiently dramatic reducers of sunlight to answer the question satisfactorily—a solar eclipse is.

Scattering layer observations were made from R.V. *Chain*, hove to about 200 miles south of Woods Hole, Mass. (37° 38' N., 69° 28' W.), in slope water in 2,105 fathoms. The observations were in the form of echosoundings made with an Alden Electronics precision graphic recorder and an EDO UQN-1b transducer and electronics (operating frequency, 12 kc/s). The depths

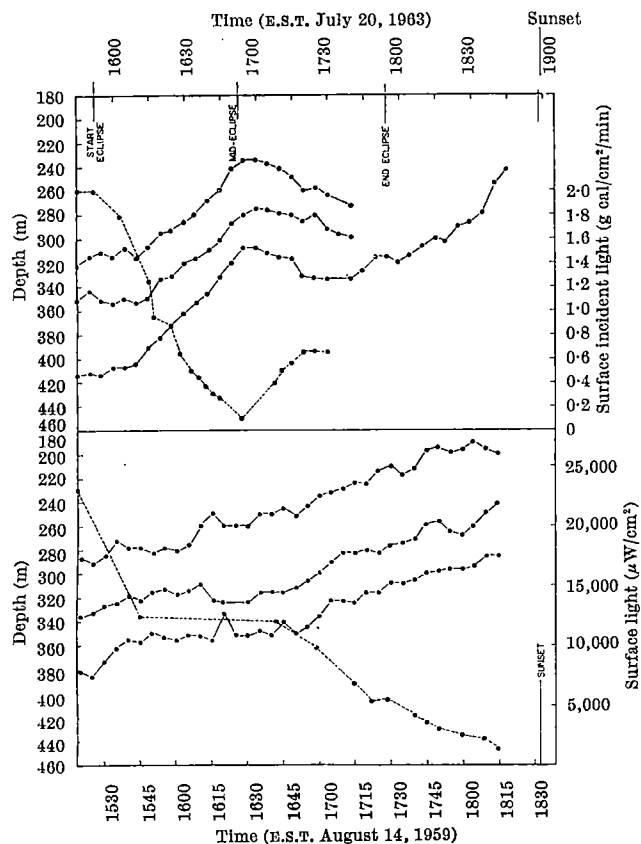


Fig. 1a (top), vertical distribution of three scattering layers and surface light intensity during the solar eclipse of July 20, 1963; b (bottom), same on a normal afternoon offered for comparison with 1a

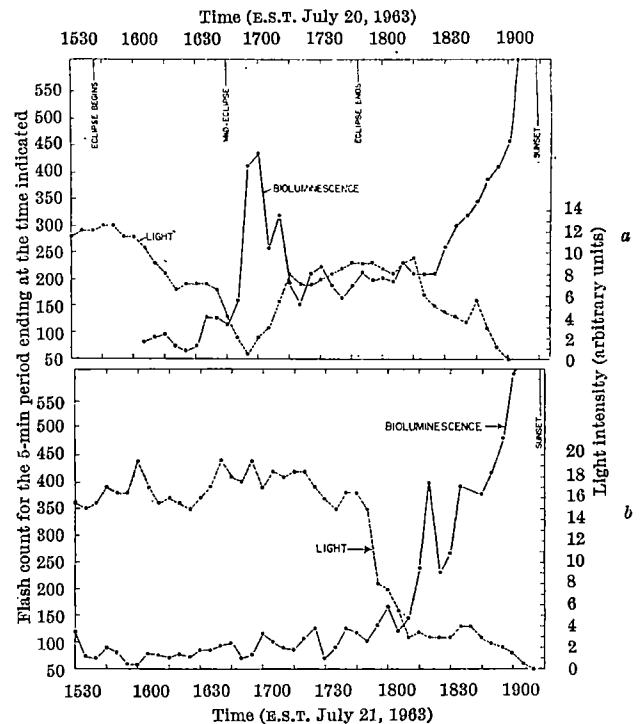


Fig. 2a, Bioluminescent flashing and surface light intensity during the solar eclipse of July 20, 1963; b, The same as 2a for the normal afternoon of July 21, 1963

plotted in our figures represent the mid-point of the densest portion of each layer and are based on a sound velocity of 4,800 ft./sec. Light readings were made using a Sekonic Auto-Leader model L-38 light-meter with 8 times neutral density filter; pointing it directly at the Sun from a point 60 ft. above the sea-surface. This light meter was calibrated against a General Electric radiation meter model 8DW 60Y2. The minimum light intensity read (very near the middle of the eclipse) was 0.1 g. cal/cm²/min. Time of mid-eclipse was 1652 E.S.T.; eclipse duration, 125 min (Nautical Almanac Office<sup>12</sup>). Ninety per cent of the Sun's diameter was obscured at mid-eclipse when the altitude of the Sun was about 22°. Sunset was at 1858 E.S.T. The sky was generally clear (a few high clouds) and the sea calm.

Bioluminescence observations were made at the entrance to Eel Pond, a sea-level, tidal pond opening on to Great Harbor, Woods Hole, by pumping water through a darkened chamber that was inspected by a photomultiplier tube. This tube was connected by appropriate circuitry to a counter that tallied and printed out counts of bioluminescent flashes every 5 min<sup>7</sup>. Light readings, not reducible to absolute levels, were continuously recorded with four International Rectifier selenium photocells (B2) connected in parallel to a Rustrak recorder. Calculated time of mid-eclipse was 1645 E.S.T., eclipse duration about 127 min<sup>12</sup>. Ninety-four per cent of the Sun's diameter was obscured at mid-eclipse when the altitude of the Sun was about 28°. Sunset was at 1914 E.S.T. The sky was overcast.

Scattering layer observations made during the eclipse, together with light readings, are plotted in Fig. 1a. We have no comparable observations at the same location for a non-eclipse day close in time, but data for a normal afternoon (August 14, 1959) from a nearby locality (38° 40' N., 68° 33' W.) are shown, together with surface light readings, in Fig. 1b. What often has been taken for a single thick scattering layer in this region generally is resolvable, with a good echo-sounder, into three or four separate layers. Both Figs. 1a and 1b show three such sub-layers, save in the late part of Fig. 1a, where, after the ship got under way at 1740, only the best developed element was detectable above background noise. It can-

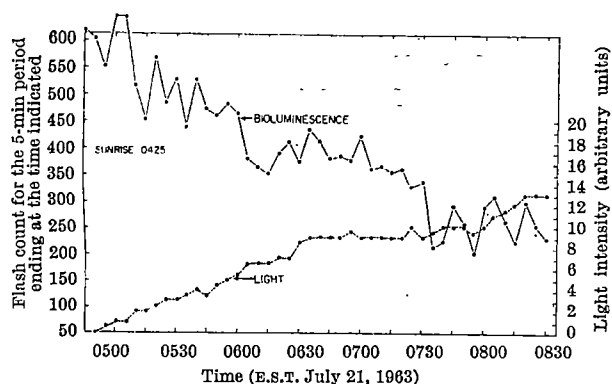


Fig. 3. The same as 2a and 2b for the normal morning of July 21, 1963

be seen that on the day of the eclipse a greater than normal rate of ascent of the layers began about 20 min after the eclipse commenced. The ascent continued until about 5 min after mid-eclipse. During this period the average rates of ascent for the three layers were 1.8, 1.6 and 2.1 m/min. With the uncovering of the Sun and increasing light the layers descended, but not so rapidly (at average rates of 0.95, 0.6 and 0.9 m/min) as they had ascended with decreasing light and not reaching, for comparable light levels, the depths they held while the Sun was being obscured.

Unfortunately, the light readings could not be continued throughout the eclipse period or after it, but the last three readings (covering the period 1720–1730) show the light level to have been unchanging. The upper two layers continued their descent during this period and for 10 min afterwards (after which time they were no longer distinguishable, as noted earlier). The deepest layer essentially stopped its descent when the light stopped increasing. After about 20 min this layer began again to ascend. During the period of 1.25–0.25 h before sunset the layer rose at a mean rate of 1.3 m/min (Fig. 1a). The rates of ascent during the like period on August 14, 1959, for the three layers shown (Fig. 1b) were 0.4, 0.6 and 0.6 m/min.

The bioluminescence observations made during the eclipse are plotted together with light readings in Fig. 2a. Similar observations for the like period on the following day, July 21, 1963, are shown in Fig. 2b. Although observation did not begin before the beginning of the eclipse, it can be seen that flash counts were normal for the time of day until about the 5-min period ending at 1635 E.S.T., or about 50–55 min after the start of the eclipse and about 10–15 min before maximum eclipse. The light level at this time was about that observed on a normal day when the flash count begins to increase with the approach of sunset. Note, that is, that the first

intersection of the light and flash-count curves on July 20 (Fig. 2a) and the intersection for July 21 (Fig. 2b) both occur at about the same point on the ordinates. The maximum flash count on July 20 occurred for the 5-min period beginning at 1655 E.S.T., the time of minimum light intensity, but 10 min after the time of mid-eclipse. That the time of minimum light intensity was not coincident with the time of mid-eclipse probably is due to the variable, and at times heavy, cloud cover. With the increase in light, flash counts diminished rapidly, but, for given light levels, never decreased during the mid-eclipse-to-sunset period to the levels observed before mid-eclipse. Note, that is, that the intersection of the light and flash-count curves occurs at a higher point on their ordinates after mid-eclipse than before it (Fig. 2a). This hysteresis, higher flash-counts at a given light level with increasing light than with decreasing light, is observed also when normal sunrise (Fig. 3) and normal sunset (Fig. 2b) are compared, but here the effect is somewhat greater than in the eclipse situation.

In summary, we can say that both the scattering layer organisms and the bioluminescent organisms responded to the eclipsing Sun much as they normally respond to the setting Sun. Their behaviour from mid-eclipse to eclipse end resembled dawn behaviour. The response of these organisms to the change from decreasing light to increasing light near mid-eclipse was rapid. Thus, it appears that the exogenous factor of changing light largely controls the behaviour examined in these organisms, overriding such endogenous rhythms as may exist.

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## INFLUENCE OF PARTICLE SIZE ON DECOMPOSITION OF RED ALDER AND DOUGLAS FIR SAWDUST IN SOIL

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**I**NTENSIFIED use of sawdust and other wood wastes as soil conditioners, especially in the Pacific north-west, has prompted a number of workers<sup>1,2-5</sup> to investigate the effects of various types of sawdust and bark mulches on soil microbial activity and plant growth. Bollen and Glennie<sup>7</sup> reviewed fundamental principles and factors associated with the use of wood wastes on the soil and the

resultant effects on microbial activity. Although particle size, including its influence on surface/volume ratio, undoubtedly affects the rate of decomposition and humification, investigations to determine this have been few. Allison and Cover<sup>1</sup> found that fine sawdust of mesh size 4–6, 6–10, and 10–20, incubated 106 days in a loam soil, respired 36.5, 42.8, and 43.6 per cent respectively of the

total carbon. The rate of oxidation of sulphur in soils has been shown to increase even more rapidly as particle size decreases<sup>17</sup>. In these and similar reports by others the size of sawdust used is given in terms of sieve mesh passed or merely as finely ground. This article reports the effect of finer particle sizes of wood on their rate of decomposition in soil.

Samples of red alder and Douglas fir sawdust in four particle size ranges of from approximately  $700 \times 3,200\mu$  to  $1.6 \times 1.7\mu$ , as viewed in two dimensions under the microscope, were obtained through the courtesy of Dr. D. W. Glennie and J. L. Ricard, Forest Research Center, Corvallis, Oregon. Analysis of the various sawdust particles is shown in Table 1. Although it is not possible to arrive at exact surface-to-volume relationships from the two-dimensional measurements, it is obvious that progressive reduction in size does increase the relative surface exposure. In addition, it must be borne in mind that subdivision of a rod into more rod-shaped particles has much less effect in increasing surface than does subdivision of a cube or sphere into more cubes or spheres.

Table 1. ANALYSIS OF RED ALDER AND DOUGLAS FIR WOOD PARTICLES

Size group	Mean size* ( $\mu$ )	Distribution of shapes Polyhedra (%)	Spheroids (%)	Total surface of addition to Warburg flasks† ( $\mu^2$ )	Total carbon (%)	Total nitrogen (%)	C/N
Red alder							
1	$660 \times 3,120$	90	10	$5.0 \times 10^3$	47.5	0.105	452
2	$106 \times 318$	54	46	$3.4 \times 10^3$	43.3	0.100	430
3	4.0	—	100	$3.2 \times 10^{11}$	44.9	0.103	436
4	1.8	—	100	$3.5 \times 10^{11}$	46.3	0.095	487
Douglas fir							
1	$725 \times 3,540$	90	10	$4.0 \times 10^3$	48.7	0.053	919
2	$105 \times 280$	73	29	$2.1 \times 10^3$	44.5	0.055	809
3	4.4	—	100	$1.6 \times 10^{11}$	47.4	0.050	948
4	1.6	—	100	$2.9 \times 10^{11}$	48.5	0.060	808

\* Lateral dimensions as viewed under microscope.

† Assuming thickness of particles to be similar to width.

Pertinent also is the content of cellulose and lignin. Representative analyses are given in Table 2. Although these data were obtained with fresh sawdust, the values for holocellulose and lignin may reasonably apply to the wood used for the Warburg experiments despite the fact that the particles were prepared from kiln-dried alder boards and stored Douglas-fir sawdust. On the other hand, the percentages of carbon and especially of nitrogen vary with age, source and storage of sawdust. In all cases, however, alder is characterized by higher nitrogen and narrower C/N ratio; alder also is higher in pentosans and lower in  $\alpha$ -cellulose.

Table 2. CELLULOSE AND LIGNIN IN FRESH SAWDUST FROM RED ALDER AND DOUGLAS FIR\*

	Holocellulose (%)	Klasson lignin (%)	Carbon (%)	Nitrogen (%)	C/N
Red alder	72.06	23.10	49.84	0.87	135
Douglas fir	64.50	27.57	51.51	0.12	429

\* On basis of unextracted dry wood.

25-g portions, oven-dry basis, of Chehalis silt loam soil which had been sieved through a 10-mesh screen were used in 125-ml. Warburg flasks. Analysis of this soil gave a pH of 6.7, 0.183 per cent total nitrogen, and 2.28 per cent total carbon. Red alder and Douglas fir sawdust of the various particle size ranges were mixed at rates equivalent to 2,000 p.p.m. carbon before addition to the flask. These additions ranged from 103 to 116 mg according to carbon content. Water content was then added to adjust moisture to 27.6 per cent, which was 50 per cent of the water-holding capacity, corresponding to approximately one-half of the field capacity. 1 ml. of 20 per cent potassium hydroxide was added to the centre well fitted with fluted filter to absorb carbon dioxide. Duplicate flasks of each treatment were attached to calibrated manometers and placed in a constant-temperature water-bath held at 30° C. The flasks were allowed to equilibrate for 5 h before the manometers were closed. Oxygen uptake was

observed for 85 h. Calculations were made according to Webley<sup>21</sup>. Carbon dioxide evolution and respiratory quotient were not determined because several investigators<sup>10,16,18</sup> have indicated possible unreliability of this procedure in short-term manometric experiments with soil.

Because some reports<sup>13,14</sup> have shown a stimulation of cellulose-decomposing micro-organisms when inorganic nitrogen is added, while others<sup>2-7,9</sup> seem to indicate that nitrogenous additions depress decomposition of cellulose, lignin, and resin substrates, the experiment was repeated with the addition of ammonium nitrate. The amount used in each case was sufficient to bring the C/N ratio of the added materials to 20/1, which is generally considered optimum for decomposition. Appropriate controls of soil only were included in each experiment.

In flasks the contents of which consumed large volumes of gas, the manometers were opened to the atmosphere and reset to the 150-mm reference point. Experiments by Gilmour *et al.*<sup>11</sup> indicate that normal uptake of oxygen starts immediately after re-setting the fluid level. This was found to be true in our experiments. During the 85-h respiration period there were no indications of oxygen depletion in any of the flasks, as would be readily shown by cessation of oxygen consumption. The results given are means for duplicate treatments.

The comparative rates of oxidation of the various sawdust particle sizes are presented in Figs. 1 and 2. The results show a definite parallelism between the particle size and microbial activity; the smaller the particle, the greater the oxidative rate, as evidenced by oxygen

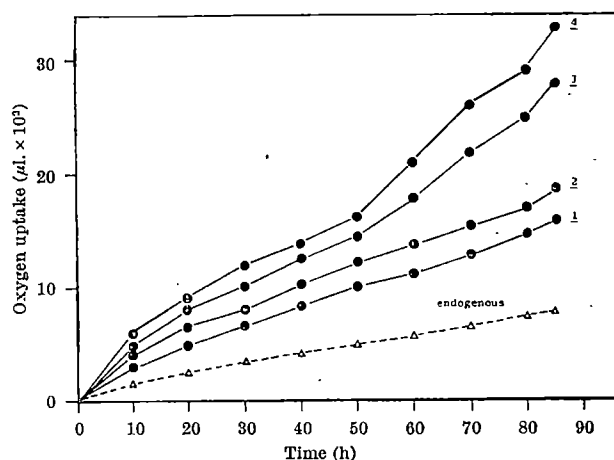


Fig. 1. Oxygen uptake by different size-groups of red alder wood particles in soil. Average particle size: 1,  $725 \times 3,540\mu$ ; 2,  $105 \times 280\mu$ ; 3,  $4.4\mu$ ; 4,  $1.6\mu$ .

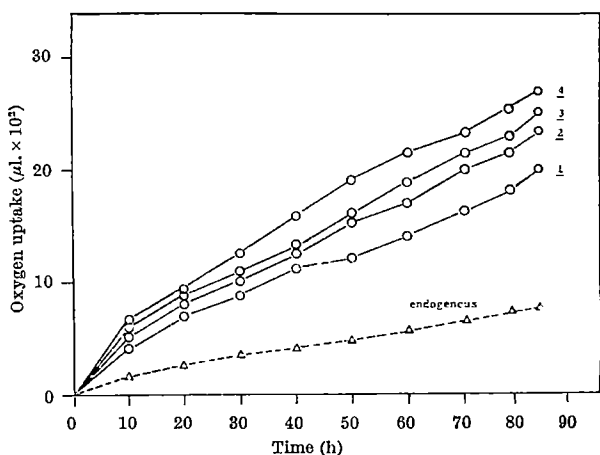


Fig. 2. Oxygen uptake by different size-groups of Douglas fir wood particles in soil. Average particle size: 1,  $660 \times 3,120\mu$ ; 2,  $106 \times 318\mu$ ; 3,  $4.0\mu$ ; 4,  $1.8\mu$ .



consumption. Particles of  $1.7\mu$  mean diameter gave the highest rate of oxygen uptake for both Douglas fir and red alder, followed in order by the mean sizes of  $4.2$ ,  $106 \times 300$ , and  $700 \times 3,200\mu$ . The increased rates of oxidation for the smaller sizes may be due in part to the increased surface in relation to volume and in part to rupturing of the lignin-cellulose bonds by the mechanical preparation of the smaller particles<sup>15</sup>. An additional advantage attributable to smaller size may be a more extensive distribution of particles in the soil mass, thus mechanically increasing exposure to micro-sites of biological activity.

The two smaller size groups of red alder sawdust (Fig. 2) were more extensively decomposed at 85 h than the Douglas fir of comparable size. However, the rate of oxidation of the red alder was greater only after 50 h. This suggests a possible difference in chemical constitution, or a difference in physical structure and exposure resulting from the milling process. An opposite effect was observed with the two larger particle sizes.

Although decreasing the particle size of the sawdust by approximately 3,000-fold increased the oxidation rate, the effect was not as pronounced as could be expected from surface/volume relationships alone. Because the reduction in size was by prolonged ball-milling, some localized heating occurred. This may have caused some polymerization of chemical constituents and possibly a resultant decrease in susceptibility to oxidation. Nevertheless, it was found that the wood particles described in Table 1 were more extensively and rapidly available to solvent extraction as particle size decreased<sup>12</sup>. Presumably this size reduction would also render these components more susceptible to microbial oxidation.

Pew *et al.*<sup>16</sup> found that 95 per cent of the carbohydrate could be removed rapidly by cellulytic enzymes if wood particles were ground to  $1\mu$  or less in a cooled vibratory ball mill. This, with other evidence<sup>15</sup>, indicates that a three-dimensional lignin network probably surrounds the cellulose polymers in wood. Such an encrusting lignin framework may effectively hinder or prevent cellulases from attacking the carbohydrate chains. Because lignin is highly resistant to microbial decomposition and is susceptible to attack by only a few specialized micro-organisms<sup>19,20</sup>, only wood particles ground small enough to disrupt most of the ligneous encrustations would expose more cellulose and permit more rapid decomposition.

The addition of ammonium nitrate with the various sawdust sizes did not stimulate oxygen uptake (Figs. 3 and 4). Comparison of the apparent oxidative rates with and without the nitrogen addition indicates that in some cases a repression occurred. The inclusion of ammonium in the nitrogen source eliminated the possibility that some

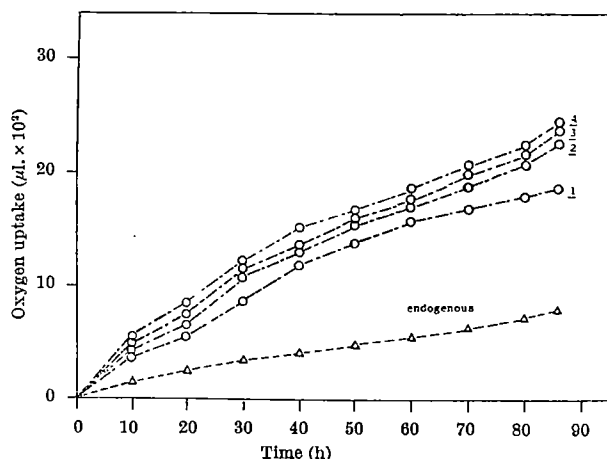


Fig. 4. Oxygen uptake by different size-groups of Douglas fir wood particles with nitrogen added in form of ammonium nitrate in soil. Average particle size: 1,  $660 \times 3,120\mu$ ; 2,  $106 \times 318\mu$ ; 3,  $4.0\mu$ ; 4,  $1.8\mu$ .

cellulose decomposers would be unable to utilize the nitrogen because of inability to synthesize nitrate reductase. It seems doubtful that a salt effect occurred due to accumulation of nitrates as suggested by Allison *et al.*<sup>2</sup>; likewise, the small amount of ammonium nitrate added to the soil, less than 0.03 per cent, could have little osmotic effect on the soil microflora. On the other hand, added ammonium nitrate may have relieved the microbes from the necessity of decomposing nitrogenous soil organic matter for their nitrogen requirements, thus decreasing the overall oxidative rate, even though the added sawdust may be more extensively attacked. In macro-respiration experiments with nine species of hardwood, not including alder, Allison *et al.*<sup>3</sup> found that in 60 days, 30 per cent of the carbon was released as carbon dioxide in the absence of fertilizer nitrogen, and 45 per cent in its presence. Comparison of Figs. 1 and 2 indicates that the addition of ammonium nitrate with the red alder wood strongly depressed uptake of oxygen in all cases after 10 h. A similar, but less marked, effect occurred with Douglas fir. An increase in evolution of carbon dioxide from organic matter of wide C/N ratio apparently will not result from addition of mineral nitrogen if available nitrogen in the soil is sufficient to care for microbial requirements.

The results indicate, within limits of the experimental method, that when sawdust is added to soil the resultant influence on microbial activity is largely dependent on the size of the particles.

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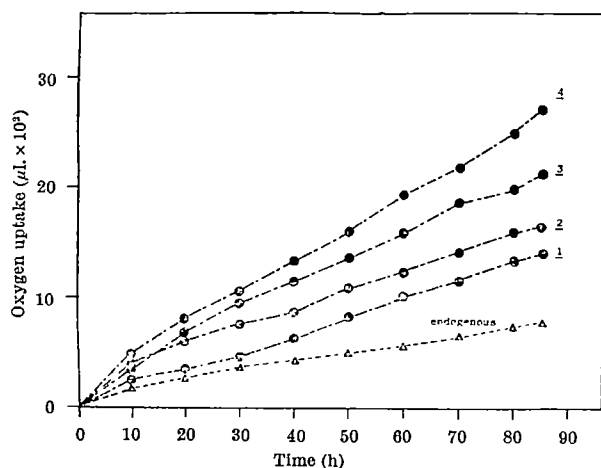


Fig. 3. Oxygen uptake by different size-groups of red alder wood particles with nitrogen added in form of ammonium nitrate in soil. Average particle size: 1,  $725 \times 3,540\mu$ ; 2,  $105 \times 280\mu$ ; 3,  $4.4\mu$ ; 4,  $1.6\mu$ .

## LETTERS TO THE EDITOR

## ASTRONOMY

**Disappearance of the Polar Cap on Mars in 1956 after a Solar Flare with Ejection of Particles**

THE reason for the extraordinary and sudden disappearance of the south polar cap on Mars during its perihelion opposition in 1956 due to outside influence, primarily that of solar activity, was considered at the time of the opposition<sup>1</sup>. It is only now that two such series of processes as the exceptionally important solar activity on the Sun towards the end of August with a large flare on August 31, 1956, of the Y type, connected with the emission of cosmic and sub-cosmic radiation<sup>2-5</sup>, and the extraordinarily frequent occurrence of intensive dust storms after August 20 with the disappearance of the polar cap on Mars during August 31–September 1, 1956 (refs. 1, 6–10), are being physically related.

The commencement of the extraordinary development on Mars was recorded at the observatory in Ondřejov, by P. Příhoda, who on the night of August 19–20 saw three projections on the terminator of the planet, obviously low clouds above Mare Australe, Argyre and Vulcani Pelagus. On August 22, Bronshten<sup>1</sup> and Sharonov<sup>10</sup> in the U.S.S.R. observed a pronounced lightening in colour in the Argyre region: this they interpreted<sup>11</sup> as being a snow (hoarfrost) deposit which was later covered with dust clouds. The formation of large light spots on the surface of Mars was found photographically by Pickering as long ago as April 9, 1890 (ref. 12), but no analogous phenomenon has as yet been observed on such a scale during this century. On the night of August 24–25, Sadil<sup>8</sup> at the Popular Observatory in Prague, as well as other observers in this region, observed a yellowish cloud, the brightness of which was comparable with a polar cap. On the following days the dust cloud spread further.

Up to September 28, 1956, the appearance of the cap was normal, but on the night of August 29–30 it became less clear, took on a yellowish tint and became bordered by an unusually contrasting polar fringe<sup>13</sup>. On blue photographs taken on August 30 it was invisible<sup>10</sup>. At 21 h U.T. on August 31 the cap disappeared<sup>14</sup> and the region became covered by a yellowish fog. Sadil<sup>8</sup>, using a red filter, observed a remnant of the cap as an insignificant brilliant spot on the days following. At the same time, only small white spots appeared at the original location of the cap<sup>15</sup>. On September 7 the cap was again visible even without a filter; its dimensions increased, and on September 15 it had attained its normal appearance and brightness<sup>8,16</sup>.

The foregoing observations indicate that the cap, or at least a large part of it, really disappeared and was renewed in the first ten days of September. A similar opinion is held by Kuiper<sup>9</sup> and Dzhipiashvili<sup>16</sup>. It is indeed difficult to explain all the phenomena described as merely phenomena occurring in the atmosphere of Mars due to internal effects. The possible influence of the exceptionally strong solar activity after August 20 and the flare on August 31, 1956, greatly facilitates an explanation of these phenomena.

Flares giving an increase in cosmic radiation that can be recorded on the Earth's surface are very exceptional phenomena; flares accompanied by sub-cosmic (proton) radiation, however, occur more frequently. A flare of importance 3 on August 31, 1956, was observed at a number of astronomical observatories including that at Ondřejov: position 14° N., 14° E., commencement 12.26; phase Y—ejection 12.40; first cosmic particles on Earth ~12.46 and increase in cosmic radiation in maximum about 2 per cent<sup>4</sup>; range of relatively slower solar protons

according to effect of polar cap absorption of cosmic radio noise 14.30; the peak integral proton intensity with energy >10 MeV was 192 (cm<sup>2</sup> sec ster)<sup>-1</sup> according to Bailey<sup>17</sup>.

Since the cloud of cosmic and sub-cosmic particles from this flare still reached the Earth on August 31, Mars must necessarily have been hit in view of its suitable spatial location with respect to the Earth and Sun at the time of opposition. Moreover, it is seen<sup>18</sup> that streams of a cloud of particles are very extensive at a distance of 1 a.u. in space, so that the probability of hitting planets is large. The situation in the interplanetary space according to measurements of cosmic radiation at Mount Washington in August 1956 was such that already in the second and third thirds of August Forbush effects had occurred. Such effects indicate the existence of an extraordinary cloud of solar particles with magnetic field in the space around the Earth. From August 31 to September 1, 1956, after the increase in cosmic radiation, a far larger Forbush effect than the foregoing occurred. Thus from September 1 to about September 7 the interplanetary space in the direction towards the Earth and Mars must have been filled with very dense clouds of slow and very fast particles from important flares on the Sun.

Short-wave radiation in the period of the large flare on August 31, and particularly the ejection of particles having velocities in a range of 10,000–100,000 km sec<sup>-1</sup>, by penetrating deep enough into the atmosphere of Mars could cause photochemical and temperature changes of such extent that some of the aforementioned extraordinary processes in the atmosphere of Mars could occur at the end of August and beginning of September 1956. Apart from the already published hypotheses, we would explain the disappearance of the cap by the fact that during extraordinary X-emission from a flare, and particularly during the penetration of the particles, an increase in temperature occurred which led to the sublimation process of evaporation of crystals of which, it is deduced, the atmospheric part of the cap consists. The interval and duration of the disappearance of the cap, about 6 days, correspond to the interval and duration of a particularly intensive Forbush effect on records of cosmic radiation<sup>4</sup>.

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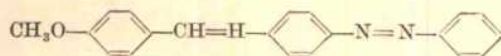
## PHYSICS

## Domain Structures in Liquid Crystals, induced by Electric Fields

THE recent report by Williams<sup>1</sup> of domain structures in *p*-azoxyanisole appeared at a time when we were obtaining similar effects with related compounds in these laboratories. Until the appearance of Williams's report, there appears to have been nothing published on electric field effects since 1943 (ref. 2). In reviewing the contradictory theories of a number of workers, Gray<sup>3</sup> has concluded that the nature of the orientating effects of applied electric fields is not clear, and the directions in which the major axes of the molecules become orientated with respect to the field are uncertain. An extensive investigation is required to resolve the existing contradictions.

We have observed large domains in liquid crystals, differing somewhat in size and structure from those reported by Williams, and produced by smaller electric fields. The samples were melted between plates of electrically conductive glass, and were usually about 0.5 mm thick. The compounds examined were those exhibiting a nematic mesophase between certain temperature limits. Compounds differed widely in the patterns observed and in their response to d.c. and a.c. fields.

The first experiments were made with *N*-*p*-methoxybenzylidene-*p*-phenylazoaniline:



with a solid to nematic transition at 185°–186° C. The compound was held a few degrees above the transition temperature and an electric field applied from a potentiometer. With a d.c. field of between 100 and 200 V/cm



Fig. 1

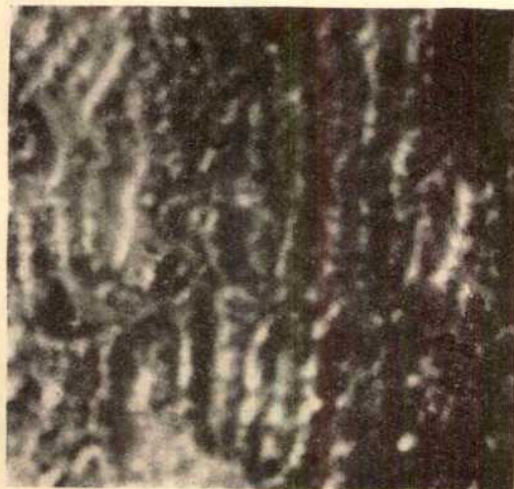


Fig. 2

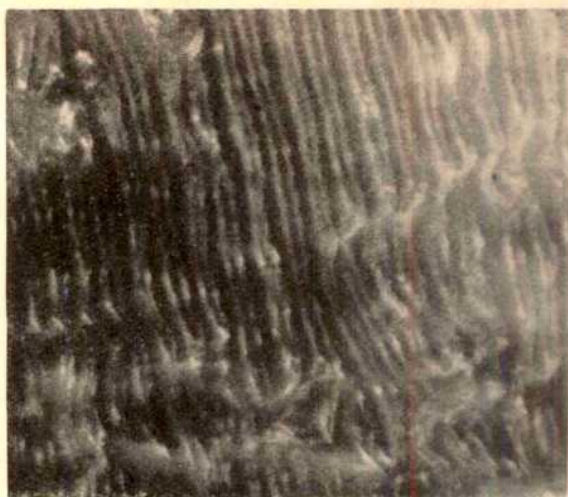
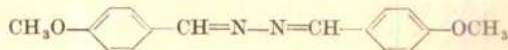


Fig. 3

a pattern of large parallel domains could be seen clearly by transmitted light with the naked eye, and was very prominent under a magnification of only four times. Fig. 1 shows the appearance of the sample at the optimum field strength of about 160 V/cm, and a magnification of 4. An enlarged view in Fig. 2, at a magnification of 25, shows the irregularities and reveals a nodular fine structure. Below a field strength of 100 V/cm, little ordered structure was observed, and above 200 V/cm the structure became finer and less well defined. At 600 V/cm all ordered structure had disappeared, giving a moving mass of small scintillating particles. No similar effects could be obtained with a.c. fields.

Comparative tests with *p*-azoxyanisole have shown that it behaves in a similar manner to that described by Williams, and gives a much finer domain structure, with no well-defined pattern visible to the naked eye.

In contrast, *p*-anisalazine:



with a transition point at 165° C, again exhibited the formation of macro-domains, which were thinner and smoother than those of *N*-*p*-methoxybenzylidene-*p*-phenylazonaniline. The optimum d.c. field strength for domain formation is about 100 V/cm, but a slightly clearer pattern is obtained from a 100-V/cm root mean square 50-c/s a.c. field. Both d.c. and a.c. fields had minimum critical values of about 70 V/cm, and the structure began to break up above 200 V/cm. Fig. 3 illustrates the structure obtained in a 100-V/cm a.c. field, at a magnification of 25 times. A curious feature is the 'pairing' effect of the lines.

The arrays of domains shown in the photographs contained units up to 5 mm long, with widths of 0.1–0.2 mm. The patterns closely resembled those obtained for domains in ferroelectric<sup>4</sup>, ferromagnetic<sup>5</sup> and ferrimagnetic materials<sup>6</sup>. The macro-domains took approximately 3 sec to form after applying the field and a similar period to disappear after switching off. While they were clearly visible by direct illumination, viewing between crossed 'Polaroids' increased the definition. Very clear patterns were also obtained in a Schlieren optical system.

It is clear that existing theories<sup>2</sup> are not adequate to explain the behaviour of mesomorphic materials in electric fields. A comprehensive theory must account for the following facts: (a) Compounds with similar structures, for example, *p*-azoxyanisole and *p*-anisalazine, give widely different types of domain pattern, at low field strengths in the region of 100 V/cm. (b) Closely related compounds, for example, *p*-anisalazine and *N*-*p*-methoxybenzylidene-*p*-



phenylazoaniline, behave differently in alternating fields, the former giving a well-defined macro-domain structure, but the latter remaining relatively unaffected. (c) The macro-domain structure, produced by low field strengths of 100–200 V/cm, breaks down completely for a small increase in the field to 500–600 V/cm, but not because of electrolysis or mass transport.

The foregoing observations were made in the course of a research programme on the switching of light beams by using small electric fields. The light transmission of a film of *N*-*p*-methoxybenzylidene-*p*-phenylazoaniline in the nematic phase showed large changes when fields were applied: a field of 2,000 V/cm decreased the transmission to about 35 per cent of its initial value. The effect was apparently produced by an increase in the light-scattering properties of the film. Under a field of 2,000 V/cm, the liquid displayed a mass of fast-moving scintillating particles.

We thank Standard Telecommunication Laboratories, Ltd., on whose behalf this work was carried out, for permission to publish it.

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### Electrification of Porous Alumina on Desorption of Gases

ELECTRIFICATION of some dusts by friction, and, hence, the association of sandstorms with static electricity, are well known; laboratory work on the subject has been summarized in two publications dealing with the study of small particles<sup>1,2</sup>. Further work has shown<sup>3</sup> that particles of sodium chloride and, hence, dry sodium chloride aerosol, may, in some circumstances, acquire strong electric charges by emission of photoelectrons. We wish to report here yet another form of electrification of some small particles. This occurs when small particles desorb gas, and it would seem that both the magnitude and the sign of electrification are associated with the chemical treatment which was given to the substance during its preparation.

The present experiments were carried out on three types of Merck activated alumina (reagent purity, marketed for use in chromatography), which are available under the names of 'Alkaline', 'Neutral' and 'Acid' alumina. Although data on the exact procedure of preparing the three varieties have not yet been released for publication (Merck, personal communication), it can be assumed that amphoteric alumina is prepared so as to contain an excess of alkali (such as Na) or acid species, which it may exchange. Hence, 10 per cent aqueous suspensions of such alumina preparations reach, with time, pH values of 9.5, 7.0 and 4.5, in the case of 'Alkaline', 'Neutral' and 'Acid' products, respectively.

The products were desiccated in an electric oven, ground in an agate mortar to pass through a -270 Mesh (ASTM), and then separated into fairly uniform fractions by centrifuging<sup>4</sup> (see Fig. 1). Those fractions which had a specific surface area of about 180 m<sup>2</sup> × g<sup>-1</sup> (as measured by the Brunauer–Emmett–Teller method at low relative pressures of nitrogen) were later evacuated in suitable 'Pyrex' vessels at 250°C for at least 24 h. When the vacuum reached a value of less than 10<sup>-4</sup> torr, the fractions were adsorbed and compressed for 60 min to about 400 torr above atmospheric pressure, with pure

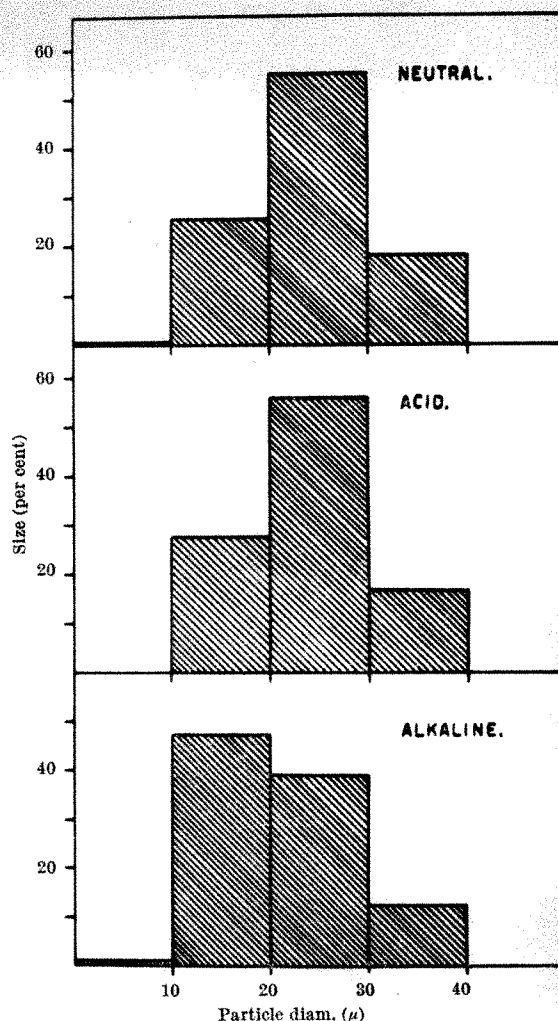


Fig. 1. Percentage particle-size, obtained by microscopy, of the three types of alumina used

nitrogen, oxygen, nitrous oxide, or dry laboratory air. This treatment appeared to make the particles electrically charged for prolonged periods of time, as revealed by tests using an electrometer, and this charge appeared to depend on the desorption of gas from the solid. Thus, in order to evaluate the effects obtained, a series of experiments was carried out using the technique described in ref. 3. Briefly, the method consisted of allowing a stream of monodisperse particles of known apparent density and size to trickle between charged plates. Calculations were made of both the initial charge and the rate of charging from the trajectory of the stream, which was photographed under suitable illumination.

It had been found earlier that the following equation held:

$$l_v = \frac{\epsilon^2 g^2}{108 E \mu \alpha} d^3 \frac{l_h}{l_v} - q_0 \frac{\epsilon g}{18 \pi \mu \alpha} \quad (1)$$

where:  $l_v$  was the vertical component, in centimetres, of the distance of fall, from the entry of particles into the electric field;  $l_h$ , the horizontal component;  $d$ , the diameter of particles (cm);  $\epsilon$ , the apparent density of particles, g × cm<sup>-3</sup>;  $g$ , the acceleration due to gravity, taken here as 980 cm.sec<sup>-2</sup>;  $\mu$ , the viscosity of the air, taken here as 185 × 10<sup>-6</sup> poise;  $E$ , the electrical field in c.g.s. units;  $q_0$ , the charge on particles before entry into electric field, in E.S.U.;  $\alpha$ , the rate of charging of particles during their fall, in E.S.U.sec<sup>-1</sup>cm<sup>-2</sup>.

Equation (1) makes it possible to estimate both  $q_0$  and  $\alpha$  from graphical plots of  $l_v$  versus  $l_v/l_h$ . We found that

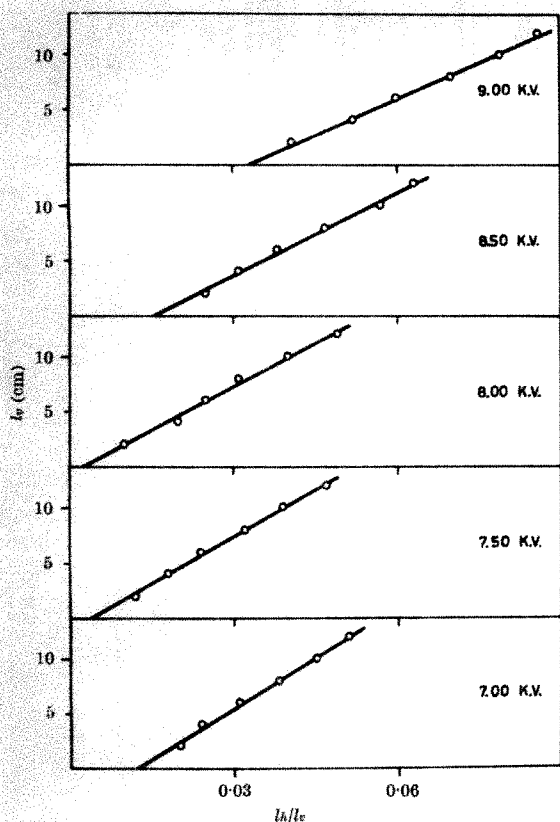


Fig. 2. Example of plots, according to equation 1 of data obtained from 8 photographs per diagram, using alternately both polarities, and average results. Each plot represents a series of experiments carried out at different values of the electric field between charged plates at a distance of 8.0 cm, and with 'Neutral' alumina which was charged with nitrogen under pressure. All results obtained are summarized in Table 1;  $\alpha$  was computed from slopes and hence  $q_0$  from intercepts

charging the substance with nitrous oxide ensures a slow prolonged evolution of gas (as observed gravimetrically) and similarly, in the cases when nitrogen was used, reproducible results could be obtained; Fig. 2 illustrates an experimental plot obtained using nitrogen gas on 'Neutral' alumina, while Table 1 gives the results obtained using both nitrogen and nitrous oxide on all types of alumina. It can be seen that, while both the 'Alkaline' and 'Neutral' species of alumina show, during the period of time immediately following decompression, a strong tendency towards positive electrification, the acid particles reveal a very small negative electric effect which, although too small to be revealed by the method described, can be qualitatively detected by means of an electrometer.

Hanle *et al.* have recently observed a process of electrical charging of solids when water of crystallization evaporates from some salts<sup>5</sup>, and Mühleisen found polarity fluctuations of electrical charges on dusts, which depended on whether the particles adsorbed or desorbed water<sup>6</sup>. A similar occurrence, which appears to be associated with the desorption of gas, is observed here and may have some

Table 1. SUMMARY OF DATA ON ELECTRIFICATION OF ALUMINA UNDER DIFFERENT CONDITIONS

Alumina	Average diameter ( $\mu$ )	Computed density ( $\text{g cm}^{-3}$ )	Gas used for compression	Average $q_0$ (E.S.U.) $\times 10^8$	$\alpha$ (E.S.U. sec <sup>-1</sup> cm <sup>-2</sup> ) $\times 10^9$
'Alkaline'	21 $\pm$ 7	1.8 $\pm$ 1.0	N <sub>2</sub>	+1.6 $\pm$ 1.0	+2.4 $\pm$ 1.0
'Neutral'	25 $\pm$ 6	1.3 $\pm$ 0.7	N <sub>2</sub>	+3.8 $\pm$ 1.0	+1.4 $\pm$ 0.5
'Acid'	24 $\pm$ 6	1.6 $\pm$ 0.8	N <sub>2</sub>	Very small negative	/
'Alkaline'	21 $\pm$ 7	1.8 $\pm$ 1.0	N <sub>2</sub> O	-0.1 $\pm$ 0.5	+1.9 $\pm$ 0.7
'Neutral'	25 $\pm$ 6	1.3 $\pm$ 0.7	N <sub>2</sub> O	+0.7 $\pm$ 0.5	+1.5 $\pm$ 0.5
'Acid'	24 $\pm$ 6	1.6 $\pm$ 0.8	N <sub>2</sub> O	Very small negative	/

Apparent density of powders was computed on the basis of an average of 25 readings of times of fall of substances examined, in glass tube 150 cm long, 4 cm internal diameter, at 25° C.

bearing, in Nature, on certain problems related to atmospheric electricity. Electrification of this type may be expected to occur on certain types of particles when they are carried upwards into regions of lower atmospheric pressure; furthermore, depending on the nature of the soil, 'desorption' of electrical charges may be expected to occur in suitable atmospheric conditions (such as, for example, fluctuations of pressure), and to be one of the causes of the 'electrode effect' (see, for example, the recent paper by Crozier<sup>7</sup>).

We thank Prov. V. Caglioti for his interest in this work, Mr. Luttazzi for his help, and 'Cassa per il Mezzogiorno' for financial assistance.

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### Single Bi-particle Elastic Collisions of Fast Protons with Metals

RECENT experiments<sup>1,2</sup> have shown that when metals are bombarded with energetic protons and ions, charged particles are emitted along with neutral particles. The present work was carried out to analyse the emitted ions and to establish that fast protons and molecular hydrogen ions make single bi-particle elastic collisions with atoms lying on a metal surface where they behave as if they are effectively isolated from the lattice.

The technique used to locate the emitted ions differed from conventional methods so far used in mass spectrometry. The ions were received on a metal collecting plate after traversing a sector magnetic field, and their position was determined by the visible light they emitted at the point of impact, where they caused metal electron plasma oscillation giving rise to soft electromagnetic radiation<sup>3</sup>.

The ion beam was obtained from a 30-Mc/s radio-frequency source and was accelerated in the Cockcroft-Walton apparatus at this Laboratory. Protons and hydrogen molecular ions, while passing through a fine canal, bored along the axis of a pure copper rod, 4 cm long and 1 cm diameter, struck atoms lying on its surface and transferred their energy and momentum to them. The maximum value of energy  $E_2$  and of momentum  $P_2$ , which are transferred in an elastic bi-particle collision by an incident ion of mass  $M_1$  to a target atom of mass  $M_2$ , are given by the equations:

$$E_2 = 4 \frac{M_1 M_2}{(M_1 + M_2)^2} \cdot E_1 \quad (1)$$

$$P_2 = 2 \frac{M_2}{(M_1 + M_2)} \cdot P_1 \quad (2)$$

where  $E_1$  and  $P_1$  are the energy and momentum respectively of the incident ions.

The ions emitted from the canal were confined by its geometry to a narrow pencil, almost parallel to incident protons, and possessed groups of particles of discrete energies. Along with the incident ion beam, they entered a brass chamber, 20  $\times$  15  $\times$  3 cm, placed about 3 cm below the canal, and described trajectories in the sector field the radii of curvature of which depended on their

charge and momentum. They fell on the metal wall of the chamber and emitted light at the points of impact; this could be observed through a glass plate which had been fixed on the opposite side of the chamber with soft 'Araldite'. The position of protons and hydrogen molecular ions was always well defined on the chamber wall, and their deflexion due to magnetic field, thus determined, agreed within probable error of measurements with our theoretical calculations.

The field of the electromagnet was calibrated with a Grassot's fluxmeter within the pole pieces and outside, extending to the copper canal and the side walls of the analyser. The chamber, together with the accelerating column, was evacuated by a fast oil pump backed by a Kinney pump through a 'Freon' cold trap, and the pressure inside it was kept as low as  $10^{-6}$  mm mercury during the experiments.

At an incident ion energy of 360 keV and with a suitable fixed magnetic field, well-defined spots could be seen on the metal wall of the analyser. These were due to singly and multiply charged copper ions, such as  $\text{Cu}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Cu}^{3+}$ ,  $\text{Cu}^{4+}$  and  $\text{Cu}^{5+}$ , which were emitted from the canal surface by fast protons and molecular hydrogen ions. The spots due to  $\text{Cu}^{2+}$  ions overlapped with those due to incident ions causing their emission, but all other spots were well separated from one another, indicating that there was no appreciable energy spread among the emitted ions belonging to a particular group and entering the analyser. The energy and momentum of these ions, as calculated from the deflexion they experienced in the magnetic field, were in agreement, within experimental error, with equations (1) and (2). Copper ions carrying more than five charges were not detected within the energy range of the incident ions investigated. At an energy of 100 keV of the incident ions there were three spots, at 200 keV five, while at 360 keV all the aforementioned species of charges appeared. This made us believe that the efficiency of emission of ions and the multiplicity of their charge rise with energy of the incident beam.

We were not able to detect any ions of impurities which, it was suspected, might be present in the discharge tube of the radiofrequency ion source, and hence we believe that all the emitted Cu ions were due to single bi-particle elastic collisions of protons and hydrogen molecular ions with Cu atoms lying on the surface of the canal. There is some uncertainty as to the nature of one or two rather faint spots which occurred in the momentum spectrum (which was spread over a length of 10–11 cm). The only plausible explanation that we can offer is that they were formed due to multiply charged ions the positive charge of which was degraded during their flight in the trajectories in the magnetic field from the canal to the collector plate. No attempt was made to detect whether complex particles<sup>3</sup>, for example of any compound of copper, were emitted in these experiments from copper surface. If they were, they overlapped with Cu ions.

The incident beam current on the copper canal and the thick metal disk which held it in position varied between 30 and 40  $\mu\text{amp}$ , while the energy of the incident ions ranged between 100 keV and 360 keV in these experiments.

We thank His Excellency Nawab Malik Amir Mohammed Khan, Governor of West Pakistan, for his encouragement to our research, and Sir John Cockcroft for his interest in these experiments.

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## GEOFYSICS

### Role of Plasma Instabilities in Auroral Phenomena

RECENTLY it was proposed that plasma instabilities are responsible for the auroral precipitation of particle trapped inside the magnetosphere<sup>1</sup>. The entire event would be a two-step process, one which takes particle inside the magnetosphere and one which dumps the trapped particles. Responsible for the second step would be plasma micro-instabilities of the type outlined by Krall and Rosenbluth<sup>2</sup>.

Against this view is the fact that the number of particles and the amount of energy contained inside the radiation belts are barely sufficient to supply one auroral event<sup>3</sup>. Moreover, micro-instabilities are not the most suitable ones for explaining gross plasma motion and can be inhibited by a number of factors<sup>4</sup>, such as the presence of a shear in the magnetic field.

The necessity of overcoming these objections leads one to look for plasma macroscopic instabilities which exist in collisionless regimes and do not respect the constraint that particles move together with the lines of magnetic field. Instabilities of this nature may be suitable for explaining the first step, as they would allow potential energy stored in the magnetic field of the thermalized solar wind to be transferred into kinetic energy. This process could occur inside the magnetopause or on the corresponding nightside.

In order to avoid having to consider the trapping and dumping phase, it is proposed that the instability excites plasma waves and then accelerates particles according to a pattern of the type considered by Stix<sup>5</sup>.

Although at the present stage it is difficult to identify one definite type of plasma instability as being responsible for the auroral process, I would mention that two classes of macroscopic collisionless instabilities have been investigated<sup>6,7</sup>. One of them<sup>7</sup> is related to the appearance of an electric field parallel to the lines of the magnetic field, so that the 'frozen-in law' does not hold, due to anisotropic pressure and ion gyro radius effects. The driving factor is a spatial gradient of the longitudinal electron pressure or a gradient of the density together with transverse pressure gradients. In the case when the equilibrium magnetic field has no shear, the growth rate of this instability is of the order:

$$\gamma \approx k \frac{p_{e\parallel}}{\rho \Omega}$$

where  $k$  is the wave number transverse to the magnetic field,  $p_{e\parallel}$  the transverse gradient of the longitudinal electron pressure,  $\rho$  the mass density and  $\Omega$  the ion gyro frequency. Assuming typically that:  $p_{e\parallel}/\rho \approx v_{the}^2$ ,  $R \approx 10^9$  cm,  $v_{the} \approx 4 \times 10^7$  cm/sec,  $B \approx 10^{-3}$  gauss,  $k \approx 10^{-7}$  cm<sup>-1</sup>, we obtain  $\gamma \approx 10^{-1}$  sec<sup>-1</sup>. For shorter wavelengths, that is  $k \approx 10^{-6}$  cm<sup>-1</sup>, the growth time becomes  $10^{-1}$  sec, which seems to be consistent with the duration of burst of auroral X-rays observed recently<sup>8</sup>.

I thank Drs. P. A. Sturrock and R. Wentworth for interesting discussions, and Drs. J. W. Chamberlain and J. I. Valerio for their comments. This work was supported by an Air Force Office of Scientific Research contract AF 49(638)-1321.

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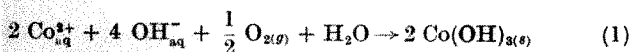
## GEOCHEMISTRY

Formation of Cobalt (III) in the Amorphous  $\text{FeOOH} \cdot n\text{H}_2\text{O}$  Phase of Manganese Nodules

RECENT discussions<sup>1</sup> on the nature of 3d transition-metal ions in sea water and in manganese nodules have rejected the possibility that oxidation of cobalt to Co (III) occurs in marine environments. A detailed electron microprobe study of manganese nodules<sup>2</sup> from diverse geographical localities supports the belief<sup>3</sup> that there is a cobalt-iron inter-element relationship in the nodules. Cobalt is enriched in the X-ray amorphous  $\text{FeOOH} \cdot n\text{H}_2\text{O}$  phase in those nodules which contain appreciable amounts of iron. The results indicate that Co (III) and Fe (III) constitute an 'isomorphous' pair in the amorphous  $(\text{Fe}, \text{Co})\text{OOH} \cdot n\text{H}_2\text{O}$  phase, which may be recrystallized to goethite on treatment with hydroxylamine hydrochloride.

Thermodynamic calculations support the hypothesis that  $\text{Co}^{2+}$  in sea water may be oxidized to  $\text{Co}(\text{OH})_3$  under certain conditions. The model on which the calculations are based is as follows:

Reaction:



$$\Delta G_{298}^0 = -52.53 \text{ k cal mole}^{-1} \text{ (ref. 4)}$$

$$\Delta S_{298}^0 = 60 \text{ cal deg mole}^{-1} \text{ (ref. 4)}$$

$$\Delta G_{275}^0 = -51.1 \text{ k cal mole}^{-1} \text{ (calc.)}$$

$$K = [(a_{\text{Co}^{2+}})^2 (a_{\text{OH}^{-}})^4 (a_{\text{O}_2})^{1/2}]^{-1}$$

Concentration of  $\text{Co}^{2+}$  in the Pacific Ocean<sup>5</sup>:  $0.38\text{--}0.67 \mu\text{g/l.}^{-1}$  or  $0.645\text{--}1.138 \times 10^{-8} \text{ m.}$

A typical depth for the formation of manganese nodules is 3,000 m (ref. 6)

pH of sea water at 3,000 m (ref. 7): 8.0;  $([\text{OH}^-] = 10^{-6} \text{ m})$

Temperature of the ocean at 3,000 m (ref. 7):  $2^\circ \text{C}$  ( $275^\circ \text{K}$ )

Average salinity of sea water at 3,000 m and  $2^\circ \text{C}$  (ref. 7): 35 g/1,000 g of sea water, corresponding to an ionic strength of approximately 0.6 m

Activity coefficient of  $\text{Co}^{2+}$  in a 0.6-M solution at  $2^\circ \text{C}$  (ref. 8): 0.49

Activity coefficient of  $\text{OH}^-$  in a 0.6-M solution at  $2^\circ \text{C}$  (ref. 8): 0.68

Partial pressure of oxygen at the sediment-water interface<sup>6</sup>: 0.25 atmosphere

Calculation: Taking  $[\text{Co}^{2+}]$  as  $10^{-8} \text{ m.}$

$$K = [(10^{-8} \times 0.49)^2 \times (10^{-6} \times 0.68)^4 \times (0.25)^{1/2}]^{-1} = 3.90 \times 10^{11}$$

$$\Delta G_{275} = \Delta G_{275}^0 + RT \log_e K = +1.24 \text{ k cal mole}^{-1}$$

Therefore, reaction (1) is unfavourable when the  $\text{Co}^{2+}$  ion concentration is  $10^{-8} \text{ M.}$  However, the reaction has a negative free energy when the  $\text{Co}^{2+}$  ion concentration exceeds  $1.3 \times 10^{-6} \text{ M}$  (Fig. 1). Furthermore, reaction (1), which is probably catalysed by  $\text{Fe}(\text{OH})_3$ , is favoured by  $\text{Co}(\text{OH})_3$  forming a solid solution with  $\text{Fe}(\text{OH})_3$ . Also, the reaction is more likely to proceed in oxidizing environments.

The question arises whether the  $\text{Co}^{2+}$  ion concentration in sea water ever exceeds  $1.3 \times 10^{-6} \text{ M.}$  The cobalt-rich manganese nodules are found on the floor of the central Pacific Ocean<sup>6</sup> in the neighbourhood of basaltic islands, atolls, and guyots. Most basalts have cobalt concentrations

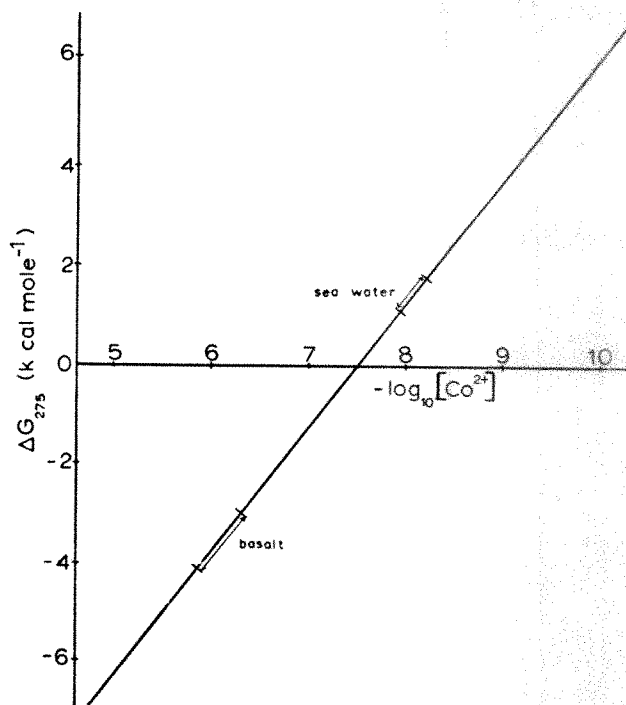


Fig. 1. Variation of  $\Delta G_{275}$  with  $\text{Co}^{2+}$  ion concentration for the reaction:  $2 \text{Co}_{\text{aq}}^{2+} + 4 \text{OH}_{\text{aq}}^{-} + \frac{1}{2} \text{O}_{2(g)} + \text{H}_2\text{O} = 2 \text{Co}(\text{OH})_{3(s)}$ . Ranges of the  $[\text{Co}^{2+}]$  in sea water and basalt are indicated

between 30 and 80 p.p.m. (ref. 10) ( $0.5\text{--}1.4 \times 10^{-6} \text{ M}$ ). Therefore, submarine vulcanism with subsequent leaching of the basaltic glass or palagonite could raise the cobalt concentration of sea water above  $1.3 \times 10^{-6} \text{ M}$  locally. This would favour reaction (1), and account for the enrichment of cobalt in the amorphous  $(\text{Fe}, \text{Co})\text{OOH} \cdot n\text{H}_2\text{O}$  phase of manganese nodules. The low cobalt content in manganese nodules which formed in areas adjacent to the American continent<sup>6</sup> may be attributed to a lack of basaltic bed-rock and to the reducing environment in the sediments.

Several simplifications are inherent in the calculations here. In particular, the effect of pressure at depth on the reaction, the variation of the ionization constant of water with temperature, and the presence of dissolved cobalt in a form ( $\text{CoSO}_4$ ,  $\text{CoCl}_4^{2-}$ , etc.) other than the simple  $\text{Co}^{2+}$  ion have been ignored. Nevertheless, the calculations demonstrate that oxidation of cobalt to a hydrated cobaltic oxide is a feasible process in certain marine environments. Similar calculations for nickel indicate that oxidation of  $\text{Ni}^{2+}$  to  $\text{Ni}(\text{OH})_3$  is unlikely ( $\Delta G_{275} = +21 \text{ k cal mole}^{-1}$  when  $[\text{Ni}^{2+}] = 10^{-7} \text{ M}$ ).

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## METALLURGY

## Grain Size of Ausformed Steel

THE grain size of the ultra-strong, ausformed steels is probably an important factor in their strength and ductility, but it is yet uncertain. The structures are so fine and complex that even the electron microscope has not hitherto revealed the grain structure clearly. However, a flexible dark field technique described previously, using the EM6 electron microscope, has been found to reveal it satisfactorily<sup>1</sup>.

Fig. 1 shows the structure, as seen under ordinary light-field conditions, of an ausformed steel, details of which are given below. The appearance can be varied a good deal by tilting the specimen, but there is usually uncertainty, as in Fig. 1, where one grain ends and another begins. Fig. 2 shows the diffraction pattern from this area; the great number of spots produced by the tiny area ( $\sim 10\mu^2$ ) bathed by the electron beam immediately makes it clear that the separate reflecting units are minute. That part of the pattern which is covered by a circular half-tone patch was aligned along the microscope axis, the objective aperture placed in position, and Fig. 2 was obtained; the patch is the objective aperture.



Fig. 1. Ausformed steel. Light-field electron micrograph ( $\times 27,000$ )

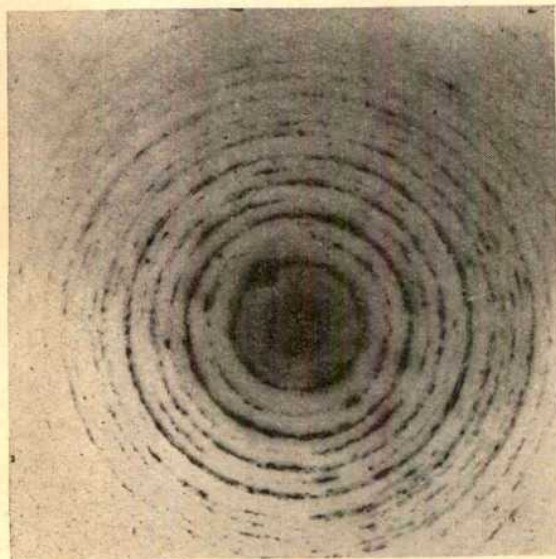


Fig. 2. Diffraction pattern of the area shown in Fig. 1

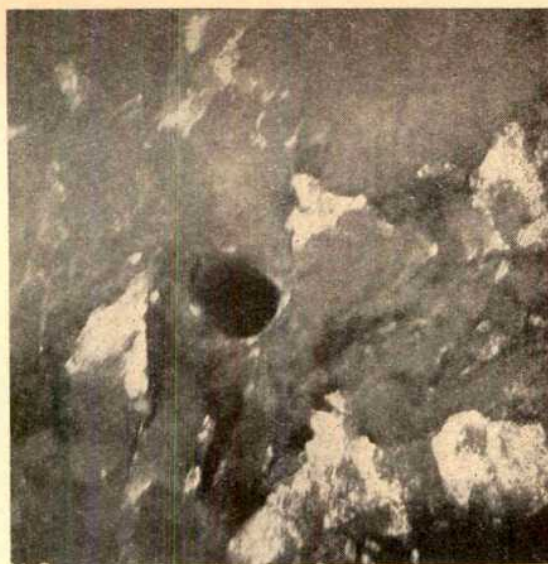


Fig. 3. Same area as shown in Fig. 1, dark-field illumination ( $\times 27,000$ )

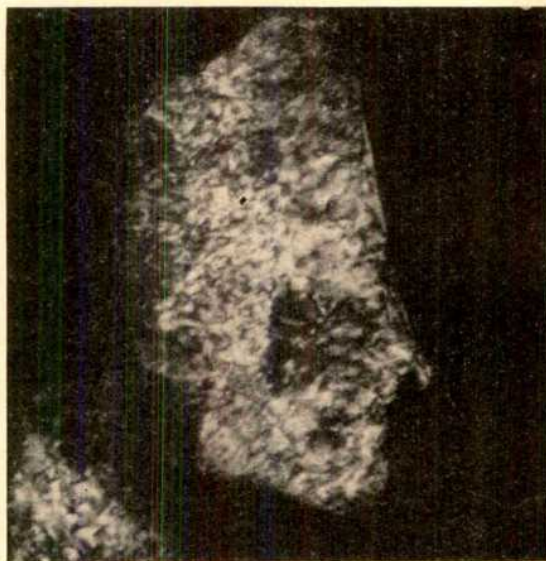


Fig. 4. Another area, dark field ( $\times 140,000$ )

With the illumination so adjusted, Fig. 3 was then photographed. Fig. 3 is therefore the dark-field counterpart of Fig. 1. In Fig. 3 there are bright areas in a generally dark ground. The bright areas are, of course, the regions which diffract the beams selected in Fig. 2. They can be seen in Fig. 3 to be contained for the most part in a few irregularly-shaped regions or grains which are patchily illuminated. If the specimen was now tilted, a bright area would typically migrate across the grain containing it during a tilt of a few degrees and then disappear, and a new bright area might flash up elsewhere beginning to outline another grain. The irregularly-shaped regions are therefore warped grains of which the ausformed steel appears to be composed. The majority had sizes of 1000–2000 Å, although some seemed to be as small as 100 Å. They are thus very small, as the diffraction pattern had suggested.

Because the illumination in the dark field condition is accurately axial in the microscope, full resolution is available. Fig. 4 is of a bright area magnified  $\times 140,000$  and shows it to contain very many dislocations indeed.

The majority of grains of the same steel in the plain martensitic condition (see below), when examined in the same way, proved to have sizes of between 2000 and 5000 Å.



The yield strength of a steel is known to be related to its grain diameter  $d$  by the Hall-Petch relationship  $\sigma_y = \sigma_i + k_y d^{-1/2}$ , where  $\sigma_i$  represents the resistance to the motion of a dislocation through the metal and  $k_y$  is a constant having a value of about 2 kg/mm<sup>2</sup>.mm<sup>1/2</sup>. Taking the average grain sizes of the ausformed and martensitic samples as 1500 and 3500 Å respectively, the superiority of the ausformed steel so assessed should be 53 kg/mm<sup>2</sup>, which compares quite well with the actual difference of 45 kg/mm<sup>2</sup> (ref. 3). Petch<sup>2</sup> has derived a relation between the tough brittle transition temperature of a steel and its grain size. According to this the ausformed material should have a transition temperature 20°–30° C lower than that of the martensitic. The actual transition temperature of the materials has not yet been determined but, as would be expected from this difference, the ausformed condition is appreciably the more ductile at room temperature, showing a reduction of area of 25 per cent against only 4 per cent for the martensitic.

With the flexible dark-field technique it is therefore possible to investigate the grain structure of ausformed steel. A thorough examination is needed before really firm conclusions can be drawn. However, so far as these results go, in the particular material examined much of the improved strength and ductility caused by ausforming is due to extra-fine grain size.

The samples of steel, and information about them, were made available by Mr. W. E. Duckworth, Dr. Irani and Mr. P. Taylor of the British Iron and Steel Research Association. The steel, which contained 13 per cent Cr and 0.3 per cent C, was austenitized at 980° C. To produce the ausformed condition it had been quenched at 500° C, rolled to 50 per cent reduction at that temperature, and air cooled to room temperature. Its tensile strength and reduction of area at room temperature were 235 kg/mm<sup>2</sup> and 25 per cent, respectively<sup>3</sup>. To produce the martensitic condition it had been water-quenched to room temperature. Its strength and reduction of area were then 90 kg/mm<sup>2</sup> and 4 per cent, respectively<sup>3</sup>.

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## MINERALOGY

### Mass Spectrometric Analysis of the Gas evolved from Some Heated Natural Minerals

It has been known since the late nineteenth century that rocks and minerals evolve gas when heated. Early workers<sup>1,2</sup> were hampered by the lack of adequate methods for analysing small quantities of gas. They attempted to offset this by using large samples, so that their investigations were mainly restricted to whole rocks<sup>3</sup>. The use of modern physical methods has made possible the accurate analysis of small amounts of gas<sup>4</sup> (< 0.1 cm<sup>3</sup> S.T.P.), and a method has been developed for using an A.E.I. MS 10 mass spectrometer to analyse the gas evolved by heated minerals.

Half-gram samples of separated minerals were heated at temperatures up to 1,100° C in a quartz tube attached by a quartz-'Pyrex' graded seal to a grease-free, 'Pyrex' high vacuum system. As the gas was evolved, it was moved by an automatic Toepler pump into a reservoir where water and carbon dioxide were condensed in a trap cooled with liquid air. A known amount of neon was added to the remaining non-condensable gases as an internal

standard. The mixture was analysed using the mass spectrometer which had been calibrated with pure gases and known synthetic mixtures. At the conclusion of the mass spectrometric analysis the non-condensable gases were pumped away and the gas reservoir isolated. The cold trap was brought up to room temperature and the pressure, due to carbon dioxide and water, measured on an oil manometer. The contribution from carbon dioxide was measured after cooling the trap with solid carbon dioxide, and the water content found by difference. Since the reservoir volume had been calibrated, the amounts of the two gases could be found. The mass spectrometer was used to examine the gas from the Shap Fell and Skaergaard minerals for sulphur compounds as well as the gases shown in Table 1. Hydrogen sulphide and sulphur dioxide were found in the gas from the biotite but not in that from the other minerals.

The duplicate determinations give some indication of the reproducibility of results. In comparing analyses it should be remembered that an error which gives a low value for water results in too high a value for carbon dioxide, and that the results for carbon monoxide are less reliable than those for other gases (see footnote to Table 1). More than four months elapsed between the two analyses for plagioclase EG-5052 and the determinations involved different mass spectrometer operating conditions and calibration data. With the exception of methane the agreement is adequate.

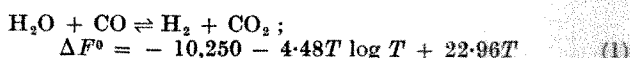
The compositions of the gases evolved by the cogenetic minerals from Sierra Leone are remarkably similar even though the amounts differ by a factor of two. This suggests that the gas composition is independent of crystal structure and chemical composition of the host mineral. It is not possible to compare directly the cogenetic orthoclase and biotite from the Shap granite because of the presence of structural water in the mica.

Plagioclases EG-5052, EG-5181 and EG-4312 from the Skaergaard layered intrusion were taken from heights of 825, 1,800 and 1,925 m respectively (measured upwards from the lowest exposed layer<sup>5</sup>). They show a trend of increasing gas content with height (that is, with progressing crystallization). This late-stage concentration of volatiles is evident petrologically in the late formation of hornblende.

Pyroxene 722X487 gave four times as much gas as the other pyroxenes and plagioclases. It is also anomalous in containing more argon than could be produced by the decay of its potassium content<sup>6,7</sup>.

It is probable that at the time of mineral formation the volatiles were in equilibrium at the prevailing temperature and pressure<sup>8</sup>. If they were trapped in fluid inclusions, reactions would have occurred to maintain equilibria as conditions changed due to cooling, but since the kinetics become increasingly less favourable as temperature falls the final composition would correspond to equilibrium at a temperature above room temperature. This process would be reversed when the mineral was heated, until at about the temperature of formation the inclusions would break open<sup>9</sup> and release the volatiles into low-pressure conditions where reaction would continue in an attempt to reach equilibrium. It is unlikely that equilibrium was reached in the experimental arrangement described here, so that the measured gas composition represents an apparent equilibrium at a temperature and pressure between those prevailing at the time of mineral formation and those in the apparatus.

From the results in Table 1 it is possible to consider two independent equilibria:



$$Q = \frac{(V_{\text{H}_2}/V_T)(V_{\text{CO}_2}/V_T)}{(V_{\text{H}_2\text{O}}/V_T)(V_{\text{CO}}/V_T)} = \frac{V_{\text{H}_2} \cdot V_{\text{CO}_2}}{V_{\text{H}_2\text{O}} \cdot V_{\text{CO}}}$$

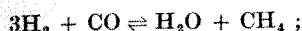


Table 1

Minerals and localities of source rocks	Analysis No.	Heating temp. °C	Heating time h	Gas composition							Total gas cm <sup>3</sup> S.T.P./g	T <sub>quib</sub> reac- tion (1) °C
				H <sub>2</sub> O %	CO <sub>2</sub> %	H <sub>2</sub> %	CO* %	CH <sub>4</sub> %	N <sub>2</sub> %	Ar %		
Gabbro, Freetown, Sierra Leone												
Plagioclase, BA-654	NS 13	975	12	70.44	9.00	12.95	7.27	0.15	0.20	Tr	0.716	1,650
Pyroxene, BA-654	NS 11	975	12	69.48	11.67	12.48	6.00	0.12	0.24	Tr	1.475	1,285
Gabbro, Skaergaard, E. Greenland												
Plagioclase, EG-5052	52	975	12	67.92	7.55	16.76	6.73	0.59	0.42	Tr	0.506	1,465
(duplicate)	NS 3	975	12	67.67	6.67	18.24	5.83	1.15	0.42	Tr	0.564	1,380
Plagioclase, EG-5181	57	975	12	53.82	29.38	13.82	2.75	0.09	0.08	Tr	0.984	595
Plagioclase, EG-4312	58	975	12	62.89	11.28	20.60	4.87	0.27	0.09	Tr	1.127	915
Gneiss, Loch Inver, Scotland												
Pyroxene, 722X487	28	1,034	2	42.70	49.93	5.94	1.29	0.09	0.06	N.D.	5.350	490
(duplicate)	33	1,034	2	44.74	47.60	5.99	1.54	0.08	0.05	Tr	5.325	530
Augite, 290	NS 4	975	12	55.81	11.54	24.81	5.70	1.49	0.33	Tr	1.458	855
Granite, Shap Fell, England												
Orthoclase, 20512	41	975	6.5	67.21	18.11	9.72	4.37	0.29	0.29	0.01	1.317	1,005
(duplicate)	43	975	6.5	67.72	18.62	9.51	3.75	0.26	0.17	0.01	1.241	945
Orthoclase, 20512	NS 9	601	12	82.32	14.77	1.93	0.67	0.18	0.09	Tr	1.058	1,070
Biotite, 20512	S 15	975	12	67.80	4.82	26.91	0.12	0.22	0.11	0.01	19.40	385

Tr = trace. N.D. = not determined.

\* During the mass spectrometric determination the ratio (amount of gas)/(amount of neon internal standard) changes due to fractionation. The carbon monoxide peak took a long time to stabilize and the initial ratio was calculated from the fall off in neon peak height using a factor of  $\sqrt{(20/28)}$  and not found by graphical extrapolation as for the other gases.



$$\Delta F^0 = -47,070 + 16.73T \log T + 3.12T \quad (2)$$

$$Q = \frac{(V_{\text{H}_2\text{O}}/V_T)P(V_{\text{CH}_4}/V_T)P}{(V_{\text{H}_2}/V_T)^3 P^3 (V_{\text{CO}}/V_T)P} = \frac{V_{\text{H}_2\text{O}} \cdot V_{\text{CH}_4}}{V_{\text{H}_2}^3 \cdot V_{\text{CO}}} \cdot \frac{V_T^4}{P^2}$$

where  $V_N$  is the volume of gas  $N$  and  $V_T$  is the total volume of gas measured at the same pressure as  $V_N$ ;  $P$  is the pressure;  $\Delta F^0$  is the change in standard free energy (data from Kubaschewski and Evans<sup>10</sup>), and  $Q$  is the proper quotient<sup>11</sup> becoming the equilibrium constant,  $K_p$ , when the reaction is at equilibrium. Equation (1) is independent of pressure and can be used to find the temperature of apparent equilibrium by comparing the experimentally determined value of  $Q$  with  $K_p$  calculated from free-energy data using the relationship  $\ln K_p = -\Delta F^0/RT$ . The values obtained are given in Table 1. If pressure is the only other unknown, then the value can be calculated from equation (2) using the value of  $T$  found from equation (1). For the Shap orthoclase this gives  $2.9 \times 10^4$  atm. at  $601^\circ\text{C}$  and  $6.7 \times 10^2$  atm. at  $975^\circ\text{C}$ . At the higher temperature reaction occurs more rapidly, so the apparent equilibrium pressure approaches more closely the pressure in the vacuum system. For the other minerals the calculated pressures vary from 0.3 atm. for pyroxene 722X487 to  $6.0 \times 10^4$  atm. for plagioclase EG-5052. This very large range, together with the fact that the temperatures calculated from equation (1) are greater than either the temperature of formation of the mineral or the heating temperature for most of the determinations, indicates that, if the measured amounts of gas are correct, then a simple two-variable model is inadequate.

It is possible that a high percentage of the evolved volatiles comes from secondary inclusions formed below the critical temperature of water, and still containing liquid water when they break open. The liquid will not be involved directly in gas phase reactions in the inclusions but will be included in the water measured by the method described here. Since only the amount of water in the gas phase should be used in calculating  $K_p$ , the value actually used is too high, and results in a high value for  $T$  calculated from equation (1), and a low value for  $T$  calculated from equation (2) at any given pressure. The high percentage of carbon dioxide from pyroxene 722X487 and plagioclase EG-5181 and the low apparent equilibrium temperatures suggest that carbon dioxide in excess of that present in the gas phase of the inclusions has been included in the total measured. This could have come from carbon dioxide dissolved in the aqueous phase, a separate carbon dioxide phase or from the decomposition of carbonates.

Investigations are being continued on minerals with observable fluid inclusions (quartz, fluorite, etc.) using

lower heating temperatures to minimize chemical reactions: eventually it is hoped to crush minerals in vacuum.

I thank Prof. L. R. Wager, Dr. N. J. Snelling and Dr. C. R. Evans for supplying separated minerals, and I acknowledge an Oxford University Burdett-Coutts studentship.

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## CHEMISTRY

### Measurement of Adsorption Isotherms by a Gas Chromatographic Technique

RECENT developments in the field of gas chromatography have included the measurement of gas-solid adsorption isotherms from the retention data<sup>1-3</sup>. A peak obtained from a single injection is used to calculate a series of retention volumes, ranging from the highest partial pressure at the peak maximum to zero partial pressure where the diffuse edge coincides with the baseline. These retention volumes are plotted against the corresponding values of partial pressure, also obtained from the chromatographic peak, to give the first derivative of the adsorption isotherm as described by Cremer and Huber<sup>1</sup>. The isotherm is obtained from it by graphical integration.

Not all workers in this field have taken into account the lateral spreading of the chromatographic peak that occurs by the process of longitudinal diffusion of the particular material within the carrier gas stream. The easiest way of correcting for this term is that of Bechtold<sup>4</sup> who used a measure of the non-diffuse edge of the peak as a correction to the diffuse edge from which the isotherm was obtained. A correction was also applied by Owens, Hamlin and Phillips<sup>5</sup>, using measurements from a second peak obtained from a chemically similar species that was not physically adsorbed on the substrate. A

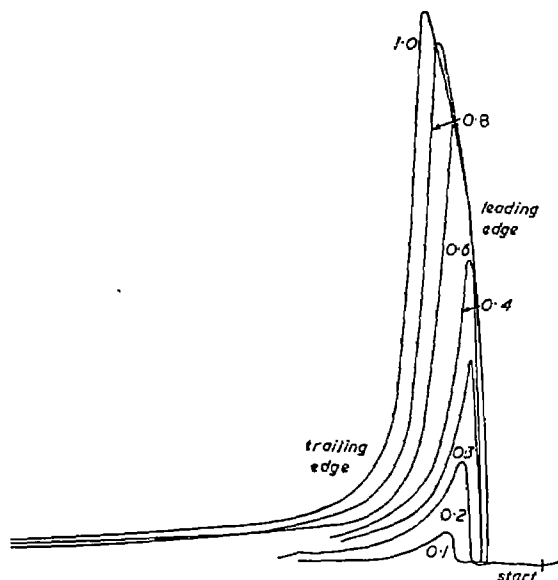


Fig. 1. Superimposed chromatograms. The numbers indicate the volume ( $\mu\text{l.}$ ) of water added

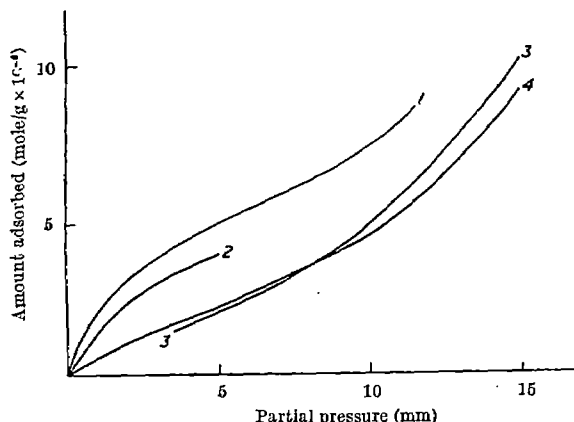


Fig. 2. Water adsorption isotherms of cleveandite: 1, using a single 0.6  $\mu\text{l.}$  water injection; 2, using a single 0.3  $\mu\text{l.}$  injection; 3, by frontal analysis; 4, using the 'peak maxima' method

the extent of longitudinal diffusion depends on the retention volume, this method of correction should require the adsorbed and the non-adsorbed species to have similar retention volumes.

As noted from the chromatographic peaks reported by most workers in this field<sup>2,6</sup>, the retention volumes of the diffuse edges are generally independent of the amount of adsorbate added, and in these cases the isotherm can be calculated from a single injection.

In obtaining water adsorption isotherms on silicate materials, we have, however, observed that the diffuse edge of the chromatographic peak changes from the leading to the trailing edge at a partial pressure of about 8 mm. This type of behaviour was previously observed by Glueckauf<sup>6</sup>, who suggested that the calculation of the isotherm should be split into two parts, corresponding to the leading and trailing parts of the diffuse edge. As the retention volumes of the diffuse edge when trailing are dependent on the amount of adsorbate added, a different isotherm will be obtained for each size of sample injection. This can be seen from Fig. 1, in which chromatograms obtained by adding varying amounts of water to cleveandite have been superimposed. It will be noted that the diffuse edges coincide when leading, but not when trailing.

Because of the difficulty of deciding which trailing-diffuse edge to accept, we have determined our isotherms

from a series of injections, taking measurements at peak maxima only and determining the retention volume for zero partial pressure by extrapolation of the curve drawn through the maxima of the superimposed peaks. The isotherm derived is shown in Fig. 2 together with one obtained by a frontal technique similar to that used by James and Phillips<sup>7</sup> and two isotherms calculated from single injections. It will be seen that a whole series of isotherms can be obtained using single injection methods. These are limited by the isotherm obtained by the peak maxima method, which itself shows good agreement with the isotherm obtained using the frontal technique.

Further work, involving the determination of isotherms of sulphur dioxide adsorption on charcoal, showed that in this case also the diffuse edges were dependent on the amount of adsorbate<sup>8</sup>, again indicating that the slope of the isotherm would vary according to the amount added, and that a 'peak maxima method' should be used in preference to a 'single injection' procedure.

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### 'Stereo-chromatography': a New Method for Chemical Identification and Preparative Isolation

SINCE the initial reports on chromatographic techniques<sup>1</sup>, and more especially after the investigations of Consden, Gordon, and Martin<sup>2</sup>, and Martin and Synge<sup>3</sup>, numerous modifications of these procedures have made possible remarkable advances in biochemical research. By evaluation of migration characteristics of unidentifiable with identifiable solutes separated by partition chromatography, identification of unknown solutes has been made possible. Similarly, by utilizing preparative 'filter paper pile' techniques, such as the chromatopile<sup>4,5</sup>, the chromatopack<sup>6</sup>, or the chromatoblock<sup>7</sup>, constituents of concentrated mixtures can be chemically resolved to yield semi-micro-quantities of pure materials. The present communication concerns the feasibility of utilizing three-dimensional, compressed, paper-pulp blocks for preparative chemical isolation and identification. This technique has three distinct advantages: (1) It makes possible the analysis of an additional parameter, the 'spreading factor' ( $R_s$ ), which can be used to differentiate chemically constituents of the solute mixture together with conventional  $R_F$  values; (2) it offers a support system capable of handling and separating exceptionally concentrated loads of solute mixtures for subsequent semi-micro-determinations; (3) it provides a closed system which should inhibit oxidations or photochemical alterations of solute zones which sometimes occur in conventional paper chromatography. One disadvantage of the technique is that it requires a solid, compressed fibre block, which, although inexpensive to fabricate, is not yet commercially available. In addition, the procedure necessitates the use of an electric band or jigsaw for serial sectioning of the chromatographic block.

Preliminary chromatographic experiments were undertaken with compressed paper-pulp blocks (purchased from the Container Corporation of America, Santa Clara, California). The blocks were fabricated by compressing raw, 3-4 per cent, paper pulp at 3,000 lb./in.<sup>2</sup> in a metal sieve box by means of a Dake hydraulic press. After compression, which removed most of the water, the blocks were oven dried for 36 h at 70° C. It was found in previous compression experiments that homogenization of the crude paper pulp in a Waring blender before compression increased the homogeneity of chromatographic blocks.

The feasibility of stereo-chromatography was demonstrated by the three-dimensional separation of indicator dyes in simple ascending chromatography. According to the procedure of Porter<sup>6</sup>, indicator dyes were used as solutes in the organic phase of an equilibrated two-phase solvent mixture obtained by mixing 40 parts of 1-butanol, 10 parts of absolute ethyl alcohol, and 50 parts of water. This mixture was separated in a separatory funnel and the hyperphase was used.

In one early exploratory experiment, 1.5 ml. of an aqueous solute mixture containing 1.5 mg bromocresol green, 1.2 mg bromthymol blue, and 1.0 mg eosin blue was injected into the centre of the base of a 25 cm × 20 cm × 7.5 cm compressed pulp block. The block was placed on the bottom of a large, closed jar containing 0.5 in. of solvent mixture and exposed for 3 h, or until the solvent front almost reached the top of the block.

At the termination of the experiment, the block was removed from the solvent and serially sectioned by an electric band saw into 0.5 cm sections. Since the block cannot be readily dried internally, it is necessary to section wet blocks in order to prevent solute zone migration which might occur during prolonged drying.

Table 1. VERTICAL AND LATERAL MIGRATION IN STEREO-CHROMATOGRAPHIC BLOCKS

Solute	$R_F$	$R_S$
Methyl orange	0.32	0.08
Methyl red	0.68	0.24
Bromthymol blue	0.79	0.35
Methylene blue	0.88	0.04
Eosin blue	0.01	0.04

After the stereo-chromatogram was sectioned, the vertical and lateral migrations of the solute zones were determined on each section by means of a metric calibrated, transparent, plastic grid. The vertical migration ( $R_F$ ) values were determined in the conventional manner. The lateral migration, or spreading factor, of the solute zones was determined by calculating the ratio of the lateral spread of the solute from the central axis of the block to the vertical migration of the solvent front.

It was observed in most stereo-chromatographic blocks that zone resolution was adequate to isolate and identify the specific indicator dyes utilized. Of greater significance, however, is the fact that differences in lateral spreading ( $R_S$  values) of the indicator dyes were observed as shown in Table 1, which includes data from four recent experiments.

The present preliminary experiments indicate that stereo-chromatography with compressed paper-pulp blocks offers a suitable technique for preparative chromatography where large amounts of solutes are to be resolved. This procedure should therefore prove useful in biological, and possibly industrial, purifications.

Although additional experiments are necessary to evaluate critically the relation of  $R_S$  to  $R_F$  values, it is noteworthy that the spreading factor,  $R_S$ , offers an additional parameter for differentiating chemical constituents. Such a parameter should be of practical importance in differentiating compounds which have similar partition coefficients, and thus produce similar  $R_F$  values (for example, leucine and isoleucine, valine and *nor*-valine), but which differ in carbon chain configura-

tion. In conventional partition chromatography, Fisher and his associates<sup>8</sup> experimentally demonstrated that a linear relation holds between the area of the spot of a test substance and the logarithm of its original concentration. Brimley<sup>9</sup> has theoretically discussed this relationship by applying equations analogous to those developed in the theory of heat flow, and by assuming that the spot moves along the paper chromatogram (conventional chromatography) by simple diffusion. However, it would seem from the present preliminary stereo-chromatograms that such a simple relationship does not apply, analogously, to the volume of solute zones and their original concentration. It appears that the greater differences in  $R_S$  than in  $R_F$  migrations for a given solute pair (bromthymol blue and methylene blue) may indicate that lateral vectorial forces which influence solute zone movement (for example, solubility and/or ordinary diffusion, eddy diffusion, and local non-equilibrium) become more apparent in horizontal spreading than in vertical migration. In the latter case the capillarity and opposing gravitational forces are predominant. In other instances lateral, diffusive forces appear less predominant (methylene blue and eosin blue).

As in all chromatographic techniques, homogeneity of the supporting medium is necessary for regular movement of solvent and solutes. The blocks in the present experiments were fairly homogeneous, but less so than ordinary commercial filter paper. Fibre size can be expected to influence solvent flow rate in stereo-chromatography as it does in paper chromatography. Similarly, the initial flow rate (first 20 min) was found to be faster than in later periods in stereo-chromatography, as it is in conventional chromatography.

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## An Oil-Wax-Surfactant System for retarding the Evaporation of Water

As a result of the growing use of fresh-water resources, much work has been done on retarding evaporation from water-storage facilities. Most of this work has centred about the use of a monolayer system, especially of long-chain fatty alcohols<sup>1</sup>. However, since the practical applications of the monolayer system show that amounts of material far in excess of the theoretical monolayer system must be used<sup>2</sup>, it becomes pertinent to examine other possible systems. It appears, however, that relatively little recent work has been done with possible systems other than the monolayer. Most investigations have been concerned with oil-surfactant systems<sup>3</sup> and one with a wax-oil system<sup>4</sup>.

I wish to report that I have found that certain petroleum oil-petroleum wax-surfactant layers are effective in retarding the evaporation of water. Some of the results are shown in Table 1. Many of these results, which were obtained on relatively small evaporation pans (about 1 sq. ft. in area), have been repeated using larger 10-ft. diameter pools with similar results. Preliminary information on testing with larger areas of water is also encouraging.



Table 1. RETARDATION OF WATER EVAPORATION BY VARIOUS SYSTEMS

Treatment	Amount of treatment* (g)	% Retardation efficiency† after 13 days outdoor exposure	% Retardation efficiency† after 20 days outdoor exposure
Long-chain fatty alcohol ‡	0.1	59.0	34.4
Oil-surfactant§	0.1	38.5	25.4
Oil-surfactant§	1.0	84.8	82.1
Oil-wax-surfactant§	0.1	79.5	48.3
Oil-wax-surfactant§	1.0	100	98.0

\* The area of the surface was 0.835 ft.<sup>2</sup>.

† Retardation efficiency was calculated as:

$$100 \times \frac{\text{Average change in depth of control} - \text{average change in depth of treatment}}{\text{Average change in depth of control}}$$

‡ Archer Daniels Midland's 'Adol 54' (commercial mixture of mainly *n*-hexadecanol and *n*-octadecanol) was used in isopropyl alcohol solution.

§ Applied in a hexane solution.

An oil-wax-surfactant system may have some advantages over a fatty alcohol system. Such systems float more easily, re-form more easily on disturbance, are biologically more inactive, less expensive, etc. However, many important problems remain to be investigated.

I am now investigating the system more closely in an attempt to gain control of the important variables. I am also co-operating with other interested groups in evaluating the practical possibilities of such a system.

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### Solubility of Polyethylene Terephthalate

It is generally considered that polyethylene terephthalate is soluble only in a restricted range of rather specialized organic liquids, such as *o*-chlorophenol, tetrachlorethane/phenol, etc. It is the purpose of this report to describe conditions under which this range may be extended.

In the course of investigations into the mechanism and kinetics of the liquid-induced crystallization of amorphous polymer it has been postulated that crystallization occurs on a progressive front accompanying the diffusion of the particular liquid<sup>1</sup>. It was further considered that if this was by a two-stage process involving first of all solubilization and then crystallization it might be possible to isolate the first of the stages by correct choice of experimental conditions. Subsequent experimental verification of this view was obtained with chloroform and *sym*-tetrachlorethane separately using samples of amorphous film (8 × 10<sup>-3</sup> cm thick) in the two liquids at approximately -40° C. On standing overnight a precipitate formed, which on evacuation of liquid compacted to a hard solid of density 1.42 g/c.c. crystalline when submitted to X-ray diffraction analysis.

Since the polymer is apparently insoluble in these liquids at room temperature, it would suggest that any solubilization which occurs is immediately followed by crystallization. It is interesting to note that in the case of the *sym*-tetrachlorethane at room temperature, splitting of the film takes place. This supports to some

degree a previous contention that in thin films phase separation may arise in the middle of the crystallized material<sup>2</sup>.

That a similar phenomenon of solution and crystallization may be associated with other amorphous but crystallizable polymers is suggested by similar results on isotactic polystyrene<sup>3</sup>.

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### Temperature Dependence of Contact Angles

THERE are very few recorded observations on the temperature coefficient of contact angles<sup>1</sup>. These suggest that the coefficient is very small, if not zero, which is at first sight surprising. Adam<sup>2</sup> has pointed out that, as a consequence, "it appears that temperature affects the surface tension and the adhesion to the solid to very nearly the same proportionate extent". He has discussed this in terms of the thermal motions of the liquid, and has suggested that "the decrease in the adhesive field of force with rising temperature is much less in solids than in liquids and is due principally or wholly to translatory motion". The implication, however, that the temperature coefficient of the free surface energy of solids is negligible because translatory motions are absent seems to require further justification.

It may be of importance to note that water was present as a liquid phase in all the cases cited by Elliott and Riddiford<sup>1</sup>, and by Adam<sup>2</sup>, since Neumann<sup>3</sup> has recently reported an appreciable decrease in the contact angles for several organic liquids against their saturated vapours, on a siliconed surface, when the temperature is raised from 12° to 28° C. Zisman<sup>4</sup> has argued that since the surface tension of a liquid decreases with rising temperature, the contact angle,  $\theta$ , should also decrease, but, as with Adam's suggestion<sup>2</sup>, this view appears to require the assumption that the temperature coefficient of the free surface energy of the solid is negligible.

The matter is one of both theoretical and practical importance. Consider Young's equation for a liquid against its saturated vapour on a solid surface:

$$\gamma_L \cos \theta = \gamma_s - \gamma_{SL} - \pi \quad (1)$$

where  $\gamma_L$ ,  $\gamma_s$  and  $\gamma_{SL}$ , respectively, are the free surface energies of the liquid, of the film-free solid and of the solid/liquid interface,  $\pi$  being the equilibrium film pressure. It is clear that the theoretical calculation of  $d \cos \theta / dT$  would require knowledge of the individual free surface energies and of their temperature coefficients.

Some progress has been made. Fowkes<sup>5</sup>, for example, has shown that internally consistent calculations of interfacial free surface energies can be made on the assumption that the energy of interaction between two phases is purely dispersive. On this valuable line of approach:

$$\gamma_{SL} = \gamma_s + \gamma_L - 2(\gamma_s^d \cdot \gamma_L^d)^{1/2} \quad (2)$$

where  $\gamma_s^d$  and  $\gamma_L^d$ , respectively, are the dispersive components of  $\gamma_s$  and  $\gamma_L$  (ref. 6) and equations (1) and (2) permit the evaluation of  $\gamma_s^d$  from contact angle measurements for cases in which  $\pi = 0$ . In certain cases,  $\gamma_s^d$  can also be evaluated when  $\pi$  is finite, and an estimate of

the temperature coefficients of  $\gamma_s^d$  and  $\gamma_L^d$  can be made<sup>7</sup>. In general, however, our lack of knowledge of  $\pi$ , and of its temperature coefficient, is a serious bar to further progress.

From equations (1) and (2):

$$\frac{d \cos \theta}{dT} = \frac{d}{dT} \left\{ \frac{2(\gamma_s^d \cdot \gamma_L^d)^{\frac{1}{2}}}{\gamma_L} \right\} - \frac{d}{dT} \left( \frac{\pi}{\gamma_L} \right)$$

$$= \frac{d}{dT} \left( \frac{W_{SL}}{\gamma_L} \right) - \frac{d}{dT} \left( \frac{\pi}{\gamma_L} \right)$$

where  $W_{SL}$  is the work of adhesion to the film-free solid, and the available data suggest that  $d(W_{SL}/\gamma_L)/dT$  is very small, at least for low-energy surfaces. On this view,  $d \cos \theta/dT \sim 0$  when  $\pi \sim 0$ . With one possible exception, the reported investigations for systems involving water are consistent with this conclusion. The possible exception is the unqualified statement by Fowkes and Harkins<sup>8</sup> that  $\theta$  has the same temperature coefficient of  $+0.06^\circ$  per degree centigrade for water both on paraffin wax and on graphite. In the first case  $\pi = 0$ , whereas there is an appreciable film pressure in the water/graphite system. It seems probable that their temperature coefficient for water on paraffin wax is too large, however, and is of the wrong sign<sup>7</sup>; the sign also seems wrong in the case of graphite. On the other hand, Neumann's results<sup>9</sup> are consistent with the present view since there can be little doubt that appreciable film pressures are present in his systems<sup>7</sup>.

On the practical side, we have made measurements using the sliding-drop technique<sup>9</sup> in order to augment the recorded investigations. These are summarized in Table 1, each value shown being the mean obtained from at least ten, and sometimes twenty, separate experiments. The scatter was less than  $\pm 2^\circ$  for the single liquid systems, the contact angle being much less reproducible for the two liquid system ( $\pm 7^\circ$ ).

Table 1. ADVANCING AND RECEDING ANGLES ON A SILICONED GLASS SURFACE

System	Temp. (°C)	S.T.*	$\theta_A$ (measured through aq. phase)	$\theta_R$
Water/saturated air	4†	75.0	104°	75°
	22	72.4	106°	78°
	75	63.5	104.5°	76.5°
2 molal NaCl/saturated air	10	77.5	104.5°	69°
	22	76.0	104.5°	69°
	75	66.8	105°	71°
0.1 M <i>n</i> -Butyric acid/saturated air	4	61.0	99°	77°
	22	58.2	99°	76°
	75	54.2	101°	87°
Water/Bayol‡	22	44.9	145°	120°
	75	42.1	160°	130°

\* S.T., surface tensions of water and aqueous solutions from *Intern. Critical Tables*; interfacial tension between water and Bayol determined at 22° and calculated at 75° by assuming  $-5.3 \times 10^{-5}$  erg cm<sup>-2</sup> deg<sup>-1</sup> for temperature coefficient.

†  $\pm 0.5^\circ$  in all cases.

‡ A light fraction paraffin oil.

In the cases of pure water and aqueous 2 molal sodium chloride, it will be seen that both  $\theta_A$  and  $\theta_R$  are sensibly independent of temperature, within the limits of experimental error. In the first case,  $\theta_A$  may be regarded as the equilibrium angle for the unpenetrated surface<sup>1,10,11</sup>, whereas  $\theta_R$  reflects the effect of penetration of water into the silicone layer<sup>11</sup>. The 2 molal sodium chloride system is evidently similar in the case of an advancing interface; the solute is negatively adsorbed at the solid/liquid and liquid/vapour interfaces, this having a small effect on  $\gamma_L$  and no discernible effect on  $\theta_A$ . On the other hand, the reduction in  $\theta_R$  as compared with pure water presumably indicates that penetration of water into the surface layer is increased by the presence of sodium chloride.

The other two systems present more difficulties. *n*-Butyric acid is positively adsorbed at both the solid/liquid and liquid/vapour interfaces; moreover, it may also adsorb at the solid/vapour interface, with the appearance

of a film pressure. The two liquid system is very different. Both the advancing and the receding cases involve, among other factors, the effect of temperature on the mutual solubility.

These results will be discussed in detail when further penetration investigations have been completed, but they serve to show that the temperature coefficient of  $\theta$  is not negligibly small for all systems. Moreover, quantitative consideration of the advancing results for pure water confirms the view that when  $\pi \sim 0$ ,  $d \cos \theta/dT \sim 0$ . Estimates of the temperature coefficients of  $\gamma_s$  ( $=\gamma_s^d$ ) and  $\gamma_L^d$  show<sup>7</sup> that  $d(W_{SL}/\gamma_L)/dT \sim 0$ . Now at 20° C,  $\gamma_L^d = 21.8$  ergs cm<sup>-2</sup> for water<sup>5</sup>, and  $\gamma_s \sim 33$  ergs cm<sup>-2</sup> for the silicone surface, both values being independent of investigations of contact angle<sup>7</sup>. Since  $\gamma_L = 72.8$  ergs cm<sup>-2</sup> at this temperature, we calculate  $\theta = 105^\circ$  from equations (1) and (2) on the assumption that  $\pi = 0$ . The agreement between this value and  $\theta_A$  for 22° C is at least a strong indication that  $\pi$  is very small at this temperature. The experimental values then suggest that  $\pi$  is negligibly small over the whole range 4°–75° C.

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## BIOCHEMISTRY

### Biological Activity of Retinoic Acid Ester in the Domestic Fowl: Production of Vitamin A Deficiency in the Early Chick Embryo

RETINOIC acid (vitamin A acid) is unable to replace retinol (vitamin A alcohol) in reproduction and vision in rats fed a vitamin A-deficient diet<sup>1,2</sup>. Results of similar tests with retinoic acid in poultry are presented in this communication.

Day-old chicks of both sexes were fed a retinol-free semi-synthetic diet supplemented with methyl retinoate (3 µg per g diet); other groups of birds were fed a similar diet either unsupplemented or containing retinyl acetate (3 µg per g diet).

All the birds fed unsupplemented diet died before the fifth week of the experiment after showing typical signs of vitamin A deficiency<sup>3</sup>. The birds fed methyl retinoate or retinyl acetate grew well, many being maintained in apparent good health on these régimes for longer than 20 months.

After reserves of retinol acquired before hatching had been exhausted at 4–5 weeks of age, the birds maintained with methyl retinoate showed a progressive failure of vision. Abnormal dilatation of the pupils in dim light was first noticed when the birds were 9 weeks old, and by 17 weeks of age their vision in bright light, as assessed by behaviour and pupil size, was severely impaired. At 20 weeks the birds were judged to be almost completely blind.

The retinae in these birds did not undergo irreversible anatomical deterioration at the same rate as has been observed in the retinol-deficient rat<sup>2</sup>. A slight reduction in density of the rod-cone layer was the only detectable histological change in the retinae of birds maintained with retinoic acid for up to 20 months, and after this time, recovery of vision, as judged from pupil responses and behaviour, was complete 7 days after dosing with retinyl acetate.

Cockerels maintained with methyl retinoate and examined at various stages of maturation had testes of normal histological appearance. Semen containing highly motile spermatozoa was regularly obtained from adult cocks and was used to inseminate normal hens and fertilize their eggs successfully. The testes of the cock are thus unlike those of the rat<sup>4</sup> in that they develop and remain healthy in the absence of retinol.

Hens maintained with methyl retinoate laid eggs at a normal rate and, after insemination with semen from normal cocks, eggs with high fertility were obtained from them. However, in these eggs normal embryonic development was seen during only the first 48 h of incubation. Embryos examined at this stage had a normal macroscopic appearance, but older embryos were abnormal in form and showed evidence of gradual disintegration. A conspicuous feature of these eggs was their failure to develop an organized circulatory system in the area vasculosa.

Some of the eggs from hens maintained with methyl retinoate were injected before incubation with solutions of retinol, retinyl acetate or retinal (vitamin A aldehyde) (0.25 or 0.5 mg) in oil. All these substances stimulated normal development in a high proportion of the eggs so injected, and in some instances normal chicks were hatched.

The failure of the embryos to develop normally in un-injected eggs from hens fed methyl retinoate is concluded to be a manifestation of vitamin A deficiency in the early embryo. Vitamin A has thus at least two important roles in avian reproduction: one is the well-known effect on egg production, and the second is this hitherto undescribed role in embryonic development. Methyl retinoate fed to the hen is able to maintain egg production but does not meet the requirements of the embryo. Hitherto, it has not been possible to demonstrate vitamin A deficiency in the chick embryo, because on deficient diets the relatively high requirement of vitamin A for egg production results in hens going out of lay before the vitamin stores in the eggs are reduced sufficiently to affect the development of the early embryo<sup>5</sup>.

Free retinoic acid was found to be extremely toxic to early chick embryos. As little as 1 µg injected into normal fertile eggs prior to incubation prevented development. The injections appeared to result in disintegration of the entire early blastoderm. Much larger quantities of retinol (> 0.5 mg) were required to produce a similar effect. Methyl retinoate was found to be similar to retinol in its relative lack of toxicity.

The toxicity of free retinoic acid and retinol in the egg is probably related to that observed in the rat<sup>6</sup>, in *Xenopus laevis*<sup>7</sup> and in chick limb rudiments in tissue culture<sup>8</sup>.

It is unlikely that the toxic action of retinoic acid is responsible for the failure of embryos to develop in eggs from hens maintained with methyl retinoate. Such embryos can be stimulated to grow normally by injecting retinol; also the two abnormalities are dissimilar both in macroscopic appearance and in time of onset. It is concluded that the early chick embryo is very susceptible to conditions of both hyper- and hypo-vitaminosis A.

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### Decomposition of Structural Polysaccharides of Bacteria by Marine Micro-organisms

THE large numbers of bacteria which are carried into the sea in sewage and land drainage survive for only a short period of time<sup>1</sup>. Both physicochemical<sup>2</sup> and biological<sup>3</sup> factors have been implicated in the bactericidal effect. Saz *et al.*<sup>4</sup> have demonstrated the presence in natural sea water of macromolecular particles with activity against *Staphylococcus aureus*. The decline of *Shigella* in natural sea water has also been correlated with the presence of antibacterial macromolecules in the water<sup>5</sup>.

While examining biological degradation of a *Flavobacterium* capsular polysaccharide<sup>6</sup>, it was observed that a bacterium isolated from soil which was active against the capsular material also caused complete liquefaction of agar. This bacterium was a short Gram-negative rod which was classified as a species of *Pseudomonas*. A bacterium isolated from soil by Sickles and Shaw<sup>7</sup> was capable of degrading capsular polysaccharide of *Pneumococcus* type III, and also decomposed agar. These observations suggested a correlation between microbial degradation of agar and decomposition of structural material of bacteria. Stanier<sup>8</sup> has demonstrated the presence in sea water of a large and varied population of agar-decomposing bacteria. Consequently an investigation was initiated to determine whether micro-organisms which degrade both agar and bacterial structural materials could be isolated from natural sea water.

Enrichment cultures were prepared using natural sea water from the Mediterranean near Ashkelon as the inoculum in ZoBell's<sup>9</sup> artificial sea water medium containing *Flavobacterium* capsules as the sole carbon source. These enrichments resulted in the isolation of a halophilic marine *Pseudomonas* capable of utilizing *Flavobacterium* capsular polysaccharide as its sole carbon source. Similar bacteria capable of growth on capsules of *Azotobacter*, *Rhizobium*, *Arthrobacter*, and cell walls of *Escherichia coli* 'B' were also isolated from sea water by enrichment on the respective structural materials. All isolates caused complete liquefaction of agar. Capsular material used in these investigations was prepared by repeated extraction with 10 per cent trichloroacetic acid at 0° C. The capsules were re-precipitated in 66 per cent ethanol following extraction of the trichloroacetic acid in ether. Cell walls of *E. coli* 'B' were prepared by extrusion in a French press. The walls were washed repeatedly in buffer.

The marine bacterium isolated from *Flavobacterium* capsule medium grew well on *E. coli* 'B' cell walls. Oxygen uptake during growth of this bacterium was measured in the Warburg microrespirometer in ZoBell's medium. Both capsules and cell walls were metabolized. During the logarithmic growth phase 75 µl. O<sub>2</sub>/h was consumed by the bacterium growing on capsular material,



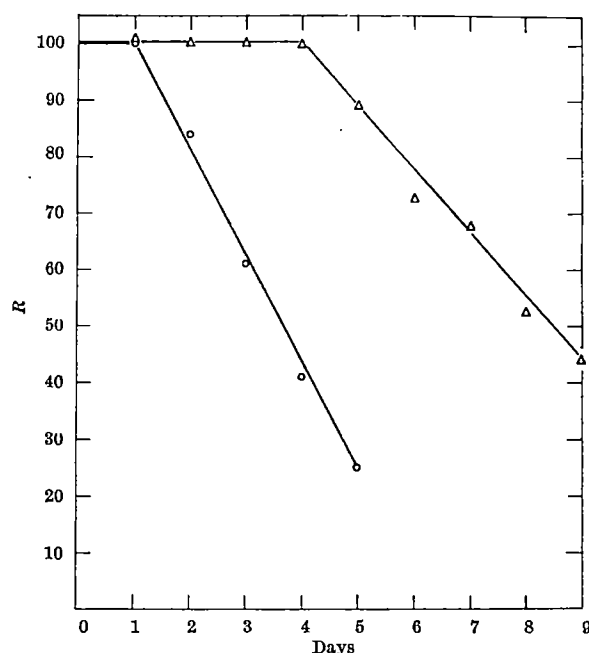


Fig. 1. Survival curves of *Escherichia coli* 'B' (O) and *Arthrobacter* (Δ) in ZoBell's artificial sea water medium in the presence of a lytic marine *Pseudomonas*. *R* represents the ratio (%) between optical density of *Esch. coli* 'B' and *Arthrobacter* samples in nutrient broth following sub-culture from ZoBell's medium inoculated with the marine *Pseudomonas* and of the optical density of samples from ZoBell's medium without the marine bacterium

and 252  $\mu$ l./h during growth on *E. coli* 'B' cell walls, following correction for endogenous respiration. No respiration was detected in flasks inoculated with a soil bacterium capable of utilizing a wide range of carbon sources, indicating the absence of non-specific contaminants in the substrate material.

Tests for enzymatic activity were carried out using culture filtrates and sonicated cells of the marine *Pseudomonas* following growth on ZoBell's medium containing nutrient broth (Difco), *E. coli* 'B' cell walls, *Flavobacterium* capsular polysaccharide and agar as the respective carbon sources. Polysaccharidase activity was detected by release of reducing sugars using the method of Somogyi<sup>10</sup> and Nelson<sup>11</sup>. Release of free amino-groups from *E. coli* 'B' cell walls was detected by the method of Moore and Stein<sup>12</sup>. Polysaccharidases active against *Flavobacterium* capsular material and agar were produced extracellularly. Detectable quantities of enzyme accumulated in the culture media after three days of incubation in shake culture. No polysaccharidase activity was detected in sonicated cells. Free amino-groups were released during decomposition of *E. coli* 'B' cell walls by sonicated cells of the *Pseudomonas*, following 24 h incubation in shake culture, indicating that the enzymatic system active against *E. coli* 'B' walls was intracellular. Preparations of the *Pseudomonas* displayed activity against *Flavobacterium* capsular polysaccharide, agar and *E. coli* 'B' cell walls only following growth on the respective substrate. These data provide evidence that the enzyme systems causing degradation of each of the four substrates were inducible.

One of the marine bacteria isolated in this investigation grew well on capsular polysaccharides of a heavily encapsulated strain of *Arthrobacter*, a bacterium commonly found in the soil. The marine organism also utilized *E. coli* 'B' cell walls. Activity of this marine bacterium against living cells of each of the two non-marine bacteria was tested by growing the bacteria together in ZoBell's artificial sea water medium fortified with 0.1 per cent peptone. Survival of the non-marine bacterium was determined by daily sub-culture to nutrient broth (Difco) prepared with tap water. The marine bacterium was unable to grow on this medium. The results shown in

Fig. 1 demonstrate the survival of *Arthrobacter* and *E. coli* 'B' in artificial sea water in the presence and absence of the marine bacterium, as expressed by the *R* value. Both non-marine bacteria were markedly suppressed by the marine bacterium. The greater resistance to lysis of the *Arthrobacter* may be attributed to its capsular layer, but it is not clear if lysis results from degradation of the capsule alone or in addition to degradation of the underlying cell wall.

The relationship between degradation of these structural materials and agarase activity, and the significance of these findings in marine ecology, are at present being investigated.

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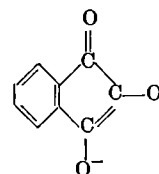
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### Free Radicals derived from Amines and Amino-acids in some Reactions of Ninhydrin

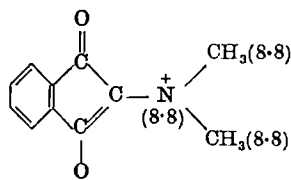
ALLOXAN and dialuric acid form free radicals in alkaline solution<sup>1,2</sup>. Ninhydrin, a chemical analogue of alloxan, has been shown to form a free radical on reaction with glycine<sup>3</sup>. The electron spin resonance (ESR) spectrum of this radical consists of 9 lines and is attributed to the two pairs of equivalent benzenoid protons of ninhydrin.

I have observed that the same radical can be produced directly by treating a molar solution of ninhydrin in methanol with 5 N aqueous sodium hydroxide. A red precipitate forms which is unstable in air. The ESR signal is associated with this precipitate. In the presence of excess alkali this precipitate turns blue and the ESR signal disappears. MacFadyen and Fowler<sup>4</sup> observed the same colour changes in alkaline solutions of hydrindantin. They also noted the sensitivity of the red compound to air. They attributed the red and blue colours to the mono- and di-valent anions of indanone-ene-diol. I have found that when hydrindantin is treated with normal potassium hydroxide, or dissolved in buffer at pH 9.0, the red solution obtained gives an intense ESR signal identical to that described above. The blue solution obtained under more strongly alkaline conditions gives no signal. It seems probable that the free radical described above is the semi-quinone anion of indanone-ene-diol.



I have also observed that this radical appears in the reaction of ninhydrin with ethylamine, *n*-butylamine, dimethylamine and diethylamine. Other free radicals

is due to the benzenoid protons of the ninhydrin portion of the following free radical; splitting constants (gauss) are shown in brackets.



The splitting constants attributed to the benzenoid protons of this radical (Fig. 1c) are 0.7 and 0.47 gauss compared to those of 0.94 and 0.74 gauss in the ninhydrin-free radical shown in Fig. 1d.

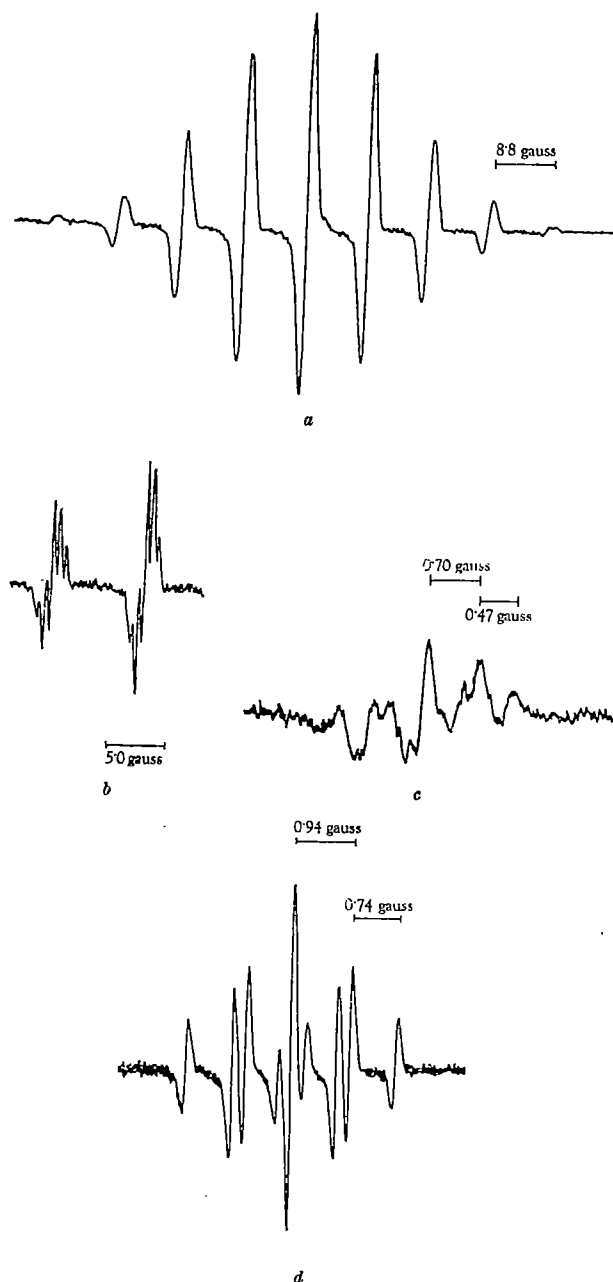


Fig. 1. *a*, First derivative of the ESR absorption of the free radical obtained from the reaction of dimethylamine with ninhydrin. *b* and *c*, Part of *a* under progressively higher resolution. *d*, First derivative of the ESR absorption of the ninhydrin-free radical

sociated with the amine can also be detected, but they decay rapidly. No free radicals were observed when tertiary amines were investigated.

The ESR spectra were studied using flat 'Pyrex' tubes inserted into an  $H_{012}$  cavity of a Hilger-Watts Microspin-band spectrometer utilizing 100-ke/s modulation. Fig. 1*a* shows the ESR spectrum obtained when equimolar quantities of dimethylamine and ninhydrin in methanol were gently warmed. The spectrum covers 70 gauss peak to peak and shows 9 equally spaced lines 8.8 gauss apart. The intensity ratios of these lines are 7:22:41:50:41:22:7:1 and correspond to those expected from the equal coupling of an unpaired electron between one nitrogen atom and six protons. Figs. 1*b* and 1*c* show part of the spectrum under progressively higher resolution. Each peak appears first as a quintet and then nine lines. It is suggested that this hyperfine structure

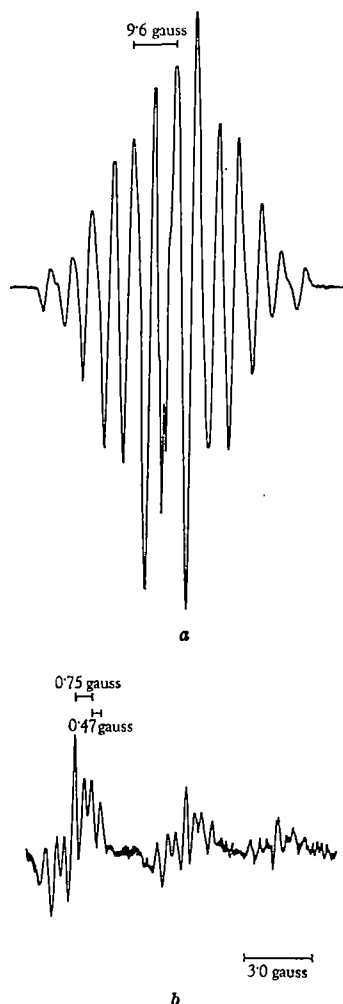
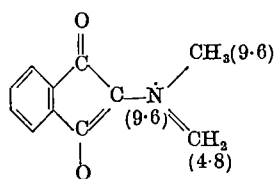


Fig. 2. *a*, First derivative of the ESR absorption of the free radical obtained from the reaction of sarcosine with ninhydrin. *b*, Three peaks in the wings of *a* under higher resolution

When ninhydrin is reacted with certain amino-acids under alkaline conditions free radicals are formed which are believed to have similar structures to that suggested above. Fig. 2*a* shows the ESR spectrum obtained when aqueous solutions of sarcosine (molar) and ninhydrin (0.3 molar) are mixed in equimolar amounts and made alkaline (pH 8.5) with normal sodium hydroxide. Thirteen equally spaced lines covering 58-gauss peak to peak are obtained in low resolution. The spectrum can be empirically attributed to coupling of an unpaired electron between the nitrogen atom and the five protons of sarcosine. The radical could have the following structure, approximate coupling in gauss:



It has not yet been possible to observe this radical without some distortion of the centre peak of the spectrum due to the presence of the unbound free radical from ninhydrin. However, the intensity ratios observed are 1:2:4:7:8:11:12:15:9:8:5:2:1 which may be compared with 1:2:5:8:11:14:14:14:11:8:5:2:1 expected for the coupling suggested.

Fig. 2b shows the appearance of the peaks in the wings of the spectrum under high resolution. It is suggested that this hyperfine splitting results from the benzenoid protons in the ninhydrin part of the complex. The centre triplet is not resolved, but the two splitting constants 0.75 gauss and 0.47 gauss are not significantly different from those observed in the dimethylamine reaction.

Other spectra have been observed from L-proline (11 lines 55-gauss total splitting) and L-hydroxyproline (12 lines 55-gauss total splitting). The reaction of aspartic acid with ninhydrin under similar conditions to those described above yields a three-line spectrum covering 13.4 gauss. This is probably due to a single nitrogen atom of coupling constant 6.7 gauss and a different type of radical structure from that suggested above is probably involved.

I thank Dr. R. Foster and Dr. R. Jameson of the Chemistry Department for their advice.

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### Tumours in the Rat after Injection of Preneoplastic and Neoplastic Nucleic Acids

SOME years ago, it was reported from our laboratories that lymphosarcomas developed at the site of subcutaneous injection of a chromatin fraction obtained from Murphy lymphosarcoma cells, and that hepatomas appeared at the site of intrahepatic injection of a similar fraction obtained from cells of a transplantable hepatoma<sup>1-3</sup>. The significance of these observations was questioned on the grounds that the possibility of the presence of intact cells in the injected material could not be excluded with certainty.

Since then, there have been several reports of induction of tumours by nucleic acids of oncogenic viruses<sup>4-10</sup>. There is evidence, too, that other types of biologically active nucleic acid molecules can penetrate somatic cells and can alter synthetic processes<sup>11-18</sup>, the nature of the observations in certain instances suggesting that exogenous DNA had entered the recipient genome. Accordingly, the concept of transfer of subcellular genetic entities from one mammalian cell to another is no longer regarded as fanciful, as it was at the time of our initial reports.

The present study was undertaken to investigate the following possibilities: (a) that the malignant potentialities of 'preneoplastic' liver cells may be transferred to normal cells by intrahepatic injection of 'preneoplastic' nucleic acids; (b) that the malignant properties of hepatoma cells

may be transferred to normal cells by incubation with hepatoma nucleic acids.

Nucleic acids were prepared from two sources: (a) the Morris hepatoma 3924C, carried in  $A \times C$  rats; (b) 'preneoplastic' liver, that is, livers of male rats that had received 2-acetylaminofluorene (0.03 per cent) in the diet for 90 days, followed by a carcinogen-free diet for 30 days, at which time (120 days) there is no detectable morphological evidence of malignancy in the liver. With this AAF dosage and the diet used, hepatomas develop ultimately in 85 per cent of male rats, the earliest in our experience having been at 156 days.

DNA was prepared by the method of Colter, Brown and Ellem<sup>11</sup>, and RNA by the method of Kirby<sup>18</sup> from tissues (liver; tumour) which had been frozen in a dry-ice/alcohol mixture immediately after removal. The remaining procedures were carried out at 4°C. The DNA was undoubtedly contaminated by a small amount of RNA, no attempt having been made to achieve purity of the preparation.

Two types of experiment were performed: (1) intra-hepatic injection of cells of regenerating liver that had been incubated with hepatoma nucleic acids; (2) intra-hepatic injection of nucleic acids of hepatoma and of 'preneoplastic' liver. In the former case, adult male  $A \times C$  rats (200 g) were partially hepatectomized<sup>19</sup> and were killed 72 h later. Regenerating liver (2 g) was disrupted in 20 ml. of Hanks's solution in a Potter-Elvehjem homogenizer with a loosely fitting 'Teflon' pestle, and was mixed thoroughly with 50 mg of hepatoma DNA or RNA. The cell suspension was incubated in a shaking incubator for 2 h at 37°C and was then centrifuged; 0.1 ml. of the packed cells was injected into the liver in 120 male  $A \times C$  rats (150 g) under ether anaesthesia. The injection site was sealed with 'Surgicel'. The rats were killed after 2-8 months.

In the second type of experiment approximately 50 mg of DNA or RNA was placed in 5 ml. saline and dissolved by vigorous mechanical stirring; 0.1 ml. (approximately 1 mg) nucleic acid was injected into either the intact liver or the regenerating liver (72 h after partial hepatectomy of 150-g male  $A \times C$  rats under ether anaesthesia. The injection site was sealed with 'Surgicel'.

Hepatoma nucleic acids were employed in 160 intact and 60 partially hepatectomized rats, which were killed 8-16 weeks later. 'Preneoplastic' liver nucleic acids were used in 110 intact and 40 partially hepatectomized animals, which were examined at laparotomy after 4 months and were killed after 6 or 8 months.

Tumours were found in two animals at the site of injection of cells of regenerating liver that had been incubated with hepatoma DNA, one 10 weeks and the other 16 weeks after injection. Both were well encapsulated and measured 1.5 cm in diameter. Their morphological characteristics closely resembled those of tumours produced by intrahepatic injection of intact cells of the hepatoma from which the DNA had been obtained. On important difference was in the time required for development of tumours of comparable size following injection of comparable numbers of hepatoma cells, which is about 3 weeks, in contrast to 10 and 16 weeks in the case of the induced tumours.

A tumour occurred in one animal in which a mixture of 'preneoplastic' liver DNA and RNA had been injected into the regenerating liver 6 months previously. It was 2.5 cm in diameter and well encapsulated, presented ventrally beneath the skin of the upper abdomen, and was adherent to the anterior surface of the liver. It was examined by a number of pathologists experienced in rodent tumours. All agreed that it was a malignant neoplasm, different from any seen previously by them in the rat, but there was no agreement as to its nature beyond this.

The most important implication of these findings is that the observed tumours represent a process of true genetic



transduction, involving genic recombination mechanisms similar to those established in the case of certain bacteria. However, other possibilities must be considered: (1) that they are spontaneous tumours, unrelated to the experimental procedure; (2) that they arose as a result of transfer, into the incubated cells or injected liver, of carcinogen (AAF) bound to the nucleic acids; (3) that they represent tumour production by a virus or by viral nucleic acid rather than by liver or hepatoma nucleic acids.

The recent report of the production of mammary tumours in rats by injection of nucleic acids and cell-free preparations of mammary cancers induced by 7,12-dimethylbenzanthracene implies viral involvement in the production of these tumours<sup>20</sup>. This possibility cannot be excluded in the present instance. However, it seems unlikely because of (a) the very low incidence of induced tumours and (b) the fact that attempts to produce tumours by intraperitoneal or intramuscular injection of cell-free saline extracts of the original hepatomas have invariably been unsuccessful.

There is no precise information as to how much, if any, AAF is bound to liver nucleic acids 30 days after discontinuing administration of the carcinogen. There is, therefore, no certainty that minute amounts may not have been introduced into the liver with the 'preneoplastic' liver nucleic acids and possibly, albeit remotely so, contributed to the development of the peculiar tumour of undetermined nature several months later. Nor can one exclude the possibility of spontaneous origin of this tumour.

In the case of the two hepatomas, however, the first of these two possible mechanisms does not apply and the second seems highly improbable because of (a) their fibrous encapsulation and localization at the site of injection, and (b) their development within only 10 and 16 weeks after the liver had been seen to be grossly normal at laparotomy. It seems reasonable to conclude that these two tumours were induced by the experimental procedure and that the probability is strong that a process of genetic transduction was involved in their pathogenesis.

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## Denaturation of Rennin ; Effect on Activity and Molecular Configuration

RENNIN has an optimum pH of 3.4 for the proteolysis of bovine serum albumin<sup>1</sup> and 3.8 for poly-L-glutamic acid<sup>2</sup>. At pH values between 5 and 7 it will coagulate milk and slowly attack casein. It has maximum stability at pH 5.4, while at values above 7 it loses activity rapidly.

Titration of a solution of rennin (crystalline enzyme prepared as described by Berridge and Woodward<sup>3</sup>, 11.5 mg/ml.) from pH 5.4 to pH 8.5 at room temperature, by means of a 'pH Stat', results in a further uptake of alkali after titration to an alkaline pH. It was found that the uptake at pH 8.5 had ceased after further addition of NaOH equivalent to 3.4 mole per mole enzyme (assumed mol. wt. 32,000). The increase in titratable groups suggests that after loss of enzyme activity there are relatively slow changes involving unmasking of groups presumably by the unfolding or splitting of the enzyme molecule. The

increase in viscosity number  $\left(\frac{V/V_0 - 1}{c}\right)$  from 5.25 at

pH 5.85-7.85 at pH 8.2 also indicates change in molecular configuration. The increases in viscosity number occur when the enzyme is initially taken to the higher pH value. On standing at pH 8.2 for 18 h and 65 h the viscosity number falls to 7.5 and 4.9 respectively. Further confirmation of possible changes, intra- and/or intermolecular, is indicated by the measurement of ultra-violet absorption which shows a 23 per cent fall in the absorption at 280 mμ, a 19 per cent fall in absorption at 290 mμ, and a 50 per cent increase in absorption at 250 mμ, after keeping a solution of the enzyme at pH 8.5 for 24 h.

Electrophoresis patterns of (a) active enzyme, (b) inactive enzyme immediately after treatment to pH 8.5, and (c) after being held at pH 8.5 for 24 h (Fig. 1) show that the active and inactive enzyme have similar mobility and that after 24 h at pH 8.5 other bands probably due to fragmentation of the enzyme appear.

Determination of the number of disulphide linkages in the enzyme molecule by the performic oxidation method

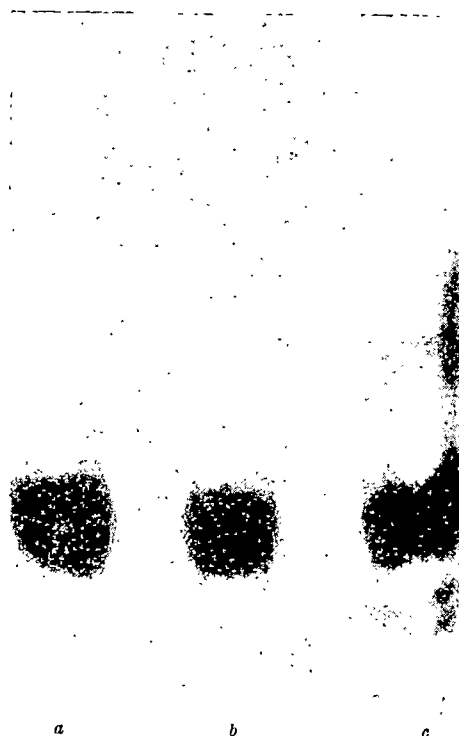


Fig. 1. Electrophoresis patterns of rennin and alkali-treated rennin in 5 per cent polyacrylamide gel, 0.005 M tris/citrate buffer pH 7.0. Run at 300 V for 5.5 h. Protein was stained with amido black. a, Active enzyme; b, inactive enzyme immediately after treatment at pH 8.5; c, after 24 h at pH 8.5.

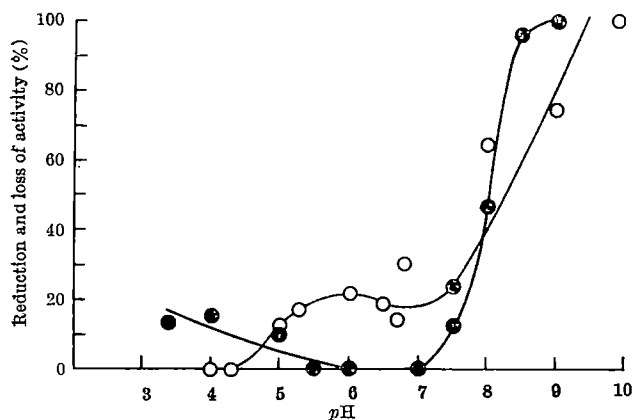


Fig. 2. The relationship of the pH of reaction with reduction of disulphide bonds and loss of activity. Open circles indicate reduction and closed circles activity. 100 per cent reduction is equivalent to one disulphide bond per enzyme molecule. The molecular ratio of  $\beta$ -mercaptoethylamine to enzyme was about 1,000 : 1

as modified by Moore<sup>4</sup> indicates three bonds per 32,000 mol. wt. No free -SH groups can be detected. However, investigation into the reactivity of the disulphide bonds shows considerable differences in their availability to reduction. When the enzyme is subjected to reduction with  $\beta$ -mercaptoethylamine at various pH (see Fig. 2) the maximum reduction occurs at pH 9.8 and is equivalent to one disulphide bond per molecule. Increasing the period of reduction from 1 h to 3 h did not increase this figure. Complete inactivation of the enzyme occurred one pH unit lower than that which gave maximum reduction. At this lower pH about 75 per cent of the possible reduction had occurred. By using sodium dodecyl sulphate dissolved to saturation in the buffer at pH 7, the quantity of disulphide reduced by  $\beta$ -mercaptoethylamine was increased to the equivalent of two linkages per molecule. Dissolving the enzyme in buffer saturated with guanidine HCl made one linkage available for reduction independent of the buffer pH. It was not possible under the conditions used to obtain reduction of all three disulphide bonds.

Enzyme activity is rapidly and irreversibly lost in the presence of urea. More than 50 per cent of the activity of *B*-rennin (isolated from crystalline rennin<sup>5</sup>) was lost in 30 min incubation at 37° in 4.6 M urea (see Fig. 3). Comparison with pepsin, an enzyme of similar molecular size containing three disulphide linkages<sup>6</sup>, shows that pepsin is more stable than rennin at the same urea concentrations. The total milk-clotting activity of commercial rennet after urea treatment is greater than the purified enzyme,

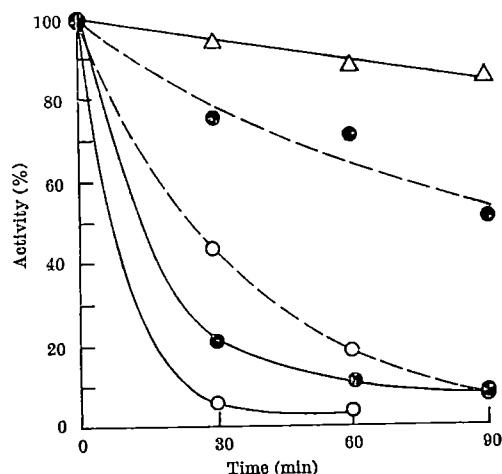


Fig. 3. Inactivation of *B*-rennin, commercial rennet and pepsin in urea at pH 5.4. ○, *B*-rennin activity; ●, rennet activity; △, pepsin activity. The broken line indicates activity in 4.6 M urea and the full line indicates activity in 6 M urea. The activity of pepsin in both 4.6 M and 6 M urea was similar

presumably owing to the presence of pepsin or other similar milk-clotting enzymes.

This series of observations shows that rennin activity is lost rapidly under relatively mild conditions such as do not denature all proteins, and that the loss is associated with minimal changes in molecular configuration. Prolonged treatment under the same conditions gives rise to further changes in configuration and finally to fragmentation of the molecule.

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## PHYSIOLOGY

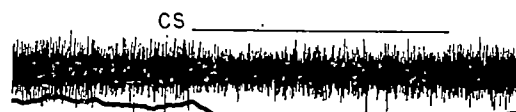
### Comparison of Multiple-unit and Electroencephalogram Activity recorded from the same Brain Sites during Behavioural Conditioning

IN an attempt to find a more sensitive measure of brain activity than that yielded by EEG recordings, and sample more extensively than is possible with single unit, micro-electrode recordings, we have examined multiple-unit activity recorded and integrated from various brain sites in cats during behavioural conditioning procedures. Multiple-unit recordings of central activity have been made in acute<sup>1,2</sup> and in chronic<sup>3</sup> preparations, but this technique has not been used in learning experiments<sup>4</sup>. In the work reported here, multiple-unit discharge was picked up by chronically implanted stainless steel insect-pin electrodes, amplified, and recorded on one beam of a Tektronix 502 oscilloscope. This unit activity was concurrently integrated and the output of the integration circuit recorded on the second beam of the oscilloscope. Simultaneously with these recordings, EEG activity was picked up through the same electrode and written out on a Grass model IIG EEG machine. In this communication a comparison will be made between the multiple-unit and EEG activity recorded from the same brain sites during the acquisition and extinction of a conditioned hindleg flexion response.

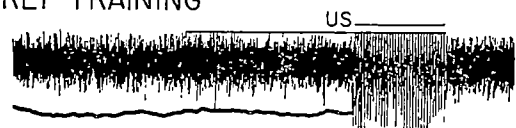
In Fig. 1, the multiple-unit activity of the medial geniculate body is illustrated on the left, while the EEG activity recorded concurrently through the same electrode is displayed on the right. Immediately below the multiple-unit activity, its integration is displayed a rise in the line of integrated activity corresponds to an increase in unit discharge, while a drop in the line indicates decreased activity. The first traces were recorded during the habituation of a chronic cat to a 1.5 sec, 1,500-c/s tone. As can be seen, a marked inhibition of geniculate unit discharge is produced by the tone and concurrently the integrated multiple-unit activity shows a marked depression. In contrast, the concomitant EEG recording indicates very little effect of tone presentation.

During subsequent training procedures, the 1,500-c/s tone (CS) was paired with a 0.5 sec, 60-c/s shock to the left hind paw (US) to establish a conditioned flexion response. In the second line of Fig. 1 traces are shown from a trial early in training prior to the appearance of overt conditioned responses. The multiple-unit activity is no longer inhibited and the integrated activity remains at a constant level during the CS presentation. Although this lack of response represents a significant shift from

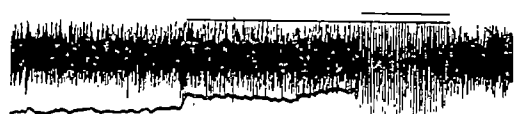
## HABITUATION



## EARLY TRAINING



## LATE TRAINING



## EXTINCTION

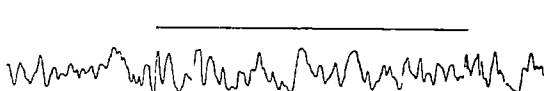
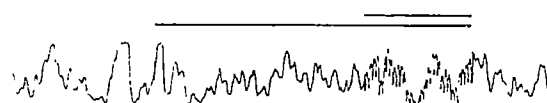
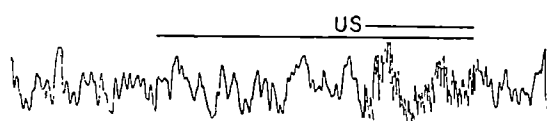
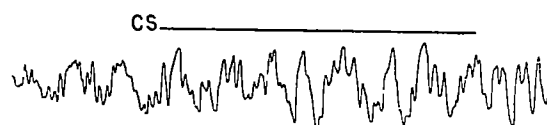
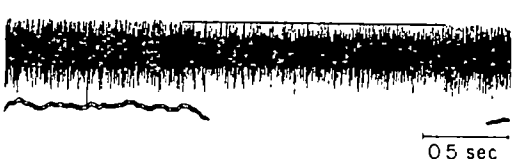


Fig. 1. Multiple-unit activity recorded and integrated from the medial geniculate nucleus (left) with concurrent recording of the EEG through the same electrode (right). Bars over traces indicate durations of 1.5 sec tone (CS: first and fourth traces) and CS paired with 0.5 sec shock to the hindpaw (US: second and third traces). The habituation excerpts are from the sixteenth habituation session. Activity during early training is shown by traces from trial 19 of the first conditioning session. The late training trial represents activity during performance of an overt conditioned response after the performance level had been maintained at 90 per cent or better for two prior sessions. The extinction trial is excerpted from the first extinction session, after disappearance of the overt response.

the inhibited unit discharge produced during habituation, no corresponding change is reflected by EEG activity.

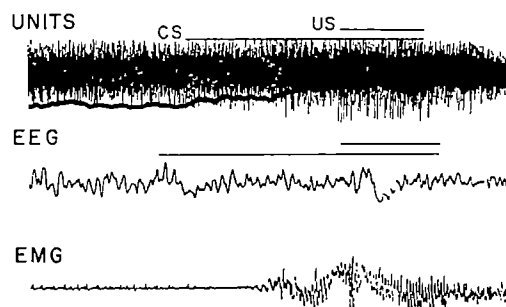
Traces recorded during a trial later in training, after overt conditioned responses had been established, are shown in the third line of Fig. 1. A sharp acceleration of unit discharge is now consistently elicited by the CS and the integrated unit activity increases correspondingly. The EEG shows a slight desynchronization, but this was constantly present in spite of the persistent unit acceleration.

In the fourth line, traces are shown from a trial during subsequent extinction, after the overt conditioned responses had disappeared. The direction of unit response was again shifted markedly. Inhibition is once more produced by the CS and, concurrently, the integrated activity drops markedly. The EEG during CS presentation, however, shows little that would distinguish it from the pre-CS period.

These sample recordings indicate that a progression of marked and oppositely directed changes in medial geniculate neuronal activity occur during various phases of behavioural conditioning and, by integration of the unit activity, these changes can be quantitated. EEG activity recorded simultaneously from the same site shows no alterations corresponding to the changes in multiple-unit activity, nor does the EEG suggest any other consistent response progression during the various training sequences. Similar results have been obtained from recordings in three other animals with histological verification of electrode placements in the medial geniculate nucleus.

In nine chronically implanted cats at present under examination, several other sites have shown the development of characteristic changes in multiple-unit activity during conditioning but no consistent or definite alterations in EEG activity at the same site. In Fig. 2, activity from the nucleus ventralis postero-lateralis (VPL) of the thalamus is represented by multiple-unit discharge in the upper trace and EEG activity, simultaneously recorded from the same electrode, in the second trace. In the third trace, EMG activity from the hamstring muscles of the

## N. VENTRALIS POSTERO-LATERALIS



## N. ANTERIOR VENTRALIS

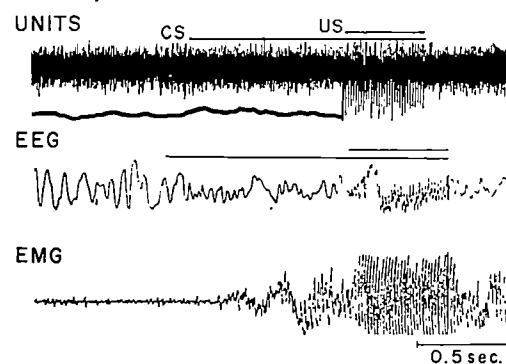


Fig. 2. Multiple-unit activity recorded and integrated with concurrent EEG from thalamic nucleus ventralis postero-lateralis during a conditioned flexion of the hindleg. Durations of 1.5 sec CS and 0.5 sec US are indicated by bars over traces. The EMG, recorded from the hamstring muscles of the responding leg, has the same time base as the EEG. Multiple-unit activity recorded and integrated with concurrent EEG from thalamic nucleus anterior ventralis during a conditioned flexion response. The EMG is recorded from the hamstring muscles of the responding leg.



'conditioned' hindleg is shown. During the conditioning trial illustrated, a marked increase in unit discharge and in the integrated activity-level is elicited by the CS concurrently with EMG activation and an overt conditioned response. The EEG, however, remains essentially unchanged during this trial as well as during other trials in which accelerated unit discharge accompanied the overt responses.

In contrast to the development of multiple-unit conditioned discharge in such thalamic sites as the medial geniculate and VPL nuclei, in other thalamic areas, such as the nucleus anterior ventralis (AV), the CS caused no change in unit activity even when overt conditioned responses were consistently being made. In the lower half of Fig. 2, the multiple-unit spike activity and integration recorded from the AV nucleus remain essentially unchanged during a CS presentation (upper trace), although EMG activation and an overt conditioned response are produced. EEG activity recorded from the same site (middle trace) shows a slight desynchronization to the CS, but this response was not constantly present.

Recordings from 23 brain sites sampled in nine chronic cats have shown that during behavioural conditioning procedures marked changes in multiple-unit activity frequently occur without any distinct or consistent change in the EEG recorded from the same site. Thus, multiple-unit recording not only provides a greater sample of spike activity than does single-unit recording, but also it would seem to be a much more sensitive index of changing neuronal activity than is the EEG. Moreover, by appropriate integration of the multiple-unit activity, a measure of neuronal activity is provided which allows a quantitative comparison of activity-levels at a particular brain site from one training sequence to the next or, during a particular training session, simultaneous comparison of activity-levels at a number of brain sites is possible.

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### Salivary Secretion of Caesium-131

In previous investigations it has been shown that potassium-42 and rubidium-86 are rapidly cleared from the blood and secreted into the saliva in identical fashion<sup>1,2</sup>. This has led to the development of techniques for the determination of exchangeable potassium with <sup>42</sup>K or <sup>86</sup>Rb, using saliva rather than urine, since urinary excretion of these two isotopes differs<sup>3</sup>. In an investigation of the applicability of the recently reactor-produced caesium-131 (physical half-life, 9.5 days; 29.4-keV X-ray) as a substitute for the short-lived gamma-emitters <sup>42</sup>K (physical half-life, 12 h) and the relatively long lived <sup>86</sup>Rb (physical half-life, 18.6 days) differences in the salivary excretion of <sup>131</sup>Cs and <sup>42</sup>K or <sup>86</sup>Rb were noted.

Tracer doses of <sup>131</sup>Cs were given to six control subjects in 50 ml. tap-water by mouth. Saliva was collected at various times thereafter starting at 14 h, the subject chewing on a small piece of paraffin for at least 2 min to establish an even flow of saliva. Samples of saliva were then collected for counting in a deep-well scintillation counter. Urine was collected for the entire period of observation to correct for excretion of <sup>131</sup>Cs.

Stable potassium (<sup>39</sup>K) in saliva was determined by flame photometry. 'Exchangeable potassium' was calculated as follows:

$$\frac{{}^{131}\text{Cs } \mu\text{c. administered} - {}^{131}\text{Cs } \mu\text{c. excreted}}{{}^{131}\text{Cs } \mu\text{c. l. saliva}/{}^{39}\text{K m.equiv./l. saliva}}$$

Table 1 shows the results of seven tests in the six control subjects. Predicted values for exchangeable potassium (Ke) were derived from Allen *et al.*<sup>3</sup> and Sagild<sup>4</sup>, who demonstrated a linear decrease in total body potassium of 17 m.equiv. a year for men and 10 m.equiv. a year for women.

Table 1. SALIVARY EXCRETION OF <sup>131</sup>Cs AND <sup>42</sup>K AND COMPARISON OF 'EXCHANGEABLE POTASSIUM' VALUES USING <sup>131</sup>Cs WITH PREDICTED VALUES USING <sup>42</sup>K

Subject, sex, age, wt., dose of <sup>131</sup> Cs	Hours after administration	<sup>131</sup> Cs, $\mu\text{c./l.}$	<sup>42</sup> K, m.equiv./l.	'Ke', m.equiv.	Ke, m.equiv. (predicted)
N. T.	14	6.85	22.4	1,448	
F, 33, 55 kg	16	4.74	15.2	1,420	
500 $\mu\text{c.}$	18	4.52	18.4	1,780	
	20	3.11	13.6	1,904	
	22	4.08	12.0	1,276	
	24	5.01	17.0	1,528	2,365 $\pm$ 170
	28	3.55	16.8	2,043	
	36			2,048	
	48			3,302	
N. T.	18	2.79	15.2	2,038	
F, 33, 55 kg	21 1/2	5.20	19.2	1,380	
400 $\mu\text{c.}$	24 1/2	6.45	24.0	1,401	2,365 $\pm$ 170
	27	4.00	16.8	1,571	
L. V.	16	2.92	22.4	2,160	
F, 24, 54.5 kg	18	2.56	21.6	2,350	
300 $\mu\text{c.}$	20	2.07	21.8	2,927	
	22	2.09	20.6	2,742	
	24	1.49	17.8	3,328	2,400 $\pm$ 170
F. W.	15	3.24	21.5	1,879	
M, 41, 70 kg	20	2.51	26.0	2,966	
300 $\mu\text{c.}$	24	2.42	23.5	2,784	3,320 $\pm$ 300
C. S.	15	1.46	16.8	4,344	
M, 31, 85 kg	18	1.37	17.6	4,846	
400 $\mu\text{c.}$	21	1.19	18.8	6,000	
	24	1.13	17.6	5,906	3,370 $\pm$ 300
F. B.	24	4.97	13.6	2,534	3,250 $\pm$ 300
M, 46, 86 kg	48			2,399	(3,390 with <sup>42</sup> K)*
1 mc.	50			3,564	(3,140 with <sup>86</sup> Rb)*
R. S.	21	1.92	25.6	4,971	
F, 63, 68.2 kg	24	2.29	25.6	4,187	2,100 $\pm$ 170
400 $\mu\text{c.}$					

\* Actual determinations.

The data show that <sup>131</sup>Cs is secreted in the saliva in an unpredictable manner as compared with <sup>42</sup>K and that at no time does there appear to be complete equilibration. The fluctuations of <sup>131</sup>Cs excretions in saliva from one collection period to the next are much greater than those of <sup>42</sup>K. An unexplained transient dip in specific activity and rise in exchangeable potassium values after 20 h was noted in four of five tests in which multiple collections were done. This is not due to diurnal variation because the tracer doses were given at various times of day.

Fig. 1 shows the specific activities:

$$\left( \frac{{}^{42}\text{K } \mu\text{c./l.}}{{}^{39}\text{K m.equiv./l.}} \right) \text{ and } \left( \frac{{}^{131}\text{Cs } \mu\text{c./l.}}{{}^{39}\text{K m.equiv./l.}} \right)$$

in two control subjects plotted against time. One of the subjects had received 400  $\mu\text{c.}$  <sup>42</sup>K and the other 400  $\mu\text{c.}$  <sup>131</sup>Cs. It is apparent that the rate of equilibration of these two isotopes in saliva is similar, so that the reduced secretion of <sup>131</sup>Cs must be due to a mechanism other than delayed equilibration.

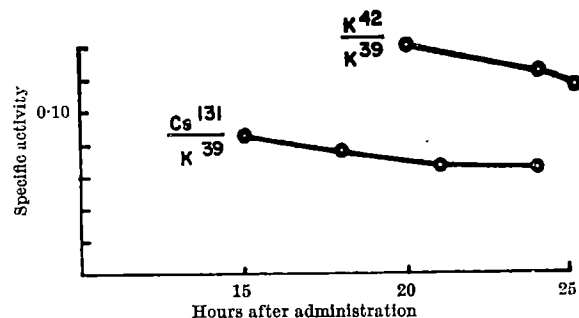


Fig. 1. Specific activities of <sup>42</sup>K/<sup>39</sup>K and <sup>131</sup>Cs/<sup>39</sup>K in two control subjects

It is concluded that the salivary glands secrete  $^{131}\text{Cs}$  qualitatively and quantitatively differently from  $^{42}\text{K}$  or  $^{86}\text{Rb}$  and that  $^{131}\text{Cs}$  cannot be used for salivary estimation of exchangeable potassium.

$^{131}\text{Cs}$  was generously supplied by Abbott Laboratories, North Chicago, Illinois.

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### Distribution of Dendrites in Retinal Ganglion and Cortical Stellate Cells

THE large multipolar ganglion cells of the retina send their dendrites in all directions over a wide area in the inner molecular layer. The general features of these cells have been investigated in many different vertebrates by numerous investigators<sup>1-3</sup>. Polyak<sup>3</sup> has given the fullest description, grouping the cells according to the extent and complexity of the dendritic processes when viewed in vertical section. Ramón y Cajal's original description grouped them chiefly according to the depth at which most of their dendrites were found in the inner molecular layer. Oshinomi<sup>4</sup>, probably because of the inherent difficulties of demonstrating the dendrites, relied solely on the size and shape of the body to place them in different categories.

It would seem that the purpose of these extensive dendritic branches is to derive impulses from areas of the retinal surface adjacent to the cell body. The extent and distribution of these branches in such an area can only be seen completely in whole mount preparations, stained by means of Ehrlich's methylene blue method. Apart from Dogiel's extensive investigations and the investigations of Kolmer<sup>5</sup>, Sorsby<sup>6</sup> and Polyak<sup>3</sup>, the retina has been little investigated by this method in recent years. Dogiel considered that the dendritic branches form a syncytial network and consequently this 'nerve net' theory precluded him from investigating the distribution of dendrites associated with individual cells. Now that it is known that the dendritic branches do not unite with each other, the determination of their distribution can provide a means of estimating the degree to which adjacent neurones are interrelated.

With this in view, Sholl<sup>7</sup> recently sought to explain the way in which incoming impulses are analysed by the visual cortex by determining the length and arrangement of the dendritic branches of the pyramidal and stellate cells found within it. Apparently these cell dendrites initially increase rapidly in numbers to a radius of about  $0\mu$  from the cell and, at greater distances, later decline slowly. Since the large multipolar ganglion cells of the retina also resemble the cortical cells, it seemed desirable to investigate the changes in their dendritic distribution to discover whether similar phases of dendritic proliferation and decay were common to other parts of the visual pathway.

To make a simple comparison between the cortical neurones and the ganglion cells of the retina, the average rate of branching of a dendrite at a given distance from the cell body was determined. This value will be referred to as the branching coefficient  $\lambda$ , being the fractional rate

at which branches were gained or lost per micron of radial distance from the cell. The branching coefficient was calculated for cortical cells from the data of Sholl, and for retinal ganglion cells it was found by measuring the dendritic processes in whole mount preparations of the rabbit retina. The positions of the dendrites were plotted by means of a camera lucida with a ruled graticule in the eyepiece of the microscope. Flatness of the field and its dimensions were checked by means of a stage micrometer.

These preliminary observations show that there are marked quantitative differences between the dendrites of cortical and retinal cells. The differences are illustrated in Figs. 1 and 2.

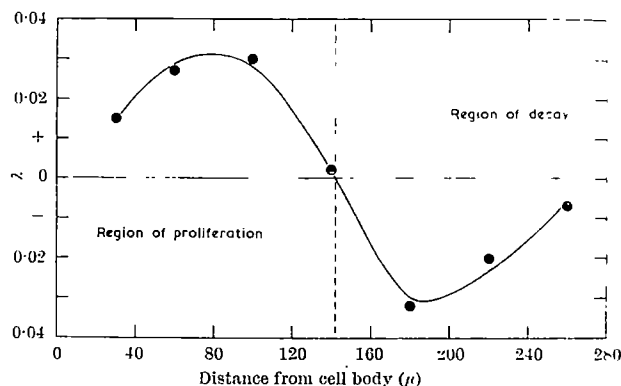


Fig. 1

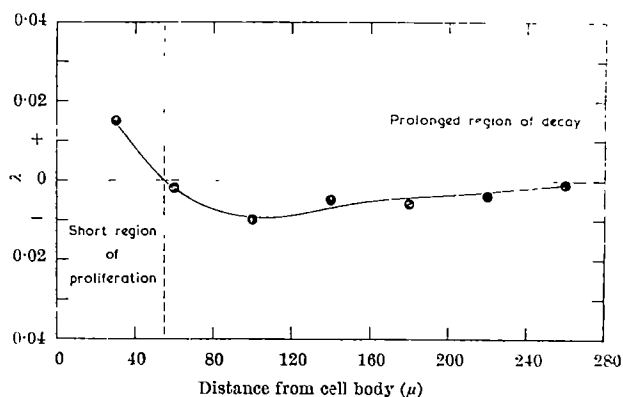


Fig. 2

At a distance of about  $30\mu$  from the cell body the fractional rate of formation of new branches is about the same in both retina and cortex. Afterwards, the cortical cells suffer an almost immediate loss of branches while the retinal cells continue to add new branches to their dendrites. Since the rate of branch formation in both regions near the cell body is similar, branching here may be controlled mainly by the cell itself. At greater distances, the influence of the cell becoming progressively weaker, other factors govern the dendritic branching and account for a decline in the cortical and a gain in the retina branches.

It will be seen from Fig. 1 that the dendrites of the ganglion cells show a continuous and rapid rate of proliferation for about half their course and thereafter lose their branches at about the same rate.

In Fig. 2 the cortical cell dendrites show a prolonged region some three times as extensive as ganglion cells where there is a slow continuous loss before they finally terminate. Furthermore, the rate of branching of retinal ganglion cells is about twice as great as cortical cells. This suggests that in the retina dendritic branches of the large multipolar ganglion cells link up with adjacent

areas to a greater extent than do the stellate cells of the visual cortex.

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### Delayed Onset of Proteinuria in Ageing Hypophysectomized Rats

THE excretion of protein in the urine of the male rat increases progressively with age<sup>1-3</sup>, the output of protein approximately doubling with each increase of 200 days in age<sup>3</sup>. This rising level of proteinuria in the rat has been associated with the gradual development of chronic nephrosis with advancing age<sup>4</sup>.

It has been shown by Goodman *et al.*<sup>5</sup> that hypophysectomy diminishes spontaneous proteinuria in the young male rat. The present investigation was undertaken to determine whether hypophysectomy performed in early life would permanently restrain the development of proteinuria in the ageing male rat.

At the age of 50 days, a group of 25 male albino rats of the Wistar strain were hypophysectomized using the parapharyngeal approach. Only hypophysectomized animals which gained less than 30 g in 300 days were used in this study. A second group of 25 male rats of the same age acted as controls. All animals were housed in air-conditioned rat quarters ( $27^{\circ} \pm 1^{\circ} \text{C}$  and  $55 \pm 5$  per cent relative humidity) and fed a pelleted diet as described earlier<sup>6</sup>. The urinary excretion of protein was determined a few days before operation and then at the ages of 250, 400, 500 and 600 days. For these estimations rats were placed individually in metabolism cages, and, after an adaptation period of three days, urine was collected for 24 h. Urinary protein was estimated using the trichloroacetic acid turbidimetric method of Henry *et al.*<sup>7</sup>.

At the age of 600 days only 6 hypophysectomized and 23 control rats survived. Protein excretion data from these animals at various ages are shown in Fig. 1. In the 23 control rats the mean protein excretion rose from zero at 48 days to 6.2 mg N per day at 600 days. However, in the 6 hypophysectomized rats no protein was excreted in urine until they were 500 days old, when trace amounts (0.1 mg/day) were detected in 3 animals. At 600 days these

3 rats were excreting 0.2, 0.4 and 1.4 mg respectively of protein N per day; the other 3 hypophysectomized rats in the group were still not excreting any protein.

Seven hypophysectomized rats died between 500 and 600 days and 4 of these excreted respectively 0.1, 0.2, 0.5 and 0.7 mg of protein N at 500 days. Of 12 hypophysectomized rats which died before 500 days two excreted 0.1 and 0.5 mg respectively of protein N at 400 days. Thus a total of 16 out of 25 hypophysectomized rats did not excrete detectable amounts of protein in their urine at any stage in their life-cycle. In some of these animals small fragments of pituitary tissue were observed in the pituitary fossa at autopsy. However, this residual tissue did not appear to be correlated with the excretion of protein.

These results, therefore, indicate that hypophysectomy, performed at 50 days of age, delays the onset of proteinuria in the male albino rat until it reaches the age of 400 days or later. This effect of hypophysectomy is similar to that of food restriction, which delays the onset of chronic nephrosis in the rat<sup>4</sup>, as well as slowing the ageing process.

This work was supported by the National Health and Medical Research Council of Australia.

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### Small $p\text{CO}_2$ Change and Neuronal Synaptic Activation

ALTHOUGH the effects of pharmacological and biochemical substances on synaptic activation have been extensively investigated, the influence of  $p\text{CO}_2$  has received little or no attention<sup>1</sup>. It had been shown previously<sup>2</sup> that large changes in  $p\text{CO}_2$  and  $p\text{O}_2$  altered synaptic activation, and the purpose of the present investigation was to examine quantitatively the effect of small changes in  $p\text{CO}_2$ . Since the impaled mammalian neurone is extremely fragile, the first investigations, lasting for several hours, were performed on a sturdier preparation—the identifiable giant neurones of *Aplysia fasciata*.

The visceral ganglia of 16 *Aplysia fasciata* were excised together with their nerves. As previously described<sup>2</sup> the preparation was investigated in a 2-ml. 'Lucite' cuvette located in a thermostated cylindrical chamber. The cuvette was filled with sea water and the nerves were mounted on silver chloride-silver hooks connected to a Grass stimulator. Glass micro-electrodes were inserted into selected nerve cells and, when the preparation was complete, the chamber was covered with a glass plate. Lateral apertures were used for the introduction of micro-electrodes and the admission of various gas mixtures so that the chamber could be successively filled with room air or with 5 per cent  $\text{CO}_2$  in air. Gas flow was regulated by a calibrated flow meter.

The pH and  $p\text{CO}_2$  in the blood of *Aplysia* were determined so that the same levels could be duplicated *in vitro* in the sea water surrounding the ganglion cells. A few seconds after the mollusc had been cut open, samples of blood were taken anaerobically with a tuberculin syringe from the largest vessel leading into the heart. The  $p\text{CO}_2$ ,

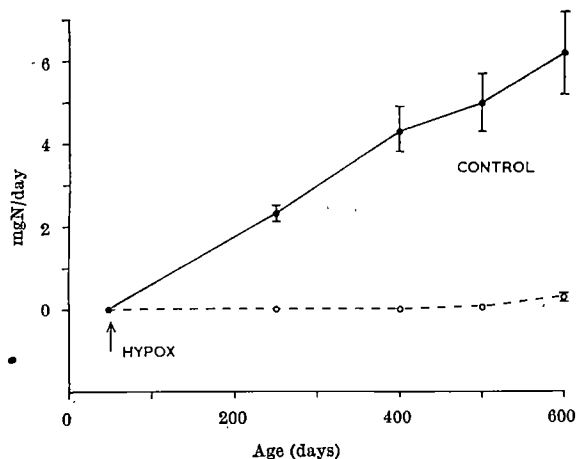


Fig. 1. Variation with age in the daily urinary excretion of protein nitrogen (mean  $\pm$  S.E.) in 6 male rats hypophysectomized at 50 days of age (broken line) and 23 controls (continuous line).



H and  $pO_2$  were analysed directly by means of a micro-electrode assembly, and the values obtained ranged between 2.5 and 4.3 mm mercury for  $pCO_2$ , while pH ranged from 7.53 to 7.83 and  $pO_2$  from 8 to 39 mm mercury. The pH,  $pCO_2$  and  $pO_2$  of the water in the cuvette were then measured during the admission of two gas mixtures (room air and 5 per cent  $CO_2$  in air). Every 30 sec, samples were taken from the cuvette under conditions of constant gas flow and when the ganglion was not in place to avoid any mechanical stimulation of the nerve cells). When gas mixtures were changed from room air to 5 per cent  $CO_2$  in air,  $pCO_2$  increased at a constant rate during the first 2 min (1.8 mm mercury every 30 sec); and the  $CO_2$  levels in the water of the cuvette during the experiment were close to those observed in the living animal (2–5 mm mercury). In several instances samples of sea water were taken with the ganglion in place and after significant alterations of synaptic potentials had been observed. Changes in  $pCO_2$  recorded at that time were similar to those observed previously.

Neurons, initially at rest and giving only excitatory post-synaptic potentials when orthodromically activated, were especially examined. After the micro-electrode had been inserted the ganglion was kept undisturbed for 10 min in order to obtain a control reference state. The leurogenital nerve was then stimulated at a frequency of 1 per sec and with an intensity sufficient to elicit on the soma only excitatory post-synaptic potentials. The amplitude of the excitatory post-synaptic potentials was set at a level higher than 10 mV because under such conditions marked local somatic responses are present. The substitution of room air with 5 per cent  $CO_2$  in the air in the chamber depolarized the soma very slightly and in 10 sec promoted the local somatic response to a pike. Fifty sec after 5 per cent  $CO_2$  had been introduced into the chamber all excitatory post-synaptic potentials elicited spikes indicating that synaptic excitability of the soma had been significantly increased. At the same time,  $pCO_2$  in the surrounding sea water had increased from 0 to 3 mm mercury, pH had dropped from 8.02 to 7.73 and  $pO_2$  had not changed.

After the 5 per cent  $CO_2$  mixture was discontinued and room air re-introduced into the chamber, there was a reversal of the described effects and only excitatory post-synaptic potentials were recorded as  $pCO_2$  returned to control levels. Fig. 1 illustrates the alteration in synaptic activity produced by a  $pCO_2$  change of from 0 to 3 mm mercury in the fluid surrounding the ganglion in a giant 1 neurone.

In conclusion, these observations suggest that small changes in  $pCO_2$  (1–3 mm mercury) affect the instantaneous excitability of the soma and thus the efficiency of its excitatory synaptic activation. Such changes in  $pCO_2$  occur spontaneously in most aerobic species and

could, therefore, markedly contribute to basic neuronal regulations.

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## PHARMACOLOGY

### A Sensitive Assay for Inhibitory Agents of Pleuropneumonia-like Organisms

RAPID methods of measuring the potential inhibition of pleuropneumonia-like organisms (PPLO) by various agents have been evolving slowly. The phenol red serial dilution broth test has been extensively used even though certain mycoplasmas do not grow well in the broth and do not produce sufficient acid to create a sharp end-point. Griffith and Black have recently devised a haemolysis-blocking test system for comparing the activities of various antimicrobial agents against *Mycoplasma pneumoniae* (Eaton agent)<sup>1</sup>. This test and similar plate tests are relatively slow, requiring 3–7 days for completion. We have attempted at this laboratory to alter the phenol red tube dilution test by various manipulations of the dye and the inoculation procedures. We have found that resazurin is preferable to phenol red, 2,3,5-triphenyltetrazolium chloride, or 2,6-dichlorophenolindophenol dyes. The test system we chose may prove to be of use in other laboratories involved in investigations of PPLO inhibition. It is a simple tube dilution sequence that gives results within 18–24 h for most PPLO and in 30–36 h for *M. pneumoniae*. This system could most likely be shortened even further by inoculum variation. For most purposes, however, it is sufficiently rapid.

The mycoplasmas were grown in Difco phenol red maltose broth with the addition of 1.5 per cent PPLO serum fraction (Difco) and 0.025 per cent thallium acetate. Alternatively, Difco PPLO broth without crystal violet and with the aforementioned additions could also be used. The inoculum was incubated at 37° C for 18–24 h and 0.05 ml. used to inoculate 2.0 ml. test medium. Difco PPLO broth, as prepared for the inoculum, was used for the tube dilution test. Resazurin was added after separate sterilization so that the final dye concentration was 0.002 per cent. (The PPLO serum fraction was also separately sterilized.) 2.0 ml. quantities of the test medium were then transferred to a number of small blood tubes. Using the normal serial tube dilution technique, the inhibition end-point was determined as the first tube containing non-reduced resazurin. Positive and negative controls were always included for direct colour comparison. The test was incubated at 37° C for 18–24 h before reading.

When the test PPLO was *M. pneumoniae*, the inoculum was grown on Difco PPLO broth, as described by Chanock *et al.*<sup>2</sup>, and only half as much resazurin was included in the test medium. The inoculum for the test was concentrated by centrifugation and the pellet obtained resuspended in a quarter of the original volume of broth. The Chanock modified Difco PPLO broth test medium was inoculated with 0.2 ml. of this 1:4 concentrated cell suspension. The tubes could be read after 30–36 h.

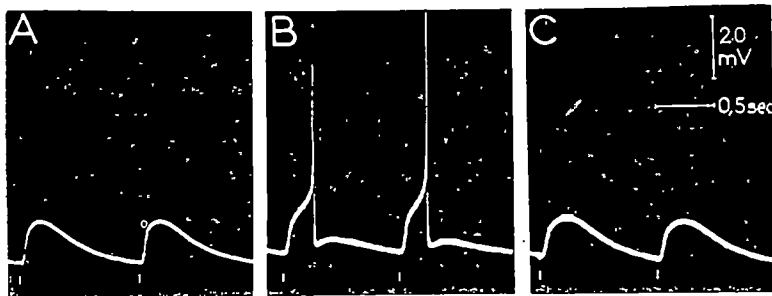


Fig. 1. Giant A-type nerve cell in the visceral ganglion of *Aplysia fasciata*, orthodromically activated by brief stimuli (1 per sec) of constant intensity to the afferent nerve fibres; temp., 26° C. A, In the presence of air, only local somatic responses were elicited superimposed on the excitatory post-synaptic potentials. ( $pCO_2$  of sea water 0.02 mm mercury (calculated); pH 8.07;  $pO_2$  89 mm mercury.) B, Thirty sec after admission of 5 per cent  $CO_2$  in air, spikes were elicited on the excitatory post-synaptic potentials. ( $pCO_2$  of sea water < 3 mm mercury; pH 7.73;  $pO_2$  88 mm mercury.) C, Fifty sec after readmission of air, there was recovery to the initial excitability level. Again, only somatic local responses followed the excitatory post-synaptic potentials. ( $pCO_2$  of sea water 0.02 mm mercury (calculated); pH 8.00;  $pO_2$  92 mm mercury)

Table 1. SUMMARY OF MINIMAL INHIBITORY CONCENTRATIONS OF ANTIBIOTICS AGAINST SIX MYCOPLASMAS (Values in  $\mu\text{g/ml}$ .)

Antibiotic	<i>M. pneumoniae</i> (FH)	S114	Culture* S103	698	S1,247	699-21
'Tylosin'	0.001	3.18	6.25	1.56	6.25	12.5
Tetracycline	0.04	1.25	1.25	1.25	1.25	1.25
'Gentamicin'	—	12.5	6.25	3.13	12.5	50.0
Erythromycin	0.001	<1.56	<1.56	<1.56	>50	>50
'Lincomycin'	—	>50	>50	<1.56	>50	>50
'Amicetin'	0.02	0.32	0.32	0.63	1.25	1.25
'Plicaceticin'	—	0.63	0.63	0.16	—	—
'Bamcetin'	—	0.16	0.16	<0.16	1.25	0.63
Trilactoylolean-	—	—	—	—	—	—
domycin	—	>50	25	6.25	>50	>50
Oleandomycin	—	>50	50	<0.78	>50	>50
'Leucomycin'	—	3.13	6.25	<0.78	25	50
Spiramycin	—	1.66	6.25	<0.78	25	25
Carbomycin	—	25	25	25	>50	>50
Cycloerine	—	>50	>50	>50	>50	>50
'Ramycin'	—	3.13	1.56	<0.78	1.56	1.56
Chloramphenicol	0.02	—	—	—	—	—
'Cephalothin'	>50	>50	>50	>50	>50	>50

\* S cultures are isolates from the nasal passages of pigs, 699 is *M. gallisepticum*, and 698 is a bovine isolate.

Table 1 summarizes some of the results obtained when various antibiotics were compared to show their relative effectiveness against mycoplasmas. Obviously, the FH strain of *M. pneumoniae* was fairly sensitive to the action of the antibiotics.

The relative activity of erythromycin against *M. pneumoniae* is in agreement with the results reported by other research workers<sup>1</sup>.

Investigations are being continued using other methods so that a very rapid test, for example, 2-4 h, may be developed. Adaptation of these dye methods may assist diagnosis—as was recently suggested by Kraybill and Crawford<sup>2</sup>.

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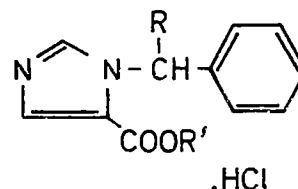
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### Propoxate (R7464): a New Potent Anaesthetic Agent in Cold-blooded Vertebrates

PROPOXATE is the proposed generic name for *dl*-1-(1-phenyl-ethyl)-5-(propoxy-carbonyl)-imidazole HCl. It is a member of a new series of 1-substituted imidazole-5-carboxylic acid esters (Fig. 1). The prototype of this series, Etimidate (proposed generic name for R7405), is an extremely potent, rapid and short-acting hypnotic agent in a variety of animal species<sup>1</sup>. Propoxate (R7464) is a potent, polyvalent and safe anaesthetic in cold-blooded vertebrates and is very soluble in fresh and salt water. Compared with 'Tricaine' (methanesulphonate of meta-amino-benzoic acid ethylester, MS222) it is about 100 times more potent, the active concentration ranging from 0.5 to 10 p.p.m. and from 50 to 1,000 p.p.m. for R7464 and MS222 respectively.

Experiments with goldfish have been carried out in 101 aerated aquaria kept at  $19^\circ \pm 2^\circ \text{C}$ . Concentrations of R7464: 1/16, 1/4, 1, 4, 16 and 64 p.p.m., were tested at various immersion times, 1/64, 1/16, 1/4, 1, 4 and 16 h. Recovery from anaesthesia was effected by transferring the fish into clean aquaria containing aerated tap water.

Our observations have led us to assign, rather arbitrarily, the following four stages of anaesthesia: stage I, increase of respiratory movements, followed rapidly by a decrease in activity and respiration; stage II, loss of equilibrium. At this point the respiratory rate is quite slow but responses to strong vibrational stimuli persist;



		R	R'
R7405	ETIMIDATE	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>
R7464	PROPOXATE	CH <sub>3</sub>	nC <sub>3</sub> H <sub>7</sub>

Fig. 1

stage III, loss of righting reflex. The fish lie on the bottom of the aquarium, respiration is superficial and irregular and they do not respond to manipulation; stage IV, respiratory arrest. This stage can be maintained for a limited period of time, without fatalities occurring.

Phases resembling those of induction of anaesthesia were observed during the recovery period, but their quantitative delineation was much more difficult. Our observations are given in Table 1.

		Induction of anaesthesia, mean values 6 animals minutes' or seconds"				Recovery from anaesthesia, approx. values minutes' or hours h				Safe immersion time (h)
R7464	Im-mersion time	I	II	III	IV	IV	III	II	I	
P.p.m.	h									
1/16	16	—	—	—	—	—	—	—	—	≥16
1/4	16	4'	35'	—	—	—	—	—	—	≥16
1	1	90"	3'	6'	—	—	?	30'	<120'	
	4	90"	3'	6'	—	—	?	105'	<140'	≥16
	16	90"	3'	6'	—	—	10'	30'	75'	
4	1/16	30"	1'	3'	—	—	8'	20'	45'	
	1/4	30"	1'	3'	—	—	12'	20'	120'	
	1	30"	1'	3'	—	—	15'	90'	150'	≥4
	4	30"	1'	3'	15'	25'	180'	165'	<18 h	
	16	30"	1'	3'	10'	120'	?	<24 h	<36 h	
16	1/16	15"	25"	50"	3'	11'	26'	45'	120'	
	1/4	15"	25"	50"	3'	6'	30'	50'	120'	≥1
	1	15"	25"	50"	3'	30'	210'	315'	420'	
	4	15"	25"	50"	3'	—	—	—	—	
64	1/64	5"	10"	25"	45"	4'	30'	90'	120'	
	1/16	5"	10"	25"	45"	6'	60'	180'	240'	≥1/4
	1/4	5"	10"	25"	45"	20'	240'	360'	450'	
	1	5"	10"	25"	45"	—	—	—	—	

I, II, III, IV: stages of anaesthesia.

The degree of anaesthesia obtained in goldfish with R7464 is closely related to the concentration used, and various and selected depths of anaesthesia can be induced with atoxic concentrations. A slight, long-lasting tranquillization, resulting in a loss of reactivity and a decreased oxygen consumption, useful for transport, can be obtained with concentrations up to 1/4 p.p.m. A somewhat deeper level of anaesthesia inducing loss of righting reflex is obtained with 1 and 4 p.p.m. At this depth routine biological investigations can be easily performed and goldfish can be removed from the water for several hours without harmful effects, the only precaution necessary being to enclose them in moist paper. The two highest concentrations shown lead rapidly to complete respiratory arrest which can be sustained for a limited period without harmful effects—1 h for 16 and 1/4 h for 64 p.p.m.

The recovery time from anaesthesia depends on the concentration and on the duration of immersion; furthermore, great individual differences in fish may occur. As a general rule, however, disregarding the duration of immersion, it may be said that the shorter the induction time of anaesthesia, the longer the recovery, and vice versa.

Similar effects to those in goldfish were obtained in unfish with 1 and 4 p.p.m. In rainbow trout and in eafish 1/4 p.p.m. was still effective and induced stage II. In young salmon 2 p.p.m. led rapidly to loss of righting reflex, and laboratory experiments performed with *Lambusia* were very promising<sup>2</sup>.

In frogs, 2 p.p.m. paralysed the hind legs after an immersion period of 30 min; 16 p.p.m. produced loss of fighting symptoms within 10 min.

Immersion of adult species of salamanders in 4 p.p.m. or 15, 30 and 60 min was carried out without fatalities; stages II and III were easily obtained after 5 and 20 min of immersion. Allowed to recover in an empty aquarium, all animals became normal after 6 h.

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### Effect of Reserpine on Atrial and Ventricular Rates in Atrial Fibrillation in Man

INVESTIGATIONS into the intrinsic rhythmicity of normal cardiac pacemakers suggest that the catecholamine content of the myocardium is an important determinant of their activity<sup>1</sup>. Reserpine depletes stores of catecholamines; in the dog heart-lung preparation after initial acceleration it slows the sinus rate<sup>2</sup>, and in the dog heart *in situ* with heart block it markedly slows the ventricular pacemaker<sup>1</sup>. In man reserpine slows the normal heart<sup>3</sup>, but since the sinus node is under extra-cardiac control this observation provides no basis for speculation concerning the mechanism of reserpine effects on the cardiac pacemaker.

Atrial fibrillation is a special circumstance in which a rapid ectopic pacemaker is relatively independent of extracardiac autonomic control. In atrial fibrillation in man, reserpine slows the ventricular rate<sup>4</sup>, but its effect on the atrial rate has not been reported. The slowing of the ventricular rate does not mean that there is necessarily a corresponding change in atrial rate, for in atrial fibrillation where the ventricle responds to atrial impulses, changes in atrial rate often result in reciprocal changes in ventricular rate, for example, after quinidine the atrial rate falls and the ventricular rate rises. In addition, ventricular rate may change as a result of effects on AV conduction. An acceleration of ventricular slowing by atropine in one patient as reported by Marangoni and Cavusoglu<sup>4</sup> does not establish increase of vagal tone by reserpine since atropine could accelerate the ventricle if reserpine had not been given.

After a two-week control period, 7 patients with atrial fibrillation, suffering from mental disease but not in congestive failure, were given reserpine in daily oral doses of 2.0 mg for one week, and 3.0 mg for the week following, after which the reserpine was withdrawn. There were no other changes in medication during the investigation period. The atrial rates were counted according to the method of Gold *et al.*<sup>5</sup> in 1-min electrocardiographic strips (sternal lead), before the administration of reserpine, twice weekly during treatment with reserpine and at the end of each week after withdrawal. Ventricular rates were determined from the same strips of electrocardiogram.

The averages of the counts were plotted. The curves (Fig. 1) indicate that the ventricular rate fell after the first week of treatment, continued below the control during the entire treatment period (but did not fall further when the dose was raised from 2.0 mg to 3.0 mg per day, indicating that the former was a ceiling dose)

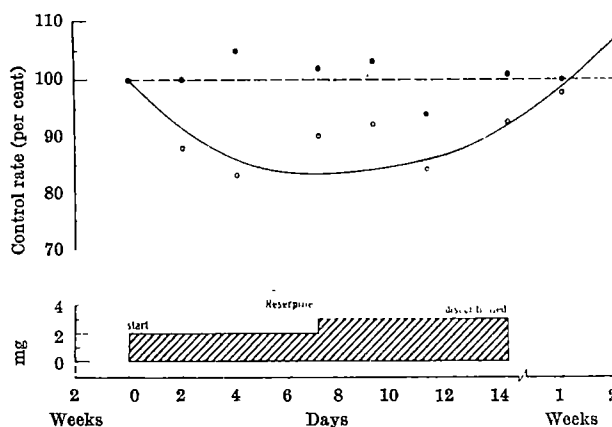


Fig. 1. Atrial and ventricular rates in seven patients with atrial fibrillation receiving reserpine. —, Ventricular rate (100 per cent = 85.7/min); ---, atrial rate (100 per cent = 40.7/min)

and rose after the first week of withdrawal, returning to control at the end of the second week. The atrial rate was not changed by the reserpine. The average rates after reserpine were paired with control rates in each case and examined by the sign test. The *P* value for the atrial slowing compared with control was 0.31 during treatment and 0.4 after treatment. The *P* value for ventricular slowing was 0.005 during treatment and 0.39 after withdrawal. Our findings are diametrically opposed to those of Arora<sup>6</sup>, who observed atrial slowing but no ventricular change after reserpine in atrial fibrillation induced by aconite in the dog heart *in situ*.

Since in our cases there was no change in atrial rate, there could have been no significant effect of catecholamine depletion on the ectopic mechanism, and since all ventricular beats were responses to atrial impulses, the only simple and reasonable explanation for the ventricular slowing is depression of AV conduction by reserpine. Nothing in this experiment bears on the mechanism of the effect; there are the possibilities of unopposed vagal activity as a consequence of catecholamine depletion, a central action to increase vagal tone, and, as already suggested by Innes, Krayer and Waud<sup>7</sup> on the basis of observations in the dog heart-lung preparation, of a direct depressant effect on AV conduction.

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### HAEMATOLOGY

#### Polypeptide Chains of Buffalo Haemoglobins

THAT many breeds of cattle exhibit polymorphism involving two haemoglobin types is now well established<sup>1</sup>. On the other hand, buffalo haemoglobin seems to be monomorphic in that all buffaloes examined possess two major haemoglobin components<sup>2,3</sup>. Bovine haemoglobins have been fairly thoroughly characterized<sup>1</sup> and it has been suggested that the two bovine haemoglobins may contain one polypeptide chain in common<sup>4</sup>.



This report describes the isolation of the two buffalo haemoglobins through carboxymethylcellulose columns, followed by evaluation of these pigments by means of resolution of the globins into their sub-units by paper electrophoresis in 6 M urea and by peptide pattern analysis of the globins and the separated polypeptide chains.

Haemolysates were prepared according to Drabkin<sup>2</sup>. The preliminary survey of the haemoglobins was carried out by paper electrophoresis in barbitone-HCl buffer, pH 8.6 ( $I = 0.05$ ). For separation into two components, the cyanomet forms of the buffalo haemoglobins were subjected to carboxymethylcellulose chromatography by a method modified from that of Huisman *et al.*<sup>3</sup>. The cellulose exchanger was prepared<sup>7</sup> from cellulose powder No. 123 (Carl Schleicher and Schüll) and was finally equilibrated with 0.01 M phosphate buffer, pH 6.0. The fast-moving component (higher anodic mobility at pH 8.6, referred to as 'buffalo-Hb-fast') was eluted by using phosphate buffer of pH 6.9, and the slower component ('buffalo-Hb-slow') at pH 7.4. The haemoglobins were dialysed prior to concentration by pervaporation. Globins were prepared according to Anson and Mirsky<sup>8</sup>. The globins were resolved by paper electrophoresis, run in 6 M urea with pyridine-acetic acid buffer of pH 6.8 according to the method of Take<sup>9</sup>, who resolved bovine globins into two sub-units moving towards the cathode, the faster

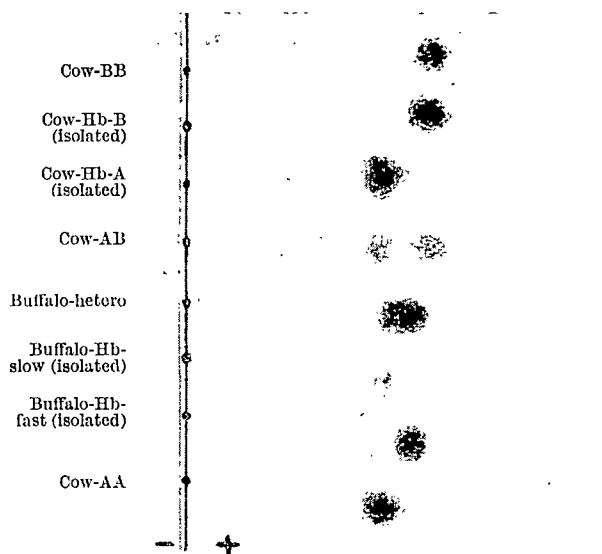


Fig. 1. Paper electrophoresis of cow and buffalo haemoglobins in barbitone-HCl buffer ( $I = 0.05$ ) at pH 8.6

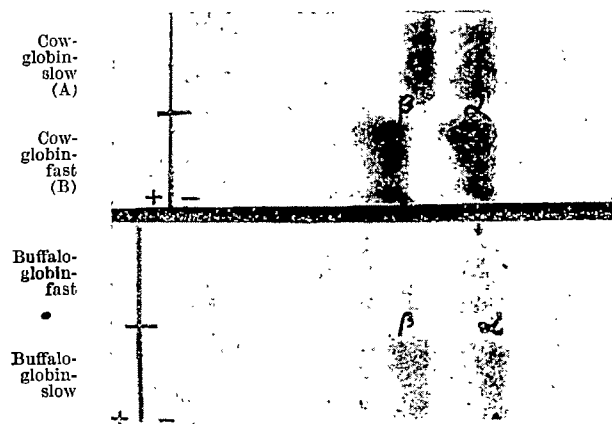


Fig. 2. Electrophoretic resolution of the globins of buffalo-Hb-fast and buffalo-Hb-slow in 6 M urea with pyridine-acetic acid buffer at pH 6.8

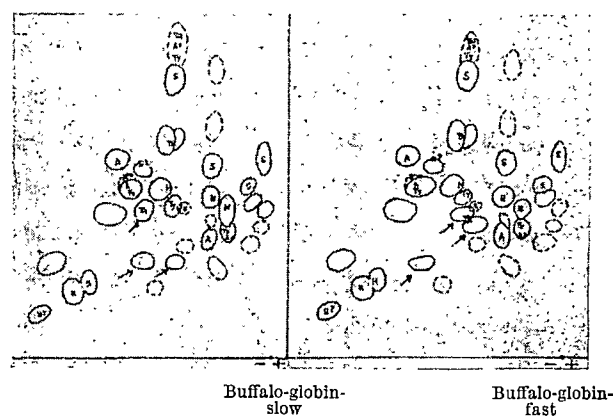


Fig. 3a. Tracings of the fingerprints of tryptic digests of the globins of buffalo-Hb-fast and buffalo-Hb-slow. Electrophoretic buffer: Michl's buffer pH 6.4. Chromatographic solvent: butanol-pyridine-acetic acid-water (30:20:6:24)

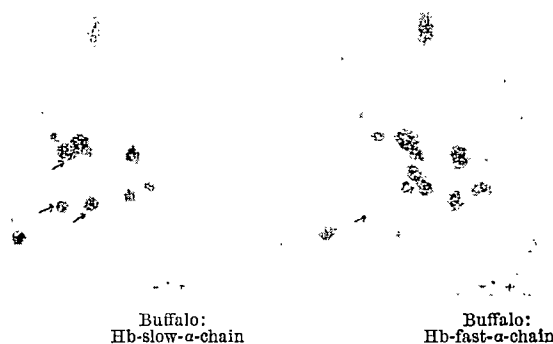


Fig. 3b. Photographic representation of the fingerprints of tryptic digests of the  $\alpha$ -chains of buffalo-Hb-fast and buffalo-Hb-slow. Electrophoretic buffer: Michl's buffer pH 6.4. Chromatographic solvent: butanol-pyridine-acetic acid-water (30:20:6:24)

component being found to correspond to the Val-Leu-chain and the slower to the Met-chain. The two polypeptide chains of buffalo globins were isolated according to the trichloroacetic acid precipitation method, as applied to bovine globins<sup>10</sup>. The purity of the chains was checked by paper electrophoresis in 6 M urea at pH 6.8. While the  $\alpha$ -chains (higher cathodic mobility) were pure, the  $\beta$ -chains (lower cathodic mobility) showed traces of the faster component. Tryptic peptides and finger-prints were prepared in the usual manner<sup>11</sup> with butanol-pyridine-acetic acid-water as the chromatographic solvent<sup>12</sup>.

The 38 samples of buffalo haemolysates examined showed two haemoglobin components in each, as against cow haemolysates (Gir breed) where polymorphism involving two haemoglobin components was observed (Fig. 1). The examination of globins of the buffalo-Hb-fast and buffalo-Hb-slow for their sub-units by electrophoresis in 6 M urea at pH 6.8, indicated that mobilities of the  $\alpha$ -chains of the two buffalo haemoglobins were different whereas that of the  $\beta$ -chains appeared similar (Fig. 2). This is in contrast to the globins of bovine Hb-A (lower anodic mobility at pH 8.6) and bovine Hb-B, which seem to share  $\alpha$ -chain in common while  $\beta$ -chains are different (see Fig. 2). The fingerprints of the globins and of the separated polypeptide chains of buffalo-Hb-fast and buffalo-Hb-slow indicated that the  $\alpha$ -chains of these two pigments (higher cathodic mobility at pH 6.8 and of comparable mobility to the Val-Leu-chain of the bovine globin) differ in three peptide spots, one involving tryptophan (Fig. 3a and 3b). The  $\beta$ -chains (of comparable mobility to the Met-chain of the bovine globin), however, did not indicate differences in their peptide patterns.

Comparison of the tryptic peptide pattern and characterization of individual polypeptide chain of bovine-Hb-A, ovine-Hb-B, buffalo-Hb-slow and buffalo-Hb-fast is being carried out.

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### <sup>32</sup>P-labelling of Nucleotides from a Soluble Erythrocyte-membrane Fraction devoid of Haemoglobin

In a previous paper<sup>1</sup>, the incorporation of radioactive organic phosphate into human red-cell ghosts was investigated in great detail. About 30 labelled phosphopeptides could be isolated, and their amino-acid patterns were determined. The rate of incorporation and its pH-dependence were also estimated. It was also shown<sup>2</sup> that the <sup>32</sup>P-incorporating membrane factor could be extracted and purified, and at least some part of the phospholipids also seemed to be metabolically active, since labelled phosphorylethanolamine and phosphorylcholine could be isolated. In addition, it was briefly mentioned that labelled trichloroacetic acid (TCA)-soluble inosine triphosphate (ATP) seemed to be formed in the labelling process. In the TCA-insoluble material, labelled ultra-violet-absorbing substances were also present.

Post *et al.*<sup>3</sup> have recently demonstrated a possible linkage between ATPase activity in the red-cell membrane and active transport of sodium, and Hoffman<sup>4</sup> has also shown that ATP might provide energy necessary for the sodium transport mechanism. Earlier investigators, including Hurley<sup>5</sup>, Gerlach *et al.*<sup>6</sup>, Tatibana *et al.*<sup>7</sup>, Bartlett<sup>8</sup> and Parsky *et al.*<sup>9</sup>, have all found that ATP in the erythrocyte content seems to be the primary acceptor when red cells are incubated with <sup>32</sup>P. Schauer and Hillman<sup>10</sup>

have found some evidence of the presence in the red-cell membrane of an ultra-violet-absorbing, acid-labile compound which they believed might be engaged in the phosphorus transport. Another possible intermediate in the process of <sup>32</sup>P transport across the membrane is ATP formed in the membrane itself. Some data have been found which support this hypothesis.

A *tris*-glycylglycine extract of human red-cell ghosts washed with water was prepared as previously described<sup>2</sup>. After isoelectric precipitation at pH 6.0 and removal of the precipitate, the solution was filtered through 'Sephadex G-200' columns. In each preparation, material from 600–700 ml. of packed red cells was used. The colourless lipoprotein fraction first eluted from the 'Sephadex' columns was used in the subsequent experiments. Incubation with <sup>32</sup>P (0.1 mc./ml.) was carried out at about 20° C for 4 h in the presence of 1 × 10<sup>-4</sup> M unlabelled Na<sub>2</sub>HPO<sub>4</sub>. Excess of free <sup>32</sup>P was removed by filtration through 'Sephadex G-25' medium-size columns.

TCA-soluble nucleotides from human erythrocyte material were prepared in the following way. Haemolysis of washed red cells was carried out by addition of 9 volumes of distilled water. The pH was adjusted to 6.0 by a flow of CO<sub>2</sub>. After storage overnight at 4° C in the cold room, the ghosts (usually from about 600 ml. of packed red cells) were removed by centrifugation in a refrigerated Stock centrifuge. The haemolysate was concentrated by evaporation *in vacuo* at 18° C to about one-tenth of the original volume. Haemoglobin and other proteins were precipitated by addition of concentrated cold TCA to a final concentration of 10 per cent. The precipitate was removed by centrifugation, and excess TCA was extracted by shaking 4 or 5 times with an equal volume of ethyl ether. The pH was adjusted to 5.5 with 5 N NaOH, and the volume of the solution to 100 ml. When not immediately used, the solution was stored in a cold room at -16° C.

The labelled <sup>32</sup>P-incorporating lipoprotein complex from about 600 ml. of packed red cells was incubated for about 30 sec with TCA-soluble nucleotides from the content of the same volume of cells. The protein was precipitated by addition of TCA. TCA was removed by extraction with ethyl ether as usual, and the solution was concentrated and run through a 'Dowex-1' (2 per cent DVB) column, mainly according to Hurlbert *et al.*<sup>11,12</sup>. The labelled nucleotide fractions were either directly analysed for phosphorus<sup>13</sup>, ribose<sup>14</sup> and base constituents<sup>15</sup> or, after preliminary purification by paper ionophoresis, scanning of the electropherograms and elution of labelled spots with ultra-violet absorbancy.

In this way, it was found that the two largest labelled ultra-violet-absorbing peaks consisted of ATP and guanosine triphosphate (GTP), respectively. Smaller

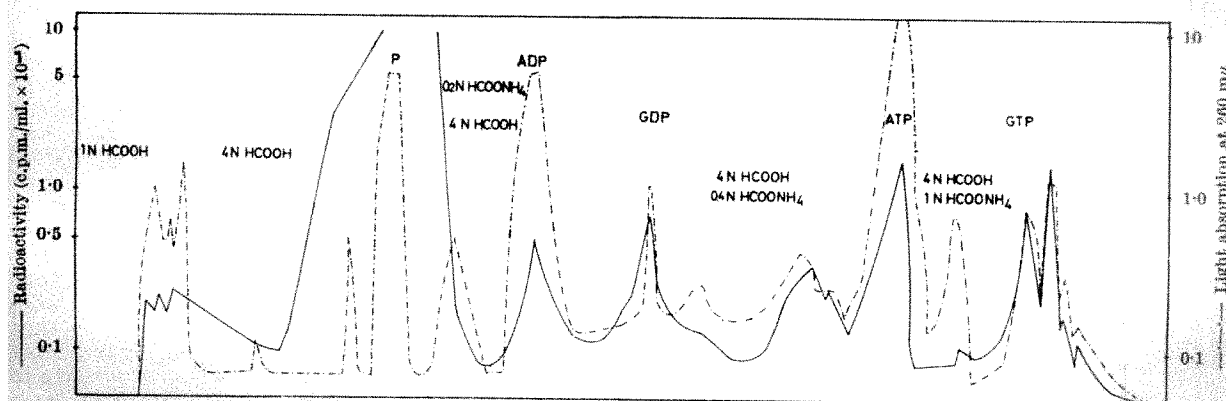


Fig. 1. Acid-soluble nucleotides from an incubation mixture of the <sup>32</sup>P-incorporating lipoprotein complex extracted from ghosts of 600 ml. of human red cells and the TCA soluble nucleotides from the content of the same volume of cells. Incubation with <sup>32</sup>P (0.1 mc./ml.) for 30 sec. The nucleotides were separated by gradient elution with the reservoir content changed in the tubes numbered as follows: 70, 1 N formic acid; 180, 4 N formic acid; 300, 0.2 M ammonium formate + 4 N formic acid; 480, 0.4 M ammonium formate + 4 N formic acid. Column dimensions 65 × 9.2 ml. Mixing volume 600 ml. and tube volumes about 20 ml. The continuous line represents radioactivity, the broken line light absorption at 260 mμ. Registration procedure as in ref. 17.

amounts of labelled adenosine diphosphate (ADP) and guanosine diphosphate (GDP) were also observed. Further on, a large labelled peak was eluted after GTP. It contained adenosine, and could possibly be identical with the ATP-2,3-diphosphoglycerate complex described by Hashimoto and Yoshikawa<sup>16</sup>. Quite unexpectedly, a large quantity of <sup>32</sup>P was eluted from the 'Dowex-1' column, despite preliminary separation by filtration on the 'Sephadex G-25' column. The reason for the appearance of this <sup>32</sup>P peak is not clear. When the labelled lipoprotein complex was precipitated directly by TCA, without previous incubation with TCA-soluble nucleotides from the red-cell haemolysate, the same labelling pattern of slightly ultra-violet-absorbing peaks appeared on the 'Dowex-1' column chromatogram<sup>17</sup>. The small amounts of material available did not permit any closer identification. Another interesting observation was made when the lipoprotein complex had been rapidly frozen to -186° C in liquid nitrogen<sup>2</sup> before incubation with <sup>32</sup>P. When the material was rapidly thawed and incubated with <sup>32</sup>P, and mixed with nucleotides as described here, it was not possible to observe any labelled nucleotide peaks in the eluate from the 'Dowex-1' column chromatography.

One of the reasons why the mechanism of ATP formation in red-cell ghosts has been difficult to investigate is the strong ATPase activity, strongly fixed to the insoluble membrane residues left after exhaustive extraction with *tris*-glycylglycine buffer in the Spinco ultracentrifuge, whereas the lipoprotein complex is devoid of such enzymatic activity.

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## IMMUNOLOGY

### Vitamin A-induced Rejection of Autografts and Homografts

HYPERVITAMINOSIS A has been shown to suppress delayed hypersensitivity reactions in the guinea-pig<sup>1</sup>. Large doses of vitamin A were also shown to cause the release of lysosomal proteolytic enzymes, both *in vitro* and *in vivo*<sup>2</sup>. In an earlier publication we suggested that proteolytic processes may be of importance in graft rejection<sup>3</sup>. In the present investigation the effects of hypervitaminosis A on transplantation of auto- and homografts in rabbits are described.

Local wild rabbits of 2.5–3 kg were used. Autografts were made on 10 rabbits by grafting pieces of full-thickness abdominal skin, 2 cm × 2 cm in area, to the rabbit's own back. Homografts of identical size were exchanged

between 14 rabbits grouped in pairs. Vitamin A palmitate, 1,700,000 units/kg, was injected intraperitoneally every second day, starting on the day of the skin grafting. As controls, autografts were performed on 10 rabbits and homografts were exchanged between five pairs of rabbits not receiving vitamin A.

Autografts in untreated rabbits were consistently successful and after 12–14 days showed evidence of permanent take. Homografts in the control animals were constantly rejected, the longest survival being 13 days.

Autografts in vitamin A-treated rabbits already appeared pale on the 6th day, at the time of removal of the compressive bandages. Between the 10th and 15th post-operative day the grafted skin was detached from the graft bed. The detachment occurred either spontaneously or at the slightest touch. The graft bed was slightly bleeding at the time of detachment and the grafted skin was whitish-grey in colour and appeared non-viable.

The fate of the homografts in the vitamin A-treated rabbits was similar to that of the autografts. On the 7th post-operative day the homografts appeared non-viable. They lost their attachment to the bed between the 7th and 9th day. In spite of the spontaneous separation from the graft bed, no haematomata, serous accumulations or pus were found beneath the graft.

Serial biopsies taken from the grafts of the vitamin A-treated rabbits showed early epidermal and dermal degenerative changes and/or necrosis despite an almost normal graft bed in which the only unusual finding was a peculiar paucity of blood vessels. The homografts of vitamin A-treated animals showed a mild round cell infiltration only. The similar fate of both auto- and homografts in the vitamin A-treated rabbits excludes the possibility that an immune mechanism is involved in this 'graft rejection'.

Unsuccessful autografts have been reported in sublethally irradiated rats, possibly due to interference with the vascularization of the graft<sup>4</sup>. Assuming that the rejection of both auto- and homo-grafts in the vitamin A-treated rabbits was also due to interference with the vascularization of the graft, the effects of vitamin A on neovascularization were studied. In these experiments, an account of which is being prepared, the effect of hypervitaminosis A on vascular neoformation in the rabbit cornea was examined. It was found that, in rabbits which received injections of 0.1 N HCl intracorneally, vitamin A palmitate administration completely prevented neo-vascularization of the cornea<sup>5</sup>. The mode of action of vitamin A in preventing vascular neoformation and consequent 'graft rejection' is not yet clear.

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## PATHOLOGY

## Staphylococcal Interference in Infections in Embryonated Eggs

THE protection afforded by infection with avirulent virus against subsequent challenge with a second lethal virus<sup>1</sup> has led to the demonstration of interferon production by infected cells *in vitro* and *in vivo*<sup>2,3</sup>. Despite clinical evidence of the importance of bacterial interference, and experimental demonstration that avirulent staphylococci are capable of inhibiting nasal colonization with virulent staphylococci<sup>4</sup>, bacterial interference has not been extensively investigated in experimental models. Investigations of experimental staphylococcal infections in embryonated eggs have demonstrated that infection with strains of staphylococci that are not virulent for eggs affords significant protection against subsequent challenge with virulent staphylococci.

Ten-day-old embryonated eggs were injected with from 10 to 100 colony forming units (c.f.u.) of avirulent coagulase-negative *Staphylococcus epidermidis* or coagulase-positive *Staphylococcus aureus* intra-allantoically, and one-half of each group were challenged with 10–100 c.f.u. of virulent *Staphylococcus aureus* 48 h later. The remainder of the eggs infected with avirulent staphylococci served as controls and were injected with 0.1 ml. of saline two days later. Virulence controls received 0.1 ml. of saline on day 10 followed by intra-allantoic injection of 0.1 ml. of a broth culture of virulent *S. aureus*, diluted to contain 10–100 c.f.u., 48 h later. Eggs were candled daily to determine viability. Table 1 demonstrates the fatality rates in each of these experimental groups. Infection with either *S. epidermidis* or virulent *S. aureus*, lines 1 and 4, produced fatality rates ranging from 18 to 20 per cent. The fatality rates observed after saline injection followed by challenge with virulent *S. aureus*, lines 3 and 6, approximated 80 per cent. Prior infection with avirulent strains, lines 2 and 5, afforded significant protection against subsequent challenge with virulent staphylococci (lines 2 and 5 versus lines 3 and 6:  $\chi^2 = 40$ ;  $P < 0.001$ ). The fatality rates of eggs infected with avirulent staphylococci followed by virulent staphylococci (28–30 per cent) did not differ materially from those observed in eggs infected with virulent strains alone. Similar protection was afforded using four other strains of avirulent *S. epidermidis* and *S. aureus* for the initial infection. Significant protection was also observed using different challenge strains of virulent *S. aureus*. In addition, prior infection with avirulent staphylococci afforded significant protection against challenge with egg virulent strains of *Salmonella typhimurium*, *Diplococcus pneumoniae* and *Streptococcus pyogenes*.

The mechanism of protection could not be explained on the basis of alterations in the chorio-allantoic fluid or exhaustion of nutrient materials essential for the growth of the challenge strain. Allantoic fluid taken from uninfected controls and from eggs infected with avirulent

staphylococci, sterilized by filtration through a Millipore filter (0.45 $\mu$ ), were inoculated with the challenge strain of *S. aureus* for determination of growth curves. The growth of virulent *S. aureus* was identical in both types of allantoic fluid, indicating that previously infected allantoic fluid was capable of supporting optimal growth of the challenge strain and that no filterable substance capable of inhibiting the extra-cellular growth of the challenge strain was produced in the allantoic fluid as a result of prior infection. *In vivo* growth of the challenge strain in the presence of active infection with a non-virulent strain varied with different strains. One strain of virulent staphylococci attained similar population densities ( $\pm 10^8$  bacteria/ml.) in the allantoic fluid of infected eggs to those observed in uninfected eggs. A second challenge strain, however, failed to multiply well in the presence of active infection with an avirulent strain.

The protective effect of active bacterial infection with avirulent staphylococci against super-infection in these experimental infections appears to be well established from these investigations. The results support the clinical observations of the protective effect of the normal body flora. In addition, the findings closely parallel the experimental demonstration of the ability of avirulent staphylococci to inhibit nasal colonization and staphylococcal infection in infants<sup>4</sup>. The mechanism of protection in this experimental system remains undefined and the subject of further investigation. Several features suggest that bacterial interference in this system reflects a phenomenon different from that of viral interference and interferon production.

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## Non-specificity of Thioflavine-T as an Amyloid Stain

It has been claimed by several workers<sup>1,2</sup> that thioflavine-T is more sensitive and consistent than congo red and methyl violet as an amyloid stain. Vassar and Culling<sup>3</sup> found no substances which gave false positive reactions apart from myeloma casts and keratin, which do not present a diagnostic problem. McAlpine, Radcliffe and Friedman<sup>4</sup> support these findings but point out that no extensive control investigation has been carried out on 'hyaline' substances with thioflavine-T.

The fluorescent technique described by Culling<sup>5</sup> was therefore adopted as a routine staining method for amyloid at the Royal Free Hospital. It was then discovered that there was often a marked variation in the intensity of

Table 1. PROTECTIVE EFFECT OF INFECTION WITH AVIRULENT STAPHYLOCOCCI AGAINST SUBSEQUENT CHALLENGE WITH VIRULENT STAPHYLOCOCCI IN EMBRYONATED EGGS

Protective strain (day 10)	Challenge (Day 12)	No. deaths/No. eggs infected	Fatalities %
<i>S. epidermidis</i> ( $\pm 100$ c.f.u.)	Saline	7/40	18
<i>S. epidermidis</i> ( $\pm 100$ c.f.u.)	Virulent <i>S. aureus</i> ( $\pm 100$ c.f.u.)	11/40	28
Saline	Virulent <i>S. aureus</i> ( $\pm 100$ c.f.u.)	31/40	78
Avirulent <i>S. aureus</i> ( $\pm 100$ c.f.u.)	Saline	8/40	20
Avirulent <i>S. aureus</i> ( $\pm 100$ c.f.u.)	Virulent <i>S. aureus</i> ( $\pm 100$ c.f.u.)	12/40	30
Saline	Virulent <i>S. aureus</i> ( $\pm 100$ c.f.u.)	32/40	80

Table 1

Case No.	Aetiology	Tissue	Congo red	Methyl violet	Thioflavine-T	Autofluorescence
1	Primary amyloidosis	Kidney	±	±	+++	—
2	Amyloidosis secondary to tuberculosis	Adrenal	++	++	+++	—
3	Amyloidosis secondary to tuberculosis	Kidney	+	±	±	—
4	Amyloidosis secondary to tuberculosis	Kidney	++	++	±	—
5	Amyloidosis secondary to tuberculosis	Kidney	++	++	+	—
6	Amyloidosis secondary to myeloma	Kidney	±	±	+	±
3	Amyloidosis secondary to tuberculosis	Spleen	++	++	—	±



Table 2. OBSERVATIONS IN TISSUES WITH 'HYALINE' SUBSTANCES

Case No.	Aetiology	Tissue	Congo red	Methyl violet	Thioflavine-T	Auto-fluorescence
7	Carcinoma of stomach	Spleen (arterioles)	+++	+	+	++
8	Malignant hypertension	Kidney tubules	-	-	++	++
9	Polyarteritis nodosa	Kidney casts	±	±	+	+
10	Polyarteritis nodosa	Spleen	±	±	±	±
	Scleroderma	Kidney casts	++	+	+	-
	Scleroderma	Arteries and arterioles	++	++	-	-
11	Scleroderma	Glomeruli	-	±	-	-
	Diabetes mellitus	Kidney casts	++	-	++	+
		Glomeruli	+	-	++	-
12	Diabetes mellitus and hypertension	Kidney arteries and arterioles	+	++	±	-
13	Diabetes mellitus and hypertension	Glomeruli with Kimmelstiel lesions	++	++	±	-
14	Coronary thrombosis and hypertension	Kidney casts	++	++	+	++
15	Experimentally produced	Fibrin clot	-	-	++	++
16	Experimentally produced	Fibrin clot with 2 per cent albumin added	-	-	++	++
	Experimental hypertension in rat	Heart (myocardium)	++	++	++	+

fluorescence from case to case. While most of the cases of amyloidosis were strongly positive and gave a brilliant greenish yellow fluorescence, a few gave only a weak fluorescence. This, however, was interpreted as positive until a negative control, a section of kidney from a case of polyarteritis nodosa, was found to be more strongly positive than some cases of known amyloidosis. Possible sources of false positive results were therefore sought. Formalin-fixed tissues were used and sections were examined unstained or stained with thioflavine-T, congo red and methyl violet. The results obtained in six cases of amyloidosis are shown in Table 1.

Sections from six kidneys and two spleens from cases with diseases associated with 'hyaline' lesions, but not amyloidosis, were similarly investigated. Sections of rat

heart muscle from a case of experimental hypertension together with two sections of fibrin clots were also examined. The results are shown in Table 2.

It will be noticed that the genuine cases of amyloidosis did not show autofluorescence except in one doubtful case. On the other hand, out of eleven false positives, seven displayed some degree of autofluorescence. It is known that formalin fixation enhances the autofluorescence of most tissue and this was particularly noticeable in 'hyaline' substances. It seems likely that this autofluorescence is only slightly affected by staining with thioflavine-T, being accentuated in some cases and somewhat quenched in others. In most cases, it came through sufficiently to result in a false positive reading.

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### Phenylalanine Tolerance Test for Heterozygous Carriers of Phenylketonuria

HSIA *et al.*<sup>1</sup> found that plasma phenylalanine levels were substantially higher after the ingestion of a standard dose, based on body-weight, of this substance by parents of phenylketonuric patients than the levels found in a comparable (age, sex, etc.) normal control group. The same observation was made by Berry, Sutherland and Guest<sup>2</sup>. Hsia *et al.* interpreted their results as indicating that a deficiency of the enzyme responsible for phenylalanine metabolism exists in the heterozygous carriers. However, if the data of Hsia *et al.*<sup>1</sup> and Berry, Sutherland and Guest<sup>2</sup> on phenylalanine concentration as a function of time are plotted<sup>3</sup>, it will be seen that the slope of the disappearance curve is essentially the same in the test group and in the control group. The data of Berry, Sutherland and Guest<sup>2</sup> actually suggest that phenylalanine is removed more rapidly by the test group than it is by normal subjects.

Since the rate of disappearance of phenylalanine (not magnitude of plasma level) is the criterion by which enzyme activity should be assessed, the findings of both groups of workers<sup>1,2</sup> cannot be used in support of the view that an enzyme deficiency exists in the heterozygous carrier. A possible reason for the differences found in plasma levels of phenylalanine in the test and control groups is that the volume of fluid (other than blood) in which this substance becomes distributed after absorption (that

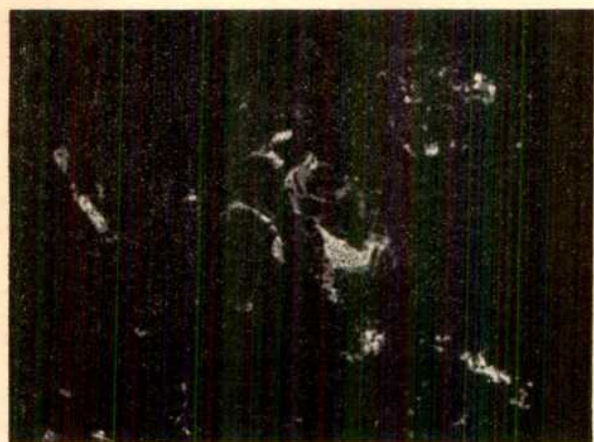


Fig. 1. Amyloidosis secondary to tuberculosis. Kidney. Thioflavine-T. Positive ( $\times 32$ )

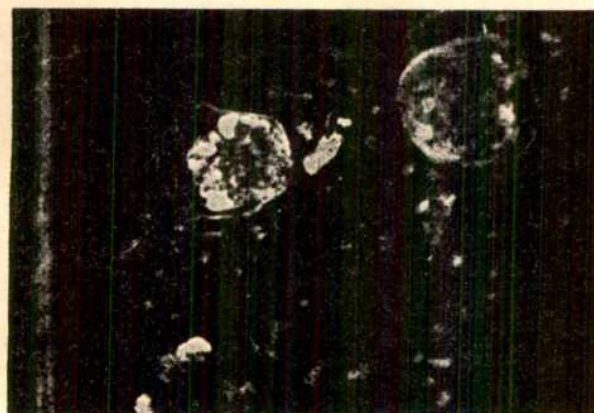


Fig. 2. Kimmelstiel-Wilson nephropathy. Kidney. Thioflavine-T. Positive ( $\times 32$ )



is, the extra vascular fluid) is less in the heterozygous carrier than it is in normal subjects.

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## BIOLOGY

### Nutrient Content of Rainwater in the Gambia

QUANTITIES of nutrients deposited by rainfall in the tropics have been measured by several workers. Venema<sup>1</sup>, in reviewing work on the nitrogen content of rain, showed wide variations from 2.2 to 41.7 lb./acre/year. He observed that total nitrogen is at its highest concentration when a period of drought is followed by heavy rainfall; when rainfall is small, its nitrogen content is high, and vice versa. Visser<sup>2</sup>, in Uganda, observed that the heavier the shower the more nitrate-N is precipitated, and stated that storms preceding heavy rain take a lot of dust into the atmosphere from which NO<sub>3</sub> is later washed. This is no doubt true of other nutrients as well. Jones<sup>3</sup> measured the nutrients added to the soil by a rainfall of 42 in. in northern Nigeria and recorded high values for nitrogen, phosphorus, potassium and sodium of 50.8, 3, 32.8 and 53.8 lb./acre respectively.

Rainwater was collected monthly from three agricultural stations in the Gambia in 1963 (Table 1).

Table 1. TOTAL ANNUAL INCREMENT OF NUTRIENTS (LB./ACRE)

Station	Miles from sea	Total rainfall	N	P	K	Ca	Na
Yundum	9	41.5	42.0	0.28	5.3	3.9	8.5
Joel	81	39.6	40.1	0.14	3.8	2.4	7.9
Roberti kunda	136	27.9	12.7	0.24	2.5	1.5	5.2

It is seen that, on the whole, the farther from the coast the less rainfall and the less nutrients added to the soil by the rain. Values for nitrogen at two stations were most as high as those quoted by Jones at Samaru, but those for phosphorus, potassium and sodium were very much lower, being in the region of one-tenth of those from northern Nigeria. Calcium, on the other hand, is higher than the value quoted by Jones of 0.9 lb./acre.

The climate of the Gambia has been described as Sahalo-megaleses<sup>4</sup>, with the annual rainfall falling in the period from mid-October, the most intense storms occurring at the beginning and the end of the rainy season. The long scorching dry season, coupled with almost complete lack of structure of upland sandy loam top soils, results in wind erosion at the beginning of the rains and dust blowing into the atmosphere. This is reflected in the actual concentrations of nutrients falling, represented on a monthly basis in Table 2, together with monthly rainfall values.

Table 2. MONTHLY RAINFALL VALUES AND CONCENTRATIONS OF NUTRIENTS

Station	Month	Rainfall (in.)	N p.p.m.	P p.p.m.	K p.p.m.	Ca p.p.m.	Na p.p.m.
Yundum	June	1.3	1.79	0.062	3.15	2.25	7.51
	July	12.4	7.95	0.050	0.70	1.05	0.76
	August	12.5	4.76	0.026	0.35	0.00	0.78
	September	10.9	1.46	0.008	0.45	0.00	0.55
Joel	October	4.5	2.02	0.024	0.37	0.25	0.63
	June	4.0	1.68	0.010	1.15	0.50	2.32
	July	13.0	4.82	0.018	0.40	0.35	0.78
	August	11.0	6.22	0.016	0.25	0.20	0.59
Roberti kunda	September	6.4	4.31	0.012	0.40	0.10	0.92
	October	5.2	2.24	0.022	0.35	0.20	0.62
	June	0.5	2.07	0.044	0.60	0.10	1.55
	July	7.4	1.96	0.062	0.42	0.50	0.93
	August	10.6	1.96	0.034	0.37	0.20	0.50
	September	4.2	1.68	0.030	0.40	0.10	0.48
	October	5.1	2.46	0.020	0.40	0.10	1.56

The concentration of nitrogen varies considerably with a marked drop at the station farthest from the coast. At Yundum, N concentration is highest in July, corresponding to a period of intense storms following a short drought of approximately 3 weeks after the first rains.

Concentrations of phosphorus are, as expected, very low; it has been stated<sup>5</sup> that samples of 'harmattan' dust collected in Northern Nigeria contain no P<sub>2</sub>O<sub>5</sub>, and the top soils of the Gambia savannah upland contain only small amounts of total phosphorus.

Generally speaking, values for potassium and calcium are highest at the beginning of the rains and indicate that the elements are either washed from, or fall in the form of, dust present in the atmosphere. The highest concentrations of sodium occur near the coast in the early rains and are related to an easterly trade wind carrying fine particles of salt water over the land.

Total additions of nutrients to the soil seem to have little agricultural significance except in the case of nitrogen, where monthly additions of 10-20 lb., during July and August, must play an important part in satisfying the pre-nodulation nitrogen requirements of the groundnut, the main cash crop of the country. The influence of rainfall nitrogen on subsistence cereal crops must be considerable, as all cereals show a marked response to nitrogenous fertilizers on the typical savannah soils of low nitrogen content.

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### High Photosynthetic Efficiency in a Low-latitude Environment

PLANT physiologists, agronomists and plant breeders over the years have been investigating the many aspects of dry matter production and the efficiency of utilization of solar energy with the view of maximum production. The factors affecting production may be subdivided broadly into genetic, edaphic and climatic factors. Climate, the most difficult to modify, frequently limits the production potential of an area. Little has been published on the maximum growth rate of individual species in different environments together with the relevant radiation values necessary to estimate photosynthetic efficiency. Also, data of this type have been obtained mainly in the middle to high latitudes in Europe and the United States.

A growth investigation at Katherine, N.T., Australia, is of interest in this regard because of the high maximum growth rate recorded in a low-latitude environment for a rain-fed crop of bulrush millet (*Pennisetum typhoides*). The climate at Katherine (132°3' E. long.; 14°3' S. lat.; 107 m alt.) has been described<sup>1</sup> as tropical savannah with a mean annual rainfall of 926 mm, 95 per cent of which falls in the five months November to March. The growth of a field crop of bulrush millet was investigated by gathering replicated sections of the above-ground portions of a bulk crop at fortnightly intervals from January to May 1964. A peak growth rate of 54 ± 5 g m<sup>-2</sup> day<sup>-1</sup> was recorded for the period April 1-15 when insolation, measured with a Kipp solarimeter, averaged 510 cal. cm<sup>-2</sup> day<sup>-1</sup>. This represents storage as dry matter in the crop of 4.2 per cent of the total incoming radiation and 9.5 per cent of the visible component (400-700 mμ), assuming a caloric content of 4,000 cal. g<sup>-1</sup>, and assuming that the visible component is 44 per cent of the total radiation. This very high growth rate for a rain-fed crop was recorded following full light interception and floral



Table 1. PEAK GROWTH RATES, AVERAGE INSOLATION AND ESTIMATES OF EFFICIENCY OF UTILIZATION OF TOTAL AND VISIBLE RADIATION

Species and country	Lat.	Peak growth rate g m <sup>-2</sup> day <sup>-1</sup>	Average insolation cal. cm <sup>-2</sup> day <sup>-1</sup>	Percentage utilization of radiation	
				(a) Total	(b) Visible (400-700μ)
<i>Pennisetum typhoides</i> (Australia)	14° S	54	510	4.2	9.5
<i>Sorghum vulgare</i> (United States) (ref. 2)	39° N	51	690	3.0	6.7
<i>Beta maritima</i> (England) (ref. 5)	52° N	31*	294	4.2	9.5
<i>Hordeum vulgare</i> (England) (ref. 4)	52° N	23	484	1.9	4.3
<i>Trifolium subterraneum</i> (Australia) (ref. 3)	35° S	23	670	1.3	2.9
<i>Brassica oleracea acephala</i> (England) (ref. 5)	52° N	21*	382	2.2	4.9

\* Includes root weight.

initiation, at a time of active internode elongation and inflorescence development. Also associated with internode elongation was a change in leaf display and a more effective distribution of the light within the crop canopy.

Compared with some high growth rates and estimates of efficiency of utilization of solar radiation recently reported<sup>2-5</sup> (Table 1), the peak growth rate of bulrush millet exceeded that of irrigated sudan grass in California, yet occurred at a lower level of insolation, which represents a considerably higher photosynthetic efficiency. Watson<sup>5</sup> recorded the same photosynthetic efficiency for the slower growing *Beta maritima*, but this was at a much lower level of insolation, where high values for photosynthetic efficiency could be expected<sup>6</sup>. The high level of photosynthetic efficiency recorded for bulrush millet was associated with growth rates almost twice as great as *Beta maritima* and moreover took account only of the top growth, whereas the roots of *Beta maritima* were included. A high light saturation value together with a high optimum temperature (30°-35° C) for net photosynthesis in bulrush millet could account for this, enabling the crop to flourish in this low-latitude environment.

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### Auxin-induced Reversal of Geotropic Response in Onion Seedlings

IN the course of an investigation concerning the germination of onion seeds and subsequent growth of seedlings in aseptic cultures, a striking and unique response to auxin was observed. In normal germination, as observed in Nature or on the basal medium (comprising Heller's minerals and 5 per cent sucrose solidified with 0.7 per cent agar), there is an intercalary elongation of the cotyledon resulting in the emergence of the root and the shoot apex. Due to a curving of the cotyledon the radicular end is carried downward and thus the seedling is established in the soil or on a synthetic nutrient medium. The free end of the cotyledon remains inserted in the seed coat and, as it elongates farther, the seed coat is lifted from the substratum (Fig. 1A).

In a nutrient medium containing auxin, however, the mode of germination is totally different. The radicle end, after its emergence from the seed, curves upward (that is, becomes negatively geotropic) instead of growing downward (Fig. 1B, C). Afterwards, rootlets appear at the tip. Concomitantly, a number of scale leaves develop at the apex resulting in the formation of an 'aerial' bulb.

High concentrations of auxins such as indolyl-3-acetic acid (IAA) inhibit the growth of seedlings, which remain

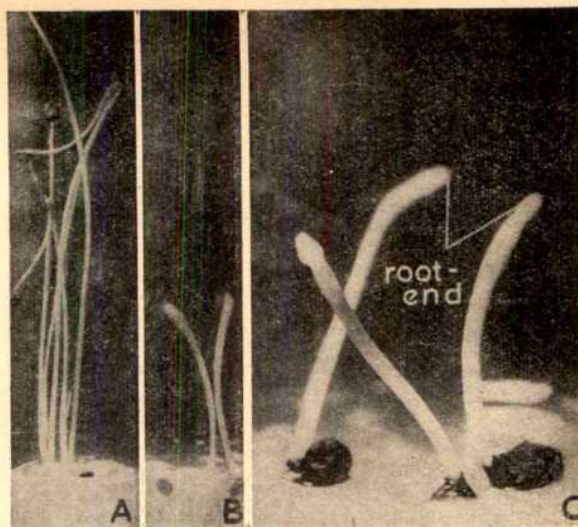


Fig. 1. Germination of seeds on basal medium (A); on basal medium containing IAA  $10^{-6}$  M (B)—note the inverted position of the seedlings; magnified view of inverted seedlings grown on basal medium containing IAA (C).

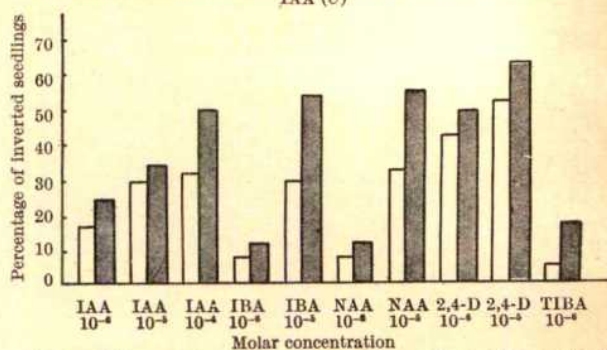


Fig. 2. Histogram showing percentage of inverted seedlings on basal medium containing different auxins and an anti-auxin—dotted bars represent the cultures grown in dark and white bars in light.

stunted. Nevertheless, the apparently negative geotropic response—in terms of percentage of inverted seedlings—is directly proportional to an increase in the concentration of auxin (log-linear to IAA between  $10^{-8}$  and  $10^{-4}$  M concentration; Fig. 2). Indolyl-3-acetic acid at  $10^{-8}$  and  $10^{-7}$  M promotes growth but the negative geotropic response is poor; at higher concentrations there is a marked response although the growth is inhibited. At  $10^{-4}$  M practically all the seedlings are inverted.

Curiously enough, if the tubes are kept in an inverted position there is no difference in the orientation of the seedlings grown on media with or without IAA. In both cases the radicular end grows away from the medium in the direction of gravity.

This response is not limited to IAA alone; other auxin like indolyl-3-butyric acid (IBA), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) influence the growth of seedlings in a similar way although the results are variable (Fig. 2). Triiodobenzoic acid (TIBA), used at concentrations of  $10^{-6}$  and  $10^{-5}$  M, does not reverse the effect of auxins. On the contrary, when used with IAA, it augments its effect.

Besides *Allium cepa*, experiments have been performed with the seeds of *A. fistulosum*, *A. hymenorrhizum*, *A. ramosum*, *A. sphaerocephalum* and *A. tuberosum*. In each case the response to auxins is the same, but the percentage of seedlings showing inverted orientation is variable. In some species the response is less marked than in *A. cepa*; in others such as *A. tuberosum* almost all the seedlings are negatively geotropic.

The tropic response of the seedlings kept in continuous light (30–40 ft.-candles) is much weaker than of those kept in complete darkness (Fig. 2).



To the best of our knowledge the experimental results described here have not been reported earlier, even though experiments closely approaching ours have been made with other materials<sup>1-3</sup>. We are unable, at present, to explain this response in terms of the Went-Cholodny theory of geotropism or in any other way, but further work is in progress to settle this question.

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### Xylem Sap Composition in Woody Plants

SAP exuded from decapitated root stumps has frequently been regarded as similar in composition to that moving in the transpiration stream<sup>1-4</sup>. This assumption is open to doubt.

During experiments on phosphorus transfer in willow I have repeatedly obtained results similar to those reported here. Rooted golden-willow cuttings (*Salix vitellina*) taken in late winter were grown in nutrient solutions containing either a normal level of phosphorus or no phosphorus. When differences in growth were apparent between the two treatments plants were transferred to a full nutrient solution containing 3 mM phosphate and sufficient <sup>32</sup>P as ortho-phosphoric acid to give an activity of 50 µc./l. At this stage stem bases were approximately 4 mm in diameter and the shoots were 2-3 ft. long with fully expanded leaves. After transfer to the radioactive solution, transpiration losses from the pots were made up frequently and plants were gathered after 1, 2 and 14 days. All leaves were removed quickly from the stems, which were then cut off at the base, and sap was extracted by Bollard's method<sup>5</sup>; this usually extracted 1-2 ml. of clear sap. A measured portion of this was spotted on to filter paper and the radioactivity was assayed, using an end-window Geiger-Müller tube. As soon as the stem was removed a glass tube was connected to the root stump and the first 0.5 ml. of the exuded sap was collected. This took only a few minutes—always less than half an hour. An aliquot of this sap was also spotted on to filter paper and assayed, as was an aliquot of the ambient solution taken when the plants were gathered. Results are given in Table 1.

I am not aware of any evidence showing that a substantial gradient of mineral concentration normally exists from bottom to top of the xylem column in transpiring plants. A more probable explanation of the results recorded in Table 1 is that when a stem is cut off there is a sudden and immense change in the phosphorus content of the xylem sap in the stump. This change could be due to the secretion of phosphorus compounds by root cells into the xylem vessels<sup>6</sup>. Some evidence for this interpretation was obtained from chromatograms made from sap samples using a 3° butanol/picric acid/water solvent, in that stem sap contained only inorganic <sup>32</sup>P, while in root

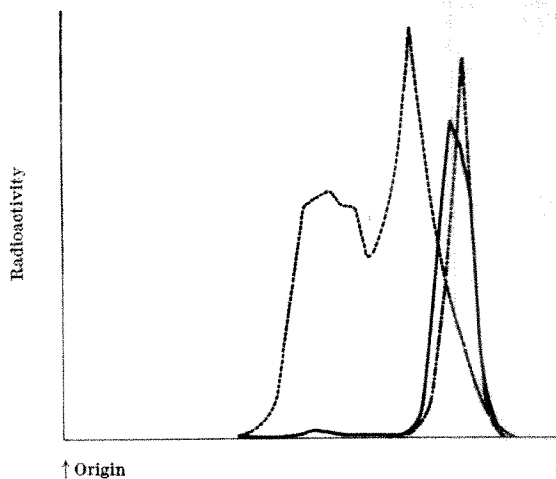


Fig. 1. Radiochromatogram of root and stem sap. In 3° butanol/picric acid/water solvent. The low  $R_F$  values in root sap are due to high electrolyte concentration in this material. —, Root exuded sap; — — —, stem xylem sap; — · — · —, standard  $H_2P^*O_4$ .

sap substantial peaks of activity were frequently found running behind the inorganic <sup>32</sup>P (Fig. 1).

The phosphorus composition of exuded root sap therefore has little connexion with that of the transpiration stream as extracted by Bollard's method.

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### Spider Genus *Dysdera* (Araneae, Dysderidae)

THE scientific study of spiders has remained peculiarly neglected. In particular the field of systematics, until recently, has relied almost exclusively on the morphological species concept of the nineteenth century. Systematic difficulties have in turn discouraged research in other branches of spider biology. This communication summarizes an investigation into the spider genus *Dysdera* (Dysderidae), undertaken to strengthen the taxonomic foundations of a difficult but phylogenetically important group.

The life-cycles of the two British species, *Dysdera crocata* and *D. erythrina*, appear to be the same. The eggs are laid in a spherical mass, only lightly stuck together, within a thick cocoon in which the parent herself is sealed. The eggs hatch in 3-4 weeks, depending on the temperature, and about a month later the parent spider tears a hole in the cocoon through which the young escape. After seven nymphal instars, occupying approximately eighteen months, the spiderlings reach sexual maturity, and may live for another two or three years.

Although many small arthropods are acceptable as food, all the evidence suggests that woodlice form the natural diet of *Dysdera*. All woodlice appear equally acceptable, but *Armadillidium* is better able to escape predation because of its ability to roll into a ball.

Most species of *Dysdera* are characterized by an extremely restricted distribution, and the two British species are rather atypical in this respect. *D. crocata* in particular is very widely distributed, and there is no doubt that this is a synanthropic species the range of which is being con-

Table 1. RADIOACTIVITY (AS C.P.M. PER µl.) OF XYLEM SAP AND NUTRIENT SOLUTION AT TIME OF HARVEST

P status of plants	Days of <sup>32</sup> P absorption	Nutrient soln.	Activity Stem sap	Root sap
Normal	1	41.4	1.9	9.9
	2	15.6	1.7	11.9
	14	11.3	4.9	34.9
Low	1	40.4	8.8	62.5
	2	30.8	10.6	31.8
	14	10.6	11.9	22.8



tinually increased by transportation. In direct competition it was found that *D. crocata* could displace *D. erythrina*, being larger and better able to withstand climatic extremes. However, in areas with dense populations of certain species of ant, *D. erythrina* had the advantage, although the reasons for this are not clear.

The external morphology of the two British species was examined from the point of view of practical systematics. First the changes associated with age were analysed, by examination of the successively cast exuviae of individual spiders. The growth of limb segments was considered in relation to the length of the carapace, and only very slight allometry found, except in the growth of tibia I, which in the males of both species, but particularly in *D. crocata*, increases considerably in length at the last moult, giving rise to a marked sexual dimorphism. The positions of the metatarsal trichobothria are constant at all stages of growth. Even in the adult the number and positions of leg spines is highly variable, and except in rare cases immature specimens cannot be identified by this means. The absence of strongly allometric growth having been demonstrated, comparisons between population samples of adults of the two species were made. Spines, which are generally lacking, only occur symmetrically on the fourth femur of *D. erythrina* very rarely, and therefore the presence of such femoral spines may be taken as indicative of *D. crocata* with a high degree of certainty. However, *D. crocata* not infrequently lacks femoral spines, and the absence of such spines is not indicative, therefore, of *D. erythrina*. The number of femoral spines in *D. crocata* is very variable.

The spacing of the eyes and the width of the eye group in relation to carapace width was analysed, and only characters of partial value were found. Anterior median eyes of *D. erythrina* are never less than one diameter apart, whereas in *D. crocata* they can be as little as 0.75 diameters apart. The lengths of limb segments and the proportions of the carapace in both species and sexes were examined, and the absence of characters of value in practical systematics demonstrated.

Because there is no epigyne in *Dysdera*, no use has hitherto been made of the female genitalia in systematic investigations. A comparative survey of the internal female genitalia in the Dysderidae and related families showed that these structures could be extremely valuable, not only for specific identification, but also as a guide to determining higher categories.

As a result of these and related investigations a new classification of the Dysderidae is proposed. The family is divided into four tribes: Dysderini, Harpactini, Orsolobini and Rhodini. The latter include *Rhode*, *Typhlorhode* and *Harpassa*, while the Orsolobini contain only the obscure South American genus *Orsolobus*. The Harpactini include *Dasumia*, *Harpactes*, *Holissus* and *Stalita*. The latter genus includes the species previously contained in *Minotauria*, *Stalitella* and *Parastalita*, which are no longer recognized. The remaining four genera, *Tedia*, *Stalitochara*, *Dysdera* and *Harpactocrates*, are placed in the Dysderini, but the status and position of *Harpactocrates* are dubious. The species at present included in *Harpactocrates* are a heterogeneous assemblage which may be clearly sub-divided into three groups, each probably deserving generic status. The first of these includes *H. gurdus*, *radulifer*, *ravastellus*, *drassoides*, *intermedius* and *appenicola*, and the second *H. cantabrorum*, *deminutus*, *ignavus*, *inaequipes*, *limbarae*, *romandiole*, *siculus*, *terualis* and *vernae*. In the third group, *H. egregius* and *storkani* are distinctively isolated, but it is probable that *H. marani* may also belong to this group. The position of *H. crassipalpis* remains uncertain; *H. saevus* and *rubicundus* belong to *Harpactes*.

The anatomy of the genitalia and accessory musculature was examined in detail, particularly in relation to function. The basic dysderoid plan consists of a single aperture leading off the epigastric furrow and opening into a bursa

copulatrix. There is usually a single median anterior spermatheca and frequently a posterior diverticulum. In *Dysdera* the spermatheca is bilobed and heavily sclerotized. It is associated with large glands the function of which appears to be, at least in part, to force the spermatozoa out of the spermatheca as the eggs pass through the bursa copulatrix. The posterior diverticulum is a thin walled muscular sac lined with chitinous protuberance through which drain mucus-secreting cells. During mating the male palpal organs are twisted simultaneously into the bursa copulatrix through the action of a pair of antagonistic muscles controlling the bulb and by the movements of the whole palp. The bulb bears down through a twisted sclerotized strip the contour of which helps to guide the bulb during insertion. The posterior apophysis of the bulb also helps to supplement this action, but its main function is to lock the palp into the female during the process of sperm transfer.

The emptying of the receptaculum seminis in the male palp was found to be under hormonal control, probably by a neurosecretory mechanism. It is brought about by the secretion of surrounding glands emptying through minute pores into the lumen of the reservoir. Hydrostatic pressure plays no part in the function of the male palp in *Dysdera*.

Spiders which had matured in the wild would not mate with individuals of the other species, but spiders that have been raised in isolation in the laboratory would, in some cases, mate readily with similarly treated individuals of the other species. In the latter matings the eggs were invariably found to be infertile. The presence of a genetic barrier to the formation of hybrids was further demonstrated by artificial insemination experiments, which also failed to produce fertile eggs.

Whereas mating may take place at any time of the year, eggs are only laid at certain seasons. In Britain and Northern Europe there is a peak laying season in May and June, but in North Africa *D. crocata* has two laying seasons, one in March and the other in August. The number of eggs produced by an individual fell at each laying, 60-40-20 being typical values for the first three cocoons. The proportion of fertilized eggs in each batch decreased if the spider had mated only once, but the numbers of spermatozoa in the female genitalia showed that in nature multiple matings are common.

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### Tantalum and Niobium in Ascidians

It is a well known and established fact that some ascidians have a peculiar ability to accumulate vanadium from sea-water. While the vanadium content does not reach 2 p.p.m./dry weight in ordinary marine animals, it exceeds 6,500 p.p.m. in some ascidians of the order Phlebobranchia<sup>1</sup>. In other orders of ascidians and holothurians this ability is less marked or lacking<sup>2</sup>. Recently it was reported that an ascidian, *Molgula manhattensis*, contains and actively incorporates niobium<sup>3</sup>. It therefore seems probable that ascidians may also be capable of absorbing tantalum. Since no information is available about tantalum in living organisms, it is of interest to know if any tantalum is contained in ascidians beside vanadium and/or niobium.

A few hundred middle-sized mature *Styela plicata* (40-45 mm in length)<sup>4</sup> were collected at Misaki Marine Biological Station, Kanagawa, Japan, in November 1961, April 1962 and July 1962. These were thoroughly cleaned with a nylon brush, and then dried in an oven at 110°C in glass vessels, and chemically analysed. Determination of tantalum and niobium was carried out by the procedure to be described here<sup>5</sup>.

A small amount (1-2 g) of finely ground material was weighed exactly and placed in a 250-ml. beaker. Thirty-ml



concentrated hydrochloric acid and 60 ml. of distilled water were added and the beaker was covered with a watch-glass. After mild heating for 30 min on a hot-plate, the sample was filtered and washed with hot dilute hydrochloric acid. The residue was transferred into a platinum crucible, ashed and ignited. Three drops of concentrated sulphuric acid were added, and the resulting mixture evaporated and further ignited. If the material as rich in silica, the silica was removed by heating the residue with three drops of sulphuric acid and some hydrofluoric acid and evaporating to dryness. The sulphur dioxide was then expelled by heating.

The residue was fused with 2 g of sodium hydrogen phosphate, then extracted with concentrated sulphuric acid, transferred to a 100-ml. volumetric flask and, after filtering if necessary, made up to 100 ml. with concentrated sulphuric acid. An appropriate aliquot was transferred to a 50-ml. volumetric flask. A solution consisting of 1.00 ml. of 30 per cent hydrogen peroxide and 25 ml. of concentrated sulphuric acid was prepared in bulk and cooled to room temperature. Twenty-five ml. of this solution was then added to the 50-ml. volumetric flask containing the aliquot of sample solution. The flask was then stoppered and the contents thoroughly mixed. A final dilution to volume was made at room temperature. The resulting solution was compared to a reference solution prepared in an identical manner and containing an equal concentration of sulphuric acid and hydrogen peroxide. Determination of tantalum and niobium was made by measuring absorption at 285 m $\mu$  and 365 m $\mu$ , respectively.

The results are summarized in Table 1. The animals collected on November 8, 1961, were divided into two lots. The difference in content of the elements is attributed to individual fluctuations. The animals collected on April 13, 1962, were mixed and powdered into a single lot from which samples were taken for repeated analyses. The same procedure was applied to the animals collected on July 8, 1962. The results of repeated analyses for each lot coincided well within the range of experimental error. On average, *Styela plicata* was found to contain 0.023 per cent (range 0.010–0.041 per cent) tantalum and 0.025 per cent (range 0.015–0.030 per cent) niobium (all values per cent dry material). Because sufficient numbers of animals were not available we cannot say whether the fluctuations represent some seasonal or developmental variation. However, our results demonstrate the accumulation of a considerable amount of tantalum in the ascidian body. This is of interest since the presence of tantalum in living organisms has not previously been reported. The vanadium content of *S. plicata* is very low: 13 p.p.m. dry material<sup>6</sup>.

According to Carlisle and Hummerstone<sup>7</sup>, the niobium content of the sea-water was 0.01–0.02  $\mu$ g/l. for an unfiltered sample collected about 40 km south-west of Plymouth, and 0.05–0.1  $\mu$ g/l. for unfiltered and 0.005  $\mu$ g/l. for filtered water sampled at Plymouth Sound. The tantalum content of the sea-water around the Pacific coast of Japan was reported by Hamaguchi *et al.*<sup>8</sup> to be  $\leq$  0.02  $\mu$ g/l. Although no other data are available concerning the tantalum and niobium content of sea-water, it is certain that both elements exist in sea-water at extremely low concentrations.

The presence of niobium in ascidians was reported earlier in *Molgula manhattensis* by Carlisle<sup>3</sup>, who also investigated the relationship between vanadium and niobium. He found niobium (25–75 p.p.m.) only in individuals with vanadium content less than 2 p.p.m.,

and not in those which contained as much vanadium as 29–98 p.p.m.

Hamaguchi *et al.*<sup>8</sup> were able to show by the quantitative neutron activation analysis method that the average tantalum content of the sea bottom clays is: 0.9 g/ton for Pacific red clay, 1.2 g/ton for Japan Sea red clay, 0.2 g/ton for Pacific foraminiferan ooze, and 0.2 g/ton for Pacific volcanic ooze. No specific accumulation of tantalum in the sea bottom clays was observed except in some red clays.

It is therefore likely that the tantalum contained in these ascidian results from active absorption by the animals. The site and the mechanism of the selective absorption of vanadium by ascidians have recently been the subject of active discussions<sup>9</sup>. It has also been shown that vanadium exists as a proteinium salt of disulphato-vanadium(III) acid<sup>10</sup> in blood cells, presumably in their vanadophores<sup>11</sup>. However, despite some attractive interpretations, we are almost completely ignorant of the part played by vanadium in the biology of ascidians. At least it seems that vanadium may be replaceable by niobium<sup>3</sup> or by iron<sup>12,13</sup>. Our results, showing the presence of tantalum and niobium in an ascidian in a quantity exceeding that of vanadium, open up a new aspect of this problem.

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## Toxicity of Rubber Stoppers to *Daphnia magna* Straus

In 1957, DeWitt<sup>1</sup> directed attention to the mortality of aquatic insects and fishes caused by latex tubing in experimental apparatus. While examining the effects of reduced oxygen concentrations on zooplankton, we found that water in direct contact with laboratory-grade rubber stoppers was acutely toxic to *Daphnia magna*.

The apparatus included a series of cylindrical glass chambers. Water was delivered and removed through glass tubes mounted in black rubber stoppers. All other connexions were made of vinyl tubing. In several preliminary experiments with first instar *D. magna*, the mortality of control animals was unusually high. Results were the same in experiments carried out to evaluate the effect of static and flowing-water conditions on heavy metal toxicity. Subsequently, we covered the black rubber stoppers with vinyl food wrap ('Saran'), and mortality among control animals was eliminated.

Bioassay of the stoppers was then undertaken. First instar *D. magna* from mixed broods were used in the assay. New rubber stoppers were sliced and placed in

Table 1. TANTALUM AND NIOBIUM CONTENT OF THE ASCIDIAN *Styela plicata* COLLECTED AT ABURATSUBO BAY, MISAKI (PER CENT OF DRY MATERIAL)

Date of collection	Ta	Nb
Nov. 8, 1961	0.028 } 0.054 } Av. 0.041	0.021 } 0.037 } Av. 0.029
April 13, 1962	0.018	0.015
July 8, 1962	0.010	0.030
Total average	0.023	0.025

culture dishes, each containing 10 animals. Except for rubber stopper slices, conditions were identical for control animals. The animals were not fed during the exposure. All experiments were carried out at 20° C.

In three separate tests, a total of 110 animals were exposed to water containing rubber slices; all were dead at the end of 24 h. In most cases, the animals were moribund in less than 8 h.

Grey neoprene stoppers were also assayed and found to be toxic. Survival time was slightly longer, but all the exposed animals were dead after 48 h. There was no mortality among control animals.

There appeared to be little value in quantifying the results further, and we have continued our experiments in apparatus equipped with ground-glass tapered joints.

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## FORESTRY

### Heterophyllous Shoots in *Betula papyrifera*

LEAVES of two morphological types occur on long shoots of tree crowns in many species of *Betula*. Those at the shoot base frequently differ from leaves closer to the shoot tip in venation, size, toothiness and other characteristics. The first group of leaves (early leaves) appears at or shortly after bud opening, and the second (late leaves) later in the growing season after the first leaves are well expanded. Early leaves are also found on short shoots, that is, shoots the internodes of which fail to elongate more than a few mm. Late leaves are lacking on short shoots. In a report on leaf dimorphism in *Populus trichocarpa*, Critchfield<sup>1</sup> stated that differences in leaf form were due to differences in ontogeny between early and late leaves. Early leaves formed in the growing season preceding their expansion. Their development was arrested and they over-wintered as embryonic leaves, continuing development the following spring. Late leaves over-wintered as leaf primordia or were not initiated until the growing season in which they expanded. Consequently their formation was not interrupted in the embryonic stage and their form differed from that of early leaves. These investigations have now been extended to an evaluation of bud contents, shoot growth, and heterophylly in *Betula papyrifera* Marsh. growing in north central Wisconsin (U.S.A.).

Buds located on twig tips and expected to produce long shoots were collected late in April, before bud opening, and again in late September during leaf fall. Buds located on lateral short shoots and expected to produce short shoots were collected in April only. Embryonic leaves and leaf primordia within these buds were counted and their lengths measured with a mm rule or ocular micrometer.

Before bud opening five branches on each of five trees were selected for shoot measurement. Internode and leaf elongation measurements were made at frequent intervals on the terminal long shoot of each branch and on the most distal short shoot of the two-year-old portion. Leaves were measured from tip to point of attachment to stipules. Internodes were numbered according to the leaf which terminated them.

The first pair of leaves on the long shoots began to unfold about May 15. They grew rapidly for two weeks, then slowed and ceased growing in the third week. The third leaf appeared when the first two were more than half grown, around May 30 on most branches. Succeeding leaves appeared at intervals of approximately five days until a mean total of 5.8 leaves per shoot had emerged. Because of the interval between the occurrence of the first

two leaves and succeeding ones, and their general firmer texture and more apparent double toothiness, leaves 1 and 2 were classified as early leaves and the remainder as late leaves.

Late leaves elongated at a rate similar to that of early leaves and their growth was usually complete in 2-3 weeks. Growth in all but one or two leaves ceased after July 29. Final mean length of early leaves, calculated from those which completed growth without serious injury, was 8.9 cm. Final mean length of late leaves was not calculated since injury due to disease and insect arrested many before growth ceased. There was a tendency for each late leaf to have a shorter final length than that formed previously on the same shoot.

Internodes 1 and 2 of long shoots seldom exceeded 1 mm in final length. The period of rapid elongation of an later internode lay within that of the leaf which terminated it, but was completed a few days sooner. Elongation of the final internode was usually complete by the time the first late leaf had ceased growing. There was a tendency for final length to decrease in succeeding internodes after internode 3. Mean final length of internodes on was 21.4 mm. Mean total shoot length was 85.5 mm only 6.3 per cent of which was made of internodes 1 and 2.

Most of the short shoot buds produced 2 leaves; a few produced 3 or 4. All these leaves resembled the early leaves formed on long shoots. Mean number of leaves produced by short shoots was 2.4. Final mean length of lateral shoot leaves was 10.2 cm, slightly longer than early leaves on long shoots. Internodes did not exceed 1 mm on lateral shoots.

Both embryonic leaves and leaf primordia were found in the spring within each bud expected to produce a long shoot. The former were clearly leaf-like, with well defined teeth, petiole and veins. The latter were small lobed structures not yet exhibiting detail. The embryonic leaves fell into two size classes with a break of 2-6 mm separating them. Large leaves had a mean length of 6.4 mm and small leaves a mean length of 1.2 mm. The large leaves were pubescent and firm in texture. Some of the largest of the small leaves were pubescent, but the majority were glabrous, translucent and delicate in texture. The leaf primordia ranged in length from 0.2 to 0.3 mm. The mean number of large leaves per long shoot bud was 2.1 and the mean number of small leaves was 2.6. Mean number of primordia was 1.1. Spring short shoot buds contained the same type of leaves but had on the average slightly more large leaves (2.7) fewer small leaves (0.9), and about the same number of primordia (1.0). Mean lengths in each class were approximately the same as in the long shoot buds. Autumn long shoot buds contained an average of 2.0 large leaves, 1.2 small leaves and 1.6 primordia. Mean lengths of these were 5.2, 1.3 and 0.5 mm, respectively (Table 1).

Table 1. NUMBERS OF EARLY AND LATE LEAVES ON SHOOTS AND CONTENTS OF SPRING AND AUTUMN BUDS OF *Betula papyrifera*

No. per shoot		Spring buds		No. per bud		Autumn buds	
Summer shoot		Embryonic Primordia		Embryonic Primordia		Embryonic Primordia	
leaves		leaves		leaves		leaves	
Early	Late	Large	Small	Large	Small	Large	Small
2.0	3.8	2.1	2.6	1.1	1.2	2.0	1.6

A comparison of bud contents with summer leaf production showed that approximately 2 large, firm-textured embryonic leaves were found in both spring and autumn buds. Their position and number corresponded to that of the early leaves expanded during the growing season. The number of late leaves produced during the summer was higher than the number of small embryonic leaves found per spring bud. Hence, late leaves developed not only from small embryonic leaves, but also from primordia present in the spring bud. Apparently early leaves over-wintered as large embryonic leaves while late leaves over-wintered either as small embryonic leaves or as primordia. Before bud break in the spring the over-

entering primordia were stimulated to continue development into embryonic leaves and were replaced by newly forming primordia. Potential late leaves were present in short shoot buds but were suppressed.

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## ENTOMOLOGY

### Structure-activity Relationships among Insecticidal Compounds derived from Chlordene

RECENT investigations<sup>1-3</sup> demonstrated the value of metabolic inhibitors such as pyrethrin synergists as tools in the investigation of structure-activity relationships among the 'cyclodienes' type insecticides. Thus, the wide variations in toxicity to the housefly found in several groups of related cyclodienes were found to be reduced in the presence of the pyrethrin synergist 'Sesamex', a phenomenon accompanied, in the case of compounds examined *in vivo*, by inhibition of metabolic conversions<sup>1,3</sup>.

The use of 'Sesamex' is further illustrated in recent investigations of chlordene (1; Fig. 1) and related compounds. Heptachlor (2), which is known to be converted to a persistent epoxide, m.p. 160°, in mammals<sup>4</sup> and the housefly<sup>5</sup>, is considerably more toxic than chlordene (1) to the housefly. Stereochemical investigations of Riemshneider<sup>6</sup>, chemical investigations of Davidow and Adamski<sup>4</sup> and inspection of molecular models suggest structure (3) for this epoxide, while the mode of formation of a second epoxide, obtained indirectly from heptachlor<sup>7</sup>, indicates that it has the structure (4). Chlordene (1) reacts with peracids to give the epoxide (5) (ref. 8) and is converted by the housefly into an epoxide-like compound<sup>9</sup> now identified as compound (5) (infra-red spectrum). Gas-liquid chromatographic analysis, on an Apiezon L-treated 'Celite' column, of acetone tissue extracts of live houseflies treated with chlordene (peak I) revealed the presence of at least two other compounds (peaks III and IV in order of retention time) in addition to the epoxide (peak II). No substances other than chlordene were found in heat-killed insects similarly treated. Compounds corresponding to peaks III and IV were isolated from insect extracts by thin-layer chromatography on alkaline alumina and compound (III) was identified (infra-red spectrum) as a 1-hydroxychlordene (6), also prepared from the 1-bromo-compound (7) by alkaline hydrolysis. Examination of acetone extracts of houseflies treated with compound (5) or (6) revealed the presence, in the case of a substance which co-chromatographed on thin-layer chromatography and gas-liquid chromatography with compound IV and suggested that this might be a hydroxy-epoxide such as compound (8), arising by oxidation of (6), hydroxylation of (5), or simultaneous hydroxylation and epoxidation of chlordene. Perbenzoic acid oxidation of compound (6) then gave a hydroxy-epoxide (8) having an infra-red spectrum identical with that of compound IV isolated from chlordene treated houseflies. Although epoxide (5) might be hydroxylated at one or more of several positions, the fact that compound IV could not be resolved and the clarity of its infra-red spectrum suggested that it was mainly compound (8); if free isomeric mono-hydroxy compounds are formed the amounts present must be small. Compounds (5) and (8) were non-toxic when injected into houseflies. Acetone extracts of houseflies treated with epoxide (5) so contained compounds which may be chlordene-diols

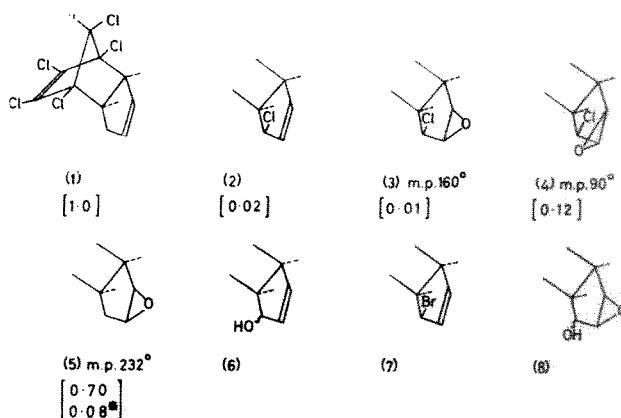


Fig. 1. Chlordene and partial structures of compounds derived therefrom by modification of the unchlorinated ring. Figures in square brackets are corresponding  $LD_{50}$ 's in  $\mu\text{g}$  per female housefly (ref. 2). \*  $LD_{50}$  following pretreatment with 'Sesamex'.

since their chromatographic behaviour was similar to that of synthetic chlordene glycols. The latter were clearly distinguished from the other compounds discussed since they remained near the point of application in most of the thin-layer systems used.

Although chlordene was recovered unchanged from heat-killed flies, rinses of the vessels which contained them showed traces of more polar compounds, and experience with similar compounds<sup>3</sup> suggested that chlordene might undergo atmospheric oxidation. This was verified by the decomposition which resulted from several hours exposure of chlordene to air and light on glass plates. Further, chlordene exposed to ultra-violet light for a few minutes gave a complex mixture of products from which compounds (5) and (6) were isolated. These results indicate the susceptibility of the chlordene molecule to oxidative conversions.

Since chlordene is converted *in vivo* into the epoxide readily obtained chemically, the structure-activity relationships in this group of compounds may be considered in detail. The apparent toxicities to the housefly of compounds (3), (4) and (5) decrease in this order (Fig. 1), the last-mentioned compound being only slightly more toxic than its precursor (1) (ref. 2). Since the *in vivo* epoxidation of heptachlor appears to be a toxication process<sup>5</sup>, it seemed that the relatively low toxicity of chlordene might result from its inability to form a stable epoxide in the housefly<sup>9</sup>. Penetration investigations with chlordene have confirmed that its rapid disappearance from housefly tissue is accompanied by a temporary accumulation of the epoxide, the levels of both compounds falling to zero within, for example, 8 h following application of 0.4  $\mu\text{g}$  chlordene per female fly. Fig. 2 shows the effect of a prior application of 'Sesamex' on the fate of chlordene epoxide (5), topically applied to the housefly. The discovery of ring hydroxylation as a detoxication route for chlordene epoxide helps to explain the stabilizing effect of 'Sesamex', an inhibitor of biological oxidations, on this epoxide *in vivo*, since the more obvious route involving hydrolytic cleavage of the epoxide ring rather than oxidation. The stabilization of compound (5) *in vivo* is accompanied by an approximately ten-fold increase in its toxicity (Fig. 1), indicating a potentially higher toxicity than that observed in normal toxicity tests. In contrast, epoxides (3) and (4) are found to accumulate in the housefly at about the same rate following their topical application and both appear to be as persistent as diel-drin<sup>10</sup>. This finding accords with the lack of any pronounced synergistic effect with 'Sesamex' and indicates that the observed toxicities of these compounds approach their intrinsic toxicities (that is, toxicities in the absence of detoxication). The intrinsic toxicity of chlordene epoxide



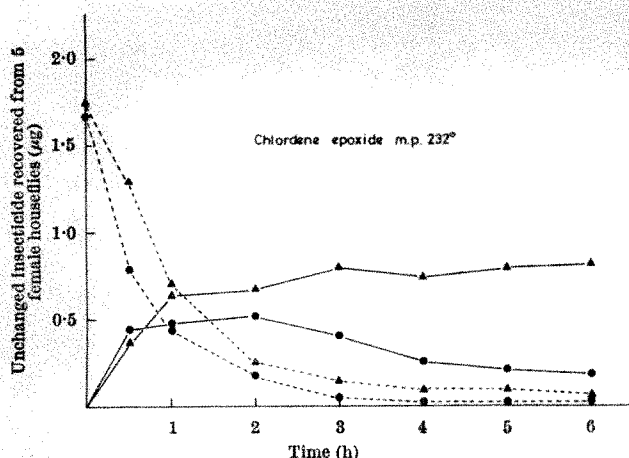


Fig. 2. Effect of pretreatment with 'Sesamex' (5 µg/fly) on the recovery, determined by gas-liquid chromatography<sup>1</sup>, of chlordane epoxide from the housefly. Dotted curves, cuticle rinses; full lines, tissue extracts. Insecticide only, ●; insecticide plus 'Sesamex', ▲. Dose, 0.37 µg/fly

appears to lie between those of the two heptachlor epoxides and may be greater than the maximum so far observed since the recoveries from insect tissue were incomplete, even in the presence of 'Sesamex'. The results suggest that the different toxicities of the heptachlor epoxides (3) and (4) may be a true consequence of their stereochemical difference, while the apparent low toxicity of chlordane epoxide results from an increased liability to detoxication arising from the absence of the additional chlorine atom present in (3) and (4). Rather than possessing a more positive role in toxicity, therefore, this chlorine atom may serve simply to shield a vulnerable position of the chlordane molecule from direct hydroxylation while also screening the epoxide rings from enzymatic attack. A toxicological investigation of the chlordane epoxide having its oxygen ring in the same configuration as that of compound (4) would be of interest in relation to this hypothesis.

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## MICROBIOLOGY

### Nomenclature of Living Parasite Material

THE term 'strain', with its connotation of ancestry, descent, race or stock<sup>1</sup>, is clearly appropriate for living material which is kept available for experimental purposes by being serially passaged in laboratory animals or in culture. Material maintained in this way, being continuously reproducing, is subject to selection. It may, therefore, alter in its biological characteristics in the process of passage and so be unsuitable for reference purposes for long-term experimentation and unrepresentative of the populations existing in Nature and from which it was derived.

It is to Weinman and McAllister<sup>2</sup> that the credit is due for directing attention to the fundamental significance of

the viable storage of parasitic organisms at low temperatures in affording material in which reproduction is arrested and biological characters are stabilized. A separate term is now required to convey this implication of the stabilization of biological characters in preserved material and to distinguish preserved from 'strain' material. The word 'stabilate' is now proposed for this purpose. A 'stabilate' may be defined as a population of an organism preserved in viable condition on a unique occasion. There will be only as many examples of a stabilate as there were individual samples of it laid down on the unique occasion. It will therefore be possible to designate each stabilate by a unique code letter or number.

We thank Prof. A. McIntosh, of the Department of English Language in the University of Edinburgh, for advice regarding the choice of a suitable word.

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### Distribution of Respiratory Quinones in 2,5-di-tert-amyl-p-benzoquinone-sensitive and -resistant Bacteria

A NUMBER of *p*-benzoquinones have been shown to inhibit bacterial growth<sup>1-3</sup> and the succinic oxidase activity of beef heart<sup>4,5</sup>. In view of the role of quinones in the electron transport and coupled phosphorylation of bacteria<sup>6-8</sup> it seemed of some interest to determine the relationship between susceptibility to *p*-benzoquinones and the distribution of respiratory benzoquinones and naphthoquinones in bacteria.

The susceptibility to 2,5-di-tert-amyl-*p*-benzoquinone (DAQ) of a number of strains of bacteria, the content of ubiquinone and vitamin K of which was known, was discovered by plating suitable dilutions of broth cultures on trypticase soy agar (Baltimore Biological Laboratory Inc.) containing a range of concentrations of DAQ and incubating at 37° for 18-36 h. The lowest inhibitory concentration was taken as that which reduced the count of readily visible colonies to less than 1 per cent compared with control plates containing no DAQ; with some bacteria colony formation was completely inhibited, while with others minute colonies could be detected on careful examination. Although it was not possible to obtain a precise estimate of the colony count with *Proteus vulgaris* due to spreading of the individual colonies, since they appeared to be no reduction in either the number of 'foci' or the overall amount of growth when plated on medium containing 300 µg/ml. of DAQ, the organism was recorded as being resistant to this concentration of DAQ. Table 1 shows that, of the small range of bacteria tested, those which contain vitamin K alone were sensitive to 30 µg/ml or less of DAQ, while those bacteria which contain (1) ubiquinone either alone or in combination with vitamin K, or (2) neither of the two respiratory quinones were resistant to at least 300 µg/ml. (the maximum concentration of DAQ which it is practicable to include in the medium).

We have previously reported that the antibacterial activity of DAQ appeared to be virtually restricted to strains of *Staphylococcus aureus*<sup>9</sup>. This was probably a reflexion of the screening method used and also the marked sensitivity of *Staph. aureus* to DAQ later demonstrated<sup>3</sup>. Although we have not investigated the respiratory quinones of *Staph. aureus*, in view of its sensitivity to DAQ it would probably contain only vitamin K; Bishop Pandya and King<sup>10</sup> have shown that *Staph. albus* contains vitamin K, but no ubiquinone.

Table 1. SUSCEPTIBILITY TO 2,5-DI-*tert*-AMYL-*p*-BENZOQUINONE AND PRESENCE OF RESPIRATORY QUINONES IN BACTERIA

Organism	Respiratory quinones*		Inhibitory conc. of 2,5-di- <i>tert</i> -amyl- <i>p</i> -benzoquinone ( $\mu$ g/ml.)
	Ubiquinone	Vitamin K	
<i>Micrococcus lysodeikticus</i> N.C.T.C. 2665	—	+	30
<i>Corynebacterium diphtheriae</i> P.W.8	—	+	10
<i>Bacillus megaterium</i> N.C.T.C. 9848	—	+	3
<i>Bacillus subtilis</i>	—	+	10
<i>Serratia lutea</i>	—	—	10
<i>Lactobacillus casei</i>	—	—	> 300
<i>Aerobacter aerogenes</i> N.C.T.C. 418	+	—	> 300
<i>Pasteurella pseudotuberculosis</i> N.C.T.C. 1101	+	—	> 300
<i>Chromobacter prodigiosum</i> N.C.T.C. 1877	+	—	> 300
<i>Achromobacter hartlebii</i> N.C.I.B. 8129	+	—	> 300
<i>Pseudomonas aeruginosa</i>	+	+	> 300
<i>Proteus vulgaris</i>	+	+	> 300
<i>Escherichia coli</i>	+	+	> 300
<i>Escherichia coli</i> K12	+	+	> 300
<i>Escherichia coli</i> B	+	+	> 300

\* Data on types of quinones present in organism 1 were taken from ref. 12, organisms 2–13 from ref. 10, and for organisms 14 and 15 from ref. 13. + Present; — not detected.

The sensitivity to DAQ of bacteria in which vitamin K is the only quinone involved in electron transport, and the relative resistance of bacteria which either possess the alternative pathway through ubiquinone or do not contain quinone-mediated electron-transport system, suggest that DAQ probably interferes with the function of vitamin K but not with that of ubiquinone. In view of the as yet incomplete knowledge of the systems of electron transport and coupled phosphorylation in bacterial cells, selective inhibition by a *p*-benzoquinone of bacteria in which electron transport is vitamin K-dependent could be of value in the further elucidation of these systems<sup>11</sup>.

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## VIROLOGY

### Activity of 6-Aminonicotinamide against *Vaccinia* Virus *in vitro*

6-AMINONICOTINAMIDE (6-AN), a potent nicotinamide antagonist<sup>1,2</sup>, was tested for its activity against vaccinia virus, viral cytopathic effects not having been demonstrated in vaccinia-infected tissue cultures in the presence of small amounts of the compound.

All experiments were performed in tube cultures of chick embryo fibroblasts (CEF), propagated in Hanks's balanced salt solution containing 0.5 per cent 'lactalbumin' hydrolysate, 0.1 per cent 'Yeastolate' and 2 per cent newborn calf serum at pH 7.2. For maintenance of cultures, Earle's balanced salt solution was substituted

and the pH increased to 7.6. Cytopathic end points were expressed as the lowest dilution which infected 50 per cent of cultures ( $TCD_{50}$ ) calculated by the method of Reed and Muench<sup>3</sup>.

Vaccinia virus, previously re-passaged in CEF, was stored in individual vials at  $-65^{\circ}\text{C}$  and titrated to determine the plaque-forming units per millilitre (P.F.U./ml.). For use in infectivity tests a multiplicity of 5 P.F.U./cell contained in 0.3 ml. was used.

In a typical experiment, 6-AN of a concentration of 10  $\mu$ g/ml. was added 3 and 6 h before, and immediately after, virus adsorption. A control series containing no 6-AN was also included.

Immediately before infection with virus, all media were removed from the tubes and stored. Adsorption of virus to the cells was allowed for 1.5 h, the tubes were drained and residual virus removed. The monolayers were then washed twice with 1.5 ml. of medium and the original media were replaced. After incubation at  $36^{\circ}\text{C}$ , random samples were removed from each group at 24-h intervals, the cytopathic effect (CPE) was assessed visually, the tubes frozen and thawed to liberate intracellular virus, and the individual group samples pooled. At least four tubes were removed for each pool.

It was noted that CPE progressed in the usual manner in tubes without 6-AN, with complete degeneration between the second and third day. In tubes containing 6-AN, however, no CPE was evident except in tubes challenged with 6-AN after virus adsorption when discrete focal areas of viral destruction were seen. It was also seen that the usual change in pH was not so marked in tubes containing 6-AN. All pooled samples were titrated simultaneously on CEF monolayers and the  $TCD_{50}$  was determined.

Fig. 1 demonstrates that the longer 6-AN was in contact with the cells before virus infection, the more inhibitory was its action, although a significant decrease in viral yield was obtained when 6-AN was added to the cultures immediately after virus adsorption. Progeny was diminished approximately 1,000-fold when 6-AN was added 6 h before virus and 100-fold when it was added after virus.

Reversal of the action of 6-AN was obtained by the addition of 100  $\mu$ g/tube of nicotinamide. When added at the same time as 6-AN, complete reversal was obtained, but when added after virus adsorption the increase in virus yield was related to time of addition and was never complete. Similarly, removal of medium containing 6-AN and replacing with fresh medium without 6-AN produced a higher yield of virus, but never as high as that of untreated cultures or of nicotinamide-treated cultures.

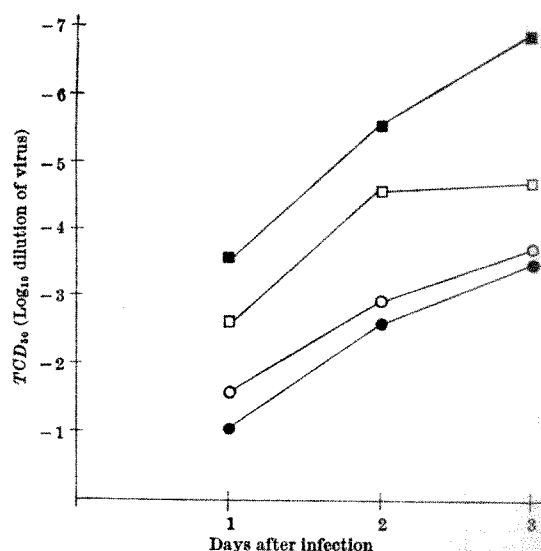


Fig. 1. ■, No 6-AN; □, 6-AN added immediately after virus adsorption; ○, 6-AN added 3 h before virus infection; ●, 6-AN added 6 h before virus infection.

Tests for direct neutralizing and toxic effects of 6-AN on vaccinia virus proved negative; this implies that its action is directed against intracellular synthesis of virus.

*Note added in proof.* Since the submission of this letter for publication, S. H. S. Lee (*J. Bact.*, **88**, 885; 1964) has reported similar results.

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### Haematological Changes in Viral (MHV-3) Murine Hepatitis

In earlier work we investigated some biochemical<sup>1</sup> and biological<sup>2</sup> aspects of hepatitis induced in mice by mouse hepatitis virus strain 3 (MHV-3).

In the present communication, in order to investigate extrahepatic pathology due to MHV-3 (ref. 3), the peripheral blood as well as the lymphopoietic and haemopoietic systems were examined during various stages of the disease. Moreover, the virus concentration in the peripheral blood, bone marrow, spleen and lymph nodes was determined.

Eighty mice of the NMRI-strain, weighing about 12 g and fed on the 'Altromin' diet, were divided into four groups of 20 animals each, indicated as A, B, C and D. Mice of groups A, B and C were inoculated intraperitoneally with 100 LD<sub>50</sub> of MHV-3 virus; mice of group D served as controls.

The number of red cells, white cells, platelets and reticulocytes in the peripheral blood was determined for eight animals from each group, picked at random among the survivors at 24 h (group A), 48 h (group B) and 72 h (group C). Also determined were the haemoglobin concentration and differential white cell count.

Five animals from each group were then killed by decapitation; the bone marrow was taken from the femoral diaphysis of each mouse; smears were prepared and the myelogram determined. Furthermore, from the animals killed 72 h after virus inoculation (group C), samples of blood, bone marrow, lymph nodes and spleen were obtained and assayed for viral content (the LD<sub>50</sub> was determined according to the method of Reed and Muench<sup>4</sup>). From the spleen and lymph nodes of these animals histological preparations were also made.

The results summarized in Table 1 show a statistically significant drop in haemoglobin, white cells, reticulocytes and platelets. The diminution progresses with time being particularly conspicuous in the terminal stage of the disease (72 h), when marked anisocytosis and poikilocytosis of erythrocytes also occur. The differential count starting at 48 h, shows a marked absolute diminution of lymphocytes and, to a lesser degree, of monocytes and eosinophilic polymorphonuclear leucocytes. These alterations, which become more evident as the disease progresses, are statistically significant. 72 h following virus inoculation, absolute neutropenia is detectable. Examination of smears reveals that granulocytes are mainly represented by juvenile elements with little segmentation of the nuclei.

Table 2 shows a diminution in the elements of the erythropoietic series as compared with the leucopoietic series. The leucopoietic series shows a marked relative increase of the more immature elements (myeloblasts and promyelocytes) compared with the more mature ones (metamyelocytes and granulocytes). Furthermore, relative increase of haemocyctoblasts is present.

A histological examination of axillary lymph node showed a marked diffuse hyperplasia of the reticuloendothelium, with subsequent obliteration of sinuses of the medulla and dissociation of follicles of the cortex. The follicles displayed a high degree of hyperplasia, being sometimes reduced to a small clump of lymphoid element at the periphery of the lymph node. The lymphoid cell showed frequent karyorrhexis and karyolysis. Marked hyperplasia of the reticuloendothelium of peripheral sinuses also occurred. The endothelium of sinuses of the medulla was hypertrophic and hyperplastic, with packing of the endothelial cells which often resemble multinucleated giant cells. Mitoses were often seen in reticular cells. Similar changes were detected in mesenteric lymph nodes.

The histological examination of the spleen showed a high degree of congestion of oedema. In the cortex there was a marked decrease in follicle volume. The follicle remains were made up mainly of cells of the lymphoblastic and histiocyte type, often nucleolated and in mitosis. Therefore, there were few mature lymphoid elements concentrated in a narrow band at the periphery of the follicles or scattered throughout the parenchyma. In the germinal centres there were frequent cellular changes with fragmentation of the nuclear membrane and chromatolysis. Scattered areas of necrosis were also evident.

The results summarized in Table 3 show that the virus is present in high concentration in the spleen and bone marrow, less in the peripheral blood and lymph nodes.

Table 1. VALUES OF HAEMOGLOBIN, RED BLOOD CELLS, LEUCOCYTES AND PLATELETS IN NORMAL MICE AND IN MICE INFECTED WITH 100 LD<sub>50</sub> OF MHV-3 VIRUS, AT DIFFERENT TIMES FOLLOWING INOCULATION

Cells/mm <sup>3</sup>	Normal mice	Infected mice					
		24 h		48 h		72 h	
		Mean values* ± S.D.	P	Mean values* ± S.D.	P	Mean values* ± S.D.	P
Haemoglobin (g%)	14 ± 0.8	13 ± 0.6	< 0.05	13 ± 0.8	< 0.05	12 ± 0.7	< 0.01
Erythrocytes	9,612,500 ± 229,850	7,787,500 ± 208,010	< 0.001	7,271,875 ± 150,450	< 0.001	6,781,250 ± 991,100	< 0.001
Reticulocytes	120 ± 15.0	86 ± 6.9	< 0.001	17 ± 6.2	< 0.001	14 ± 3.2	< 0.001
White cells	6,937 ± 1,545	4,562 ± 1,050	< 0.001	3,031 ± 633	< 0.001	2,562 ± 311	< 0.001
Neutrophils	2,119 ± 515.9 (30.55%)	2,645 ± 939.0 (57.98%)	> 0.05	1,739 ± 479.7 (57.37%)	> 0.05	1,530 ± 259.0 (59.72%)	< 0.05
Eosinophils	109 ± 88.6 (1.57%)	34 ± 17.7 (0.74%)	< 0.05	18 ± 9.0 (0.59%)	< 0.05	11 ± 10.3 (0.43%)	< 0.05
Basophils	19 ± 20.2 (0.27%)	9 ± 11.0 (0.20%)	> 0.05	6 ± 5.1 (0.20%)	> 0.05	4 ± 5.4 (0.16%)	> 0.05
● Lymphocytes	4,421 ± 881.6 (63.73%)	1,759 ± 402.8 (38.56%)	< 0.001	1,189 ± 244.3 (39.23%)	< 0.001	975 ± 176.4 (38.06%)	< 0.001
Monocytes	269 ± 153.6 (3.88%)	115 ± 54.1 (2.52%)	< 0.05	79 ± 49.9 (2.51%)	< 0.01	42 ± 24.8 (1.64%)	< 0.01
Platelets	1,012,000 ± 18,420	657,000 ± 23,790	< 0.001	460,000 ± 22,600	< 0.001	373,000 ± 16,030	< 0.001

\* From eight determinations, each on a different animal.

For cell counts and haemoglobin determination the tail venous blood was used. Differential white cell counts were made on 500 elements.



Table 2. BONE MARROW PICTURE IN NORMAL MICE AND IN MICE INFECTED WITH 100 LD<sub>50</sub> OF MHV-3 VIRUS AND KILLED AT 72 H

Per cent	Normal mice Mean values* ± S.D.	Infected mice Mean values* ± S.D.	P
Haemocytoblasts	1.36 ± 0.59	3.38 ± 0.81	< 0.01
Proerythroblasts	1.44 ± 0.26	1.14 ± 0.63	> 0.05
Basophilic erythroblasts	13.22 ± 1.60	5.16 ± 0.64	< 0.001
Polychromatic erythroblasts	3.83 ± 0.81	2.56 ± 0.69	> 0.05
Orthochromatic erythroblasts	5.12 ± 1.09	3.38 ± 0.67	< 0.05
Myeloblasts	5.78 ± 1.00	25.40 ± 2.06	< 0.001
Promyelocytes	5.69 ± 0.84	15.30 ± 1.69	< 0.001
Myelocytes	15.54 ± 1.46	16.22 ± 1.49	> 0.05
Metamyelocytes	21.30 ± 1.64	13.24 ± 1.96	< 0.001
Granulocytes	23.46 ± 1.45	12.46 ± 1.98	< 0.001
Megakaryocytes	0.40 ± 0.46	0.34 ± 0.11	> 0.05
Lymphocytes	1.32 ± 0.44	0.74 ± 0.33	< 0.05
Monocytes	0.68 ± 0.28	0.40 ± 0.22	> 0.05
Plasmacytes	0.39 ± 0.19	0.24 ± 0.11	> 0.05

\* From five determinations each on a different animal. In each smear 1,000 cells were counted (May-Grünwald-Giemsa staining).

The peroxidase reaction was used to differentiate haemocytoblasts from myeloblasts. Only the 72-h values are reported in the table, as they were the most significant.

Table 3. VIRUS CONCENTRATION IN PERIPHERAL BLOOD, BONE MARROW, SPLEEN AND AXILLARY LYMPH NODES OF MICE INFECTED WITH 100 LD<sub>50</sub> OF MHV-3 VIRUS 72 H PREVIOUSLY

Materials	LD <sub>50</sub>
Blood	10 <sup>-5.25</sup>
Bone marrow	10 <sup>-4.16</sup>
Spleen	10 <sup>-6.10</sup>
Axillary lymph nodes	10 <sup>-4.15</sup>

From the results presented here, the following conclusions can be drawn: (1) The hypoplasia of the erythroid series detected in the bone marrow, and particularly the considerable pathological changes of these elements, might be responsible for the marked and progressive anaemia, reticulocytopenia and diminution of haemoglobin noted in the peripheral blood. (2) The marked maturative delay and pathological changes observed in the myelopoietic series of bone marrow and the histological damage present in cells of the lymphopoietic regions of the organism (spleen and lymph nodes) might account for the leucopenia. (3) The alteration of the maturative process of megakaryocytes in the bone marrow might explain the marked thrombopenia of the peripheral blood.

The high virus concentration in the bone marrow and lymphopoietic tissues suggests that the aforementioned changes might be produced by an active multiplication of the virus in these organs.

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## GENETICS

### Inheritance of Self-incompatibility and Brown Keel Tip in *Lotus corniculatus* L.

*Lotus corniculatus* is a segmental allotetraploid<sup>1,2</sup> which frequently exhibits chromosomal tetrasomic inheritance<sup>3-7</sup>. We have investigated the inheritance of pubescence, a chlorophyll deficiency, cyanogenesis, flower colour, streaks on the corolla, keel tip colour and self-incompatibility. We obtained evidence for chromosomal tetrasomic inheritance with each characteristic studied. However, we also found a number of crosses which do not give good fits to those expected with tetrasomic inheritance and feel these warrant further study.

Our most extensive investigation is of self-incompatibility, a continuation of work described previously<sup>8</sup>. We developed a technique, based on that of Picard and

Demarly<sup>9</sup>, that permits us to distinguish between compatible and incompatible matings. This technique involves growing pollen on an agar medium which is pretreated with stylar extracts. Two plant families were studied. One consisted of 19 S<sub>1</sub> progeny and their relatively self-fertile parent. These were tested in all possible combinations. The other consisted of cross-progeny of two unrelated plants. Two cross-progenies of eight plants each (the one reciprocal to the other) were tested in all possible combinations. The self-progeny group of plants gave a reasonably good fit to the pattern expected if self-incompatibility is determined by S-alleles inherited tetrasomically at a single locus. The two cross-progenies gave reasonably good fits to the pattern expected if self-incompatibility is determined by S-alleles inherited disomically at two loci. If we allow for the two different segregation patterns in the two families, our data accord with the hypothesis that we are dealing with a *Nicotiana*-type gametophytic oppositional incompatibility system. We have to allow for compatibility due to dominance in the pollen grain. When a recessive S-allele present in the pollen is matched in the style but a dominant allele in the same pollen grain is not matched, the pollen is compatible.

These studies suggest the following hypothesis on which further studies may be based. The four chromosomes carrying the S-alleles are homologous in the self-progeny, so we get random pairing by twos to give chromosomal tetrasomic inheritance. The four chromosomes carrying the S-alleles in the cross-progeny consist of two structurally differentiated pairs to give disomic segregations at two loci. If this is true, we should be able to find plants which will produce progeny that segregate tetrasomically in some crosses and disomically in others, and there should be intermediate segregations.

This hypothesis should also be tested with other segregating characters. A procedure that may be used is to cross a quadruplex with a nulliplex plant and examine segregations after back-crossing to the nulliplex. If there is a tendency for the two chromosomes from one parent to pair together (that is, two carrying the dominant allele), there should be an increase in simplex progeny compared to the 1 duplex : 4 simplex : 1 nulliplex expected with random chromosomal tetrasomic inheritance. Conversely, a tendency for one chromosome from one parent to pair with one from the other should result in a decrease in simplex progeny. The progeny genotypes may range from all simplex to 1 duplex : 2 simplex : 1 nulliplex, and expected phenotypic ratios for complete dominance may range from all the dominant phenotype to 3 dominant : 1 recessive. These extremes correspond to ratios expected with disomic inheritance at two loci. If we get the extreme condition and one pair of chromosomes is homozygous and the other heterozygous at this locus, we would expect simple disomic segregation ratios. However, a small degree of tetrasomic pairing would be expected to break down disomic patterns. If this hypothesis were valid, we would expect duplex siblings to differ from each other when back-crossed to the same recessive parent. Also, we must allow for the possibility that the degree of preferential pairing may be influenced by environmental effects so that test-crosses made at different times may segregate differently. On the other hand, test-crosses made to different nulliplex plants at one time should not differ significantly.

It is obviously very difficult to test an hypothesis as flexible as this. It is possible to reconcile most of the data we have obtained so far with this hypothesis. Other published data, such as that of Buzzell and Wilsie<sup>6</sup>, also appear to fit this hypothesis. It accounts for the excess of brown keel tip they obtained when they backcrossed duplex with nulliplex plants. It does not account for the deficiency of brown keel tip they observed with simplex crosses. However, we have evidence that supports their postulate that another locus is segregating restraint<sup>10</sup> or

suppressor genes that reduce the proportion of brown keel tip plants. Our evidence is that at least one locus that is involved in the expression of cyanogenesis is also involved in the expression of keel tip colour. Plants recessive for the allele at the suppressor locus are both acyanogenetic and yellow keel tip. This suggests that the duplex  $\times$  nulliplex segregations they present underestimate the proportion of plants duplex and simplex at the brown keel tip locus. An increase in this ratio is in agreement with the hypothesis for preferential pairing presented here, and disagrees with the postulate that ratios of less than 5:1 are a result of equational chromatid segregation. The observed low frequency of quadrivalents per cell<sup>3</sup> is also evidence against equational chromatid segregations.

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### Taste Sensitivity to Phenyl-thio-carbamide and Endemic Goitre among Pewenche Indians

THE tasting thresholds to phenyl-thio-carbamide (PTC) and the clinical characteristics of the thyroid gland were determined in 88 women and 70 men belonging to the Pewenche branch of the Araucanian Indians. Most of the subjects were born (and all of them lived) in the geographically isolated Pedregoso Valley in the Chilean Andes, 38° 32' S. The total population of the valley is of about 1,000 persons.

According to Nagel *et al.*<sup>1</sup>, who examined blood groups and haptoglobin types, this community represents a genetic isolate, and exhibits a high incidence of endemic goitre. Preliminary knowledge of the fertility trends of the subjects and of their pedigree relationships makes it possible to estimate the mean inbreeding coefficient,  $\alpha$ , as 0.0105. The mean age of the Indians studied was 35.4 years with a standard deviation of 16.38, ranging from 7 to more than 70 years.

Table 1 shows the percentage distribution of the types of thyroid gland morphology, either normal or goitrous; the last category has been subdivided into diffuse and nodular varieties. Table 2 summarizes the distributions of the observed tasting thresholds to PTC for women and men separately obtained by means of the Harris and Kalmus technique<sup>2</sup>. The recommended corrections for age and sex<sup>3</sup> did not prove helpful at this level of analysis, for, in spite of some smoothing of the curves, the antimode zone remained unaltered. The proportions of non-tasters did not change either. The total non-taster percentage (3.2) is comparable with that found in other Amerindian populations<sup>4,5</sup>. The proportions of non-tasters among the three types of thyroid gland forms (Table 1), given by only five subjects, are not significantly different if tested for independence ( $\chi^2_{(2)} = 0.668$ ;  $0.70 < P < 0.75$ ).

Other results should be compared with the present ones. A significant association has been shown between endemic goitre and the frequency of non-tasters to PTC in a recent survey made in Israel with children of Ashkenazic origin, 8-18 years old<sup>6</sup>. This correlation was not found in a

Table 1. MORPHOLOGY OF THYROID GLAND AND PERCENTAGE OF NON-TASTERS IN 158 PEWENCHE INDIANS

	Women		Men		% of non-tasters
	No.	%	No.	%	
Normal thyroid	6	6.8	20	28.6	3.8
Diffuse goitre	33	37.5	26	37.1	1.7
Nodular goitre	49	55.7	24	34.3	4.1
Total	88	100	70	100	3.2

Table 2. TASTING THRESHOLDS TO PHENYL-THIO-CARBAMIDE IN 158 PEWENCHE INDIANS DIVIDED ACCORDING TO SEX

	Solutions														Total	% of non tasters		
	<1	1	2	3	4	5	6	7	8	9	10	11	12	13	14			
Men	3							1	3	5	20	28	8	1		1	70	4.3
Women	1		1					3	10	26	31	13	2	1			88	2.8
Total	4		1					1	6	15	46	59	21	3	1	1	158	3.2

population of White and Negro-Mulatto boys of the same age in Brazil<sup>7</sup>. On the other hand, sporadic goitres of various kinds have been shown to be related to the non-taster character in repeated observations made in English people<sup>8,9</sup>. Such relationships, for both endemic and non-endemic goitre, appear to be conflicting, as was shown by Fraser<sup>10</sup>.

While our subjects are not comparable with those from Israel from the point of view of age and race, perhaps the main difference is that ours come from a genetical isolate whose inhabitants have been submitted to selective goitrogenic influences for many generations. If this were so, part of the observed variation in the thyroid gland response in endemic goitre zones might be better explained by other factors of population structure in addition to the specific PTC genes effect.

Relationships of the PTC trait with tuberculosis<sup>11</sup> diabetes<sup>12</sup> and paralytic poliomyelitis have been explored<sup>13</sup>. Although some authors have not found the first two associations<sup>8,14</sup>, these results could reflect the simultaneous operation of multiple selective factors. That the PTC polymorphism is unstable is clear from a recent analysis on the White to Negro gene flow in the United States<sup>15</sup>. In most of these investigations the impact of recent immigration is apt to conceal the slower dynamics of a selective factor or to create the false impression of an association.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, March 8

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 4.30 p.m.—Annual General Meeting. 5.30 p.m.—Dr. Mary Hesse: "The New Problem of Induction".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. Peter Scott: "The Population Structure of Australian Cities".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. H. E. M. Barlow, F.R.S.: "Screened Surface Waves and Some Possible Applications".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. T. Barnard: "Micro-paleontology; Past, Present, and Future".\*

SOCIETY OF CHEMICAL INDUSTRY (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. M. Stanley: "Some Achievements in Petroleum Chemicals" (Seventh Castner Memorial Lecture).

## Tuesday, March 9

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. E. H. Thompson: "What is Photogrammetry?"\*

UNIVERSITY OF LONDON (at the London School of Economics, Houghton Street, London, W.C.2), at 5 p.m.—Prof. P. E. de Josselin de Jong (Leiden): "Modern Trends in Social Anthropology in Holland". (Studies in Social Anthropology, 1.)\*

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Mrs. Graham Spry: "The Expeditions of John Palliser, Explorer of Western Canada, 1847-1861".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. B. A. Berdal and Mr. K. G. Kiel: "Skogfoss Hydroelectric Power Station".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. F. J. Wilkins, Mr. T. A. Deacon and Mr. R. S. Becker: "A Multijunction Thermal Converter—an Accurate d.c./a.c. Transfer Instrument".

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the National College of Rubber Technology, Northern Polytechnic, Holloway Road, London, N.7), at 5.30 p.m.—Mr. P. McL. Swift: "Oil Extension of Natural Rubber"; 7 p.m.—Dr. D. C. Blackley: "Aspects of the Science and Technology of Rubber Latices—Some Recent Developments of Current Interest".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 6, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Developments in the Field of Medical Engineering" initiated by Dr. E. G. Melrose and Dr. S. R. Montgomery.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Sir Lawrence Bragg, O.B.E., F.R.S.: "Waves". (Afternoon lecture for Sixth Form Boys and Girls in Schools from London and the Home Counties. To be repeated on March 10, 16 and 17.)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. A. M. Case: "Demography and the Cancers". (Last of sixteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)\*

UNIVERSITY OF LONDON (in the Manson Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. N. Jenkins: "Recent Trends in Dental Caries".\*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. V. E. Gough: "Tyre and Vehicle Vibration".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Brighton College of Technology Chemical Society, at Brighton College of Technology, Lewes Road, Brighton, Sussex), at 7 p.m.—Prof. J. A. V. Butler, F.R.S.: "Nucleic Acids".

## Tuesday, March 9—Thursday, March 11

INSTITUTE OF METALS (at Church House, Great Smith Street, Westminster, London, S.W.1)—Spring Meeting.

## Wednesday, March 10

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 3 p.m.—Meeting on "The Economics of Automation".

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, S.W.3), at 5 p.m.—Dr. K. F. W. Hinson: "Lungs and Asbestos".\*

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Dr. T. F. Gaskell: "North Sea Exploration".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. King: "Electronic Circuits—Past, Present and Future".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. E. S. Forster: "Industrial Applications of Thyristors (Silicon Controlled Rectifiers)".

UNIVERSITY OF LONDON (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 5.30 p.m.—Dr. A. R. Ten Cate: "Histochemical Techniques in the Study of the Cellular Biology of Dental Tissues". (Last of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)\*

INSTITUTION OF ELECTRONICS AND RADIO ENGINEERS, ELECTRO-Acoustics GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. F. H. Brittain: "Problems in Listening".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. D. R. Newman: "Aerodynamics and Performance Demands on Aircraft Engineering" (James Clayton Lecture).

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6.30 p.m.—Eighteenth Conversation.

OFFICE OF HEALTH ECONOMICS (at the Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1), at 8.30 p.m.—Mr. S. C. Jones: "Government Relations with a Research Based Industry".\*

## Wednesday, March 10—Saturday, March 13

ROYAL SOCIETY AND THE GENETICAL SOCIETY (in the Botany Lecture Theatre of the Biological Sciences Building, University College, Gower Street, London, W.C.1)—Symposium on "From Mendel's Factors to the Genetic Code".

## Thursday, March 11

BRITISH JOINT CORROSION GROUP (at the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 10 a.m.—Inaugural Meeting. Dr. Ulric R. Evans: "Some Problems Facing the British Joint Corrosion Group", followed by Discussion on "The Influence of Metal Structure on Corrosion".

MINERALOGICAL SOCIETY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

UNIVERSITY OF LONDON (at the London School of Economics, Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Prof. P. E. de Josselin de Jong (Leiden): "Islam and Culture Change in South-East Asia". (Studies in Social Anthropology, 2.)\*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Need or Otherwise for the Provision of Stabilizing Windings on Star/Star Connected Transformers", opened by Mr. A. J. Wakeling.

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Two Point Boundary Value Problems in Control", opened by Dr. C. Storey.

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Gear Pumps Versus Vane Pumps".

## Friday, March 12

SOCIETY FOR WATER TREATMENT AND EXAMINATION (at the Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1) from 9.30 a.m. to 5.30 p.m.—Annual General Meeting followed by a Symposium on "Consumer Complaints".

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.50 a.m.—Symposium on "The Physiology of Roots".

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Meeting on "Height Measurement in Supersonic Aircraft".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Steam-Turbine Designer and the Metallurgist".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. F. Bergel, F.R.S.: "Whence and Whither Chemotherapy?"

## Monday, March 15

BRITISH SOCIETY FOR INTERNATIONAL HEALTH EDUCATION (at the Drapers' Hall, Throgmorton Street, London, E.C.2), at 5 p.m.—The Lord Cohen of Birkenhead: "Problems in Health Education".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Irreversible Effects Resulting from Breakdown in Transistors and Rectifiers" opened by Mr. C. F. Drake and Mr. A. F. Newall.

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mrs. Alban Caroe (Gwendoline Bragg): "The Royal Institution in Sir William Bragg's Time".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or an ASSISTANT (qualified to give instruction in the field of medieval philosophy) IN PHILOSOPHY—The Secretary of the University Court, The University, Glasgow (March 13).

LECTURERS (with good academic qualifications in physics, preferably including a higher degree) IN PHYSICS for duties in the University Departments of Surgery at the Royal Infirmary; Practice of Medicine at the Royal Infirmary; Surgery at the Western Infirmary; and Practice of Medicine at the Western Infirmary—The Secretary of the University Court, The University, Glasgow (March 13).

LECTURER IN STATISTICAL MATHEMATICS—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (March 15).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (March 15).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (March 15).

TUTORIAL RESEARCH STUDENT IN MATHEMATICS—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (March 15).

LECTURER IN APPLIED MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (March 16).

ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS AND MATHEMATICAL PHYSICS—The Registrar, University College of South Wales and Monmouthshire, Cardiff (March 19).



LECTURER (with special qualifications in soil biochemistry and microbiology) in SOIL SCIENCE—The Registrar (Room 22, O.R.B.), The University, Reading (March 20).

ASSISTANT LECTURER or LECTURER (or, in special cases, Senior Lecturer) in MATERIALS SCIENCE—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Brighton, Sussex (March 31).

CHAIR OF BOTANY in the FACULTY OF SCIENCE of the UNIVERSITY of MALAYA—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, March 31).

DEMONSTRATOR (honours graduate in zoology, entomology, or agricultural zoology, including 1965 graduates) in AGRICULTURAL ZOOLOGY in the DEPARTMENT OF AGRICULTURAL SCIENCES, School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham (March 31).

CHAIR OF GENETICS—The Secretary, The University, Aberdeen (April 2).

UNIVERSITY LECTURER or DEPARTMENTAL DEMONSTRATOR in PHYSICAL CHEMISTRY—Dr. Lee's Professor of Chemistry, Physical Chemistry Laboratory, The University, South Parks Road, Oxford (April 3).

LECTURER or ASSISTANT LECTURER in PHARMACOLOGY, University of the West Indies—Secretary to the Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (April 5).

SENIOR LECTURERS or LECTURERS (3) in FORESTRY in the School of General Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 5).

ASSISTANT EXPERIMENTAL OFFICER (botanist or agricultural botanist) to assist in work on factors affecting the yield of field crops—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (April 15).

RESEARCH STATISTICIAN, SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with a good honours degree in mathematics or statistics and preferably postgraduate experience and some knowledge of agriculture or biology)—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (April 15).

CHAIR OF PSYCHOLOGY—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (April 24).

ASSISTANTS in CHEMISTRY—The Secretary of the University Court, The University, Glasgow (April 30).

LECTURER (with a special interest and research experience in electrochemistry) in PHYSICAL CHEMISTRY—The Secretary of the University Court, The University, Glasgow (April 30).

ROCHE POST-DOCTORAL FELLOW in PHYSICAL CHEMISTRY, with special reference to crystal and molecular structure studies or organic compounds—The Secretary of the University Court, The University, Glasgow (April 30).

JUNIOR and SENIOR GEOLOGISTS—The Director, Mines Branch, Department of Lands and Mines, Province of New Brunswick, Fredericton, N.B., Canada.

LECTURER (preferably with experience in electronic and microwave physics) in PHYSICS—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

LECTURERS and ASSISTANT LECTURERS in CHEMISTRY—The Registrar, The University, Manchester, 13, quoting Ref. 29/65/Na.

MASTER to teach Mathematics up to advanced level and university scholarship standard (half the teaching at least being with Vith Forms); and a MASTER to teach Biological subjects up to advanced level and university scholarship standard (with a substantial amount of Vith Form work)—The Headmaster, King's College School, Wimbledon, London, S.W.19.

MICROBIOLOGIST (under the age of 30 years and preferably with virological training) in the VIRUS VACCINE DEPARTMENT, to undertake the testing of new immunological products—The Secretary, The Lister Institute of Preventive Medicine, Elstree, Herts.

POST-DOCTORAL RESEARCH FELLOW (preferably with practical experience of N.M.R., or an interest in theoretical calculations) in THE DEPARTMENT of ORGANIC CHEMISTRY, for work on high-resolution N.M.R., with Dr. R. J. Abraham—The Registrar, The University, Liverpool, 3, quoting Ref. CV/47/9/N.

PRINCIPAL LECTURER in POLYMER PHYSICS—Clerk to the Governors, National College of Rubber Technology, Northern Polytechnic, Holloway, London, N.7.

PRINCIPAL LECTURER in ZOOLOGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

RESEARCH FELLOW in the DEPARTMENT of MATERIALS, to work in the field of high-temperature deformation in metals—The Registrar, The College of Aeronautics, Cranfield, Bedford.

RESEARCH FELLOW (with a Ph.D. or equivalent research degree with some experience in cryogenics) in LOW-TEMPERATURE PHYSICS to study the effect of high magnetic fields on nuclear and electronic interactions in magnetic metals at very low temperatures—The Deputy Secretary, The University, Southampton.

SENIOR RESEARCH ASSOCIATE (science graduate holding M.Sc., Ph.D. or equivalent qualifications with some experience in protein chemistry) in the DEPARTMENT of CLINICAL BIOCHEMISTRY, for research on isoenzymes in cancer—Prof. A. L. Latner, The Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne, 1.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization. Fire Research Technical Paper No. 10: Design of Roof-Venting Systems for Single-Storey Buildings. By Dr. P. H. Thomas and P. L. Hinkley. Pp. vi + 45. (London: H.M. Stationery Office, 1964.) 3s. 6d. net. [1112]

The Creation of New Universities. By C. I. G. Bosanquet and A. S. Hall. (Based on a Conference at the University of Keele, 14th to 16th July, 1964.) Pp. 14. (Keele: The University, 1964.) [1112]

The British Council. Annual Report 1963-1964. Pp. vii + 107 + 12 plates. (London: The British Council, 1964.) 2s. 6d. [1112]

Imperial College of Science and Technology (University of London). Fifty-seventh Annual Report of the Governing Body, 1963-64. Pp. 142. (London: Imperial College of Science and Technology, 1964.) [1112]

Annual Report of the Advisory Council on Scientific Policy, 1963-1964. Pp. vi + 48. (Cmd. 2538.) (London: H.M. Stationery Office, 1964.) 4s. net. [1112]

Ricardo and Co., Engineers (1927), Ltd. Report and Accounts, 30th June, 1964. Pp. 10. (London: Ricardo and Co., Engineers (1927), Ltd., 1964.) [1112]

Ciba (A.R.L.), Ltd. Technical Notes, No. 263 (November, 1964): Araldite in Electron Microscopy. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1964.) [1112]

General Register Office. Census 1961: England and Wales. Age, Marital Condition and General Tables. Pp. xv + 108. (London: H.M. Stationery Office, 1964.) 22s. net. [1112]

Ministry of Agriculture, Fisheries and Food. Animal Disease Surveys. Report No. 3: Disease, Wastage and Husbandry in the British Dairy Herd, 1958-59. By F. B. Leech, M. P. Vessey and W. D. Macrae. Losses of Breeding Ewes in England and Wales in 1958-59. By F. B. Leech and M. P. Vessey. Analysed Results of Post-Mortem Examinations of Sheep 1959-61. By L. E. Hughes. Rhinitis of Swine 1956-57. By J. T. Done, Marlon D. Richardson and Catherine N. Hebert. Pp. 48 + 4 plates. 8s. 6d. net. Report No. 4: Brucellosis in the British Dairy Herd. By F. B. Leech, M. P. Vessey, W. D. Macrae, J. R. Lawson, D. J. MacKinnon and W. J. B. Morgan. Pp. iii + 42. 5s. 6d. net. (London: H.M. Stationery Office, 1964.) [1112]

The Institution of Electronic and Radio Engineers. The Annual Report of the Council for the year ended 31st March 1964. Pp. 22. National Prosperity and the Engineer. By Colonel G. W. Raby. (Presidential Address.) Pp. 11. (London: The Institution of Electronic and Radio Engineers, 1964.) [1112]

### Other Countries

Bulletin of the American Museum of Natural History. Vol. 128: The Birds of Costa Rica: Distribution and Ecology. By Paul Slud. Pp. 1-430 + 2 plates. (New York: American Museum of Natural History, 1964.) 10 dollars. [1612]

National Academy of Sciences—National Research Council. Publication No. 1199: Laboratory Animals, Part 2—Animals for Research. Fifth edition. Pp. v + 89. (Washington, D.C.: National Academy of Sciences—National Research Council, 1964.) 2 dollars. [1612]

Publikationer fra det Danske Meteorologiske Institut. Meddelelser, Nr. 18: Danish Visual Aurora Observations, 1957-60. By Knud Lassen, O. Rud Laursen and Johannes Olsen. Pp. 76. (Charlottenlund: Danske Meteorologiske Institut, 1964.) [1612]

United States Department of Health, Education and Welfare: Public Health Service. Publication No. 1242: Androgenic and Myogenic Endocrine Bioassay Data. Issue 1, Entry Nos. 1-1697. Edited by Arthur G. Hilgar and Donald J. Hummel. Pp. 243. (Bethesda, Maryland: National Cancer Institute, National Institutes of Health, 1964.) [1612]

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais. Dundo—Lunda—Angola. Museu do Dundo. Publicacoes Culturais, No. 63: Subsídios para o Estudo da Biologia na Lunda—Estudos Diversos (XXV). Pp. 131 (4 plates). (Lisboa: Companhia de Diamantes de Angola, 1964.) [1612]

Nineteenth Annual Report of the Council of the Queensland Institute of Medical Research for the year ended 30th June, 1964. Pp. 19. (Brisbane: Government Printer, 1964.) [1612]

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 114: Fauna of the Devonian Formation, District of Mackenzie. By D. J. McLaren and A. W. Norris. Pp. 74 + 17 plates. 3.25 dollars. Bulletin 118: Hydrogeochemistry of Uranium in the Bancroft-Haliburton Region, Ontario. By J. A. Chamberlain. Pp. ix + 19 + 1 plate. 1 dollar. Bulletin 119: The Jurassic Faunas of the Canadian Arctic-Cadoceratoniae. By Hans Frebold. Pp. 27 + 20 plates. 2.50 dollars. Paper 64-26: Pre-Mississippian Rocks of Nansen Sound Area, District of Franklin. By H. P. Trettin. Pp. v + 21. 35 cents. (Ottawa: Queen's Printer, 1964.) [1712]

United States Department of the Interior: Geological Survey. Geophysical Abstracts, No. 214, November 1964. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others. Pp. iii + 855-939. 35 cents. Bulletin 1161-B: Geology of the Linville Falls Quadrangle, North Carolina. By John C. Reed, Jr. Pp. iv + 53 + plates 1-8. (Washington, D.C.: Government Printing Office, 1964.) [1712]

Smithsonian Institution, Bureau of American Ethnology. Bulletin 191: Anthropological Papers, Numbers 68-74. Pp. iii + 425 + 104 plates. (Washington, D.C.: Government Printing Office, 1964.) 2.25 dollars. [1712]

Centre de Documentation Economique et Sociale Africaine (CEDESA). Enquêtes Bibliographiques, Nr. 10: Fertilité des Sols et Éléments de Sociologie Rurale en Afrique au Sud du Sahara. Par Dr. J. Lebrun et P. C. Lefevre. Pp. xvii + 182. (Bruxelles: Centre de Documentation Economique et Sociale Africaine, 1964.) 500 francs. [1712]

Smithsonian Miscellaneous Collections, Vol. 146, No. 4: Evolutionary Trends in the Avian Genus *Clamator*. By Herbert Friedmann. Pp. 127. (Publication No. 4532.) (Washington, D.C.: Smithsonian Institution, 1964.) [1712]

Japan: National Institute of Genetics. Annual Report, No. 14, 1963. Pp. vii + 139. (Mishima, Shizuoka-ken: The National Institute of Genetics, 1964.) [1712]

Rubber Research Institute of Malaya. Annual Report, 1963. Pp. 118. (Kuala Lumpur: Rubber Research Institute of Malaya, 1964.) 3 Malayan dollars. [1712]

The Western Australian Museum. Report of the Museum Board for the year ended 30th June, 1964. Pp. 46. (Perth: Western Australian Museum, 1964.) [1712]

Canada: Department of Mines and Technical Surveys. Mineral Resources Division and Geological Survey of Canada. Map 900A: Principal Mineral Areas of Canada. Fourteenth edition. Geological Maps. Map 1121A: Griffls Lake, New Quebec. Map 1149A: Cape St. Lawrence, Nova Scotia. Map 1150A: Cape North, Nova Scotia. (Ottawa: Director, Geological Survey of Canada, 1964.) [1712]

The American Ephemeris and Nautical Almanac for the year 1966. Pp. vii + 508. (Washington, D.C.: Government Printing Office, 1964.) 3.75 dollars. [1712]

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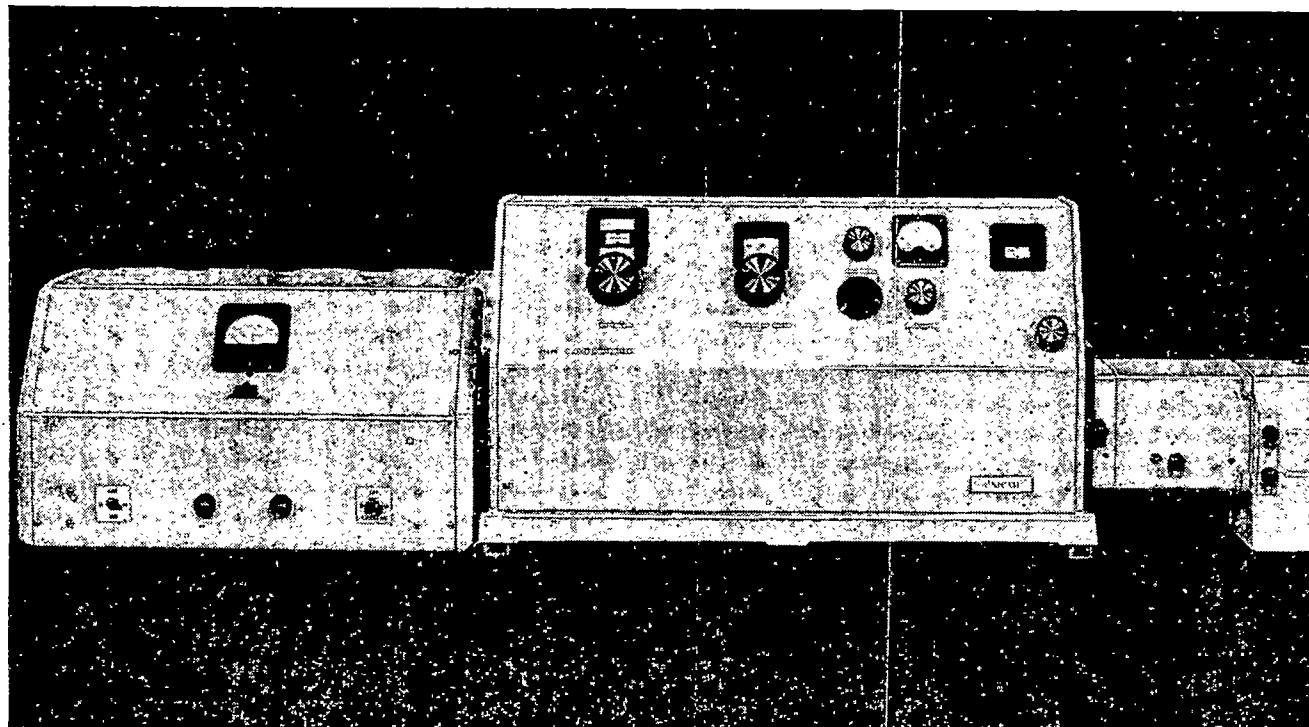
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# LETTERS TO THE EDITOR

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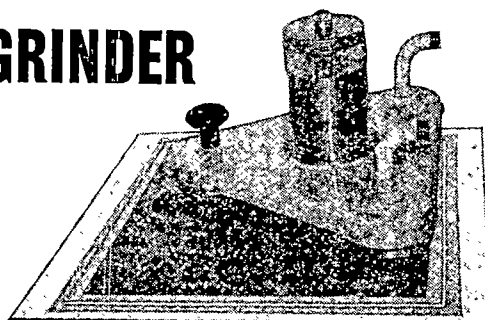
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## SCIENTIFIC RESEARCH AND TRAINING IN AFRICA

AT the Lagos Conference on the organization of research and training in Africa, at which some 28 countries were represented, recommendations were adopted outlining a plan for scientific research and training in relation to the conservation and utilization of natural resources. Many of them are unexceptionable; collectively, they appear somewhat unrealistic. There is little evidence that the wise counsel of Dr. S. Dedijer on undeveloped science in undeveloped countries has yet fallen on receptive ears. The Plan\*, as now published by the United Nations Educational, Scientific and Cultural Organization, in association with the United Nations Economic Commission for Africa, will do little to encourage those who have stoutly championed the continuance of the Overseas Research Council and advocated much more generous support from Britain in that field.

First, dealing with national scientific policy, the Plan recommends continued and large-scale efforts by the African Governments to promote scientific and technical research on natural resources. For this purpose systematic campaigns in each country are recommended to direct attention to the essential part which science and technology can play in solving social and economic problems. Also recommended are the mobilization of all possible means to deal with a shortage of scientific and technical staff and the establishment of a true corps of permanent research workers, including the creation or reinforcement of suitable national scientific structures. These Government measures for the exploitation and utilization of natural resources should include measures for conserving both fauna and flora.

The second section of the Plan, dealing with the organization and implementation of policies of research on natural resources, suggests the establishment of appropriate scientific research committees to determine the research objectives, and the budgeting arrangements within the national planning bodies. It recommends, moreover, that neighbouring countries should co-ordinate their research policies in matters of common interest in this field, exchanging information and co-ordinating efforts to facilitate the implementation of joint projects and the joint use of research institutions. Next, it recommends the establishment, at national or sub-regional levels, of specialized institutes for scientific and technical research on natural resources, possibly by the development or combination of existing institutions for this purpose. The number of inter-African documentation centres should be increased at least three-fold. At the continental level, it recommends a Scientific and Technical Committee on Natural Resources in Africa. This Committee would be specifically concerned with: (1) harmonizing the methods, terminology and utilization of basic maps for study and research on natural resources; (2) facilitating comparison between the results obtained in national research programmes; (3) ensuring that national research programmes would be compared as a matter of course in

disciplines where this was essential for the advancement of knowledge, and arranging continental programmes in the earth sciences and life sciences, and establishing relations with the international scientific community in matters of common concern. The African countries are further recommended to note international research programmes and consider how they may support and participate in such programmes as the International Hydrological Decade, the Upper Mantle Project of the International Union of Geodesy and Geophysics, the International Biological Programme, and the International oceanographic research programmes co-ordinated by the International Oceanographic Commission.

The third section, on scientific and technical personnel, proposes to develop the supply of research workers required on a ratio of 1 : 2 : 3 for postgraduates, graduates and technicians, and recommends that Unesco should study the distribution of levels of research workers in the English and French-speaking countries, as well as survey the total number of research workers at present working in Africa. For the period 1965-80 a target of 200 scientific workers and university teachers per million inhabitants is suggested. There are recommendations regarding the development and content of educational systems, including a study of the use of audio-visual techniques for secondary and higher education and of the availability of suitable material. Co-operation between universities and research institutes should include the training of scientists of all levels. The increased part which research institutes could play in this training should be taken into account when considering the expansion of the institutes. On the recruitment and status of scientists, African workers are alone considered, but there is a wise recommendation on the importance of efforts directed to increase the understanding by the general public of the value of the contributions of science in Africa.

The recommendations relating to finance and research economics, set out in the fourth section, include the establishment in each country of a National Scientific Research Council with an appropriate budget and the planning of research projects for at least three years. The present cost of a research team in Africa, consisting of an experienced research worker assisted by a young graduate or senior technician, is estimated at 22,000-24,000 dollars and 50,000 dollars for a research worker with 3 graduate assistants and 2 technicians per year. This section recognizes the value of co-operative research association in certain sectors of the economy as well as the wisdom of encouraging research by private organizations. Criteria are then set forth for determining the national research budget, without implying any order of priority. This contemplates an allocation of some 0.5 per cent of the gross national product to the research sector or 6 per cent of the total investment budget.

The final section on international co-operation is concerned first with the Organization of African Unity and Unesco, secondly with inter-African co-operation and finally with international co-operation. The hope is expressed that Unesco and other international organizations will give technical and other support to the projects

\* United Nations Educational, Scientific and Cultural Organization, in association with the United Nations Economic Commission for Africa. *Outline of a Plan for Scientific Research and Training in Africa*. Pp. 25. (Paris: United Nations Educational, Scientific and Cultural Organization, 1964.) 1 franc; 1s. 6d. See also p. 1165 of this issue of *Nature*.



of the Scientific Commission of the Organization of African Unity. Next, it recommends that each African state should admit Africans of other national states to its institutes or training centres, and that the frequent exchange of professors and research workers should be studied and operated. In view of the shortage of institutions and of personnel, it recommends that arrangements should be made for the geographical distribution of projects among African institutions to avoid duplication of effort. Finally, it 'recommends' that Unesco, the Economic Commission for Africa and other United Nations agencies, inter-governmental and non-governmental bodies, and member states of Unesco outside Africa, should continue to expand their collaboration with African countries in scientific research and training through the provision of scientific and technical assistants.

The *naïveté* of this recommendation is in keeping with the thinly veiled nationalism of the whole Plan and the reluctance to admit the sources of assistance in the past, or the real weaknesses in the present situation which make the Plan so depressing. Fairly enough, the Conference emphasizes that international co-operation must respect the scientific independence of each country, and that action taken from outside should be based on a request from that country, and that all programmes should be related to the National Development Plan if such exists, and be co-ordinated with other efforts in this field. It is equally sound that so far as possible technical assistance of any kind should now be channelled through international agencies such as Unesco or other specialized agencies of the United Nations. Nevertheless, at a time when the financial stability and even the future of the United Nations itself are endangered, and even doubtful, it would seem prudent for the African countries not to desecrate exclusively such channels.

What matters is that these emerging and struggling scientific communities where they exist in African countries should develop their own independent and professional links with the scientific community elsewhere, as Dr. Moravcsik so wisely suggests, and that the Governments of the African countries should consider the full implications of Dr. Dedijs's far-seeing article. Science is unlikely to develop as an autonomous and growing institution in the new states, whether in this field of natural resources or elsewhere, unless the excesses of nationalism are kept, to some extent, in check, and free interchange of scientists and interflow of information are allowed in accordance with proved principles which have stood the test of time in the Western world.

That much appears to be fully recognized by M. S. Adiseshiah in the considered appreciation of this Plan which, under the title "The Planned Development of Scientific Research in Africa", he contributes to *Impact of Science on Society* (14, 137, No. 3; 1964). Apart from scientific manpower, which is the key element, he points out that it is doubtful whether the present allocation to research in Africa even reaches the present 0.2 per cent of the gross national product in India. At the outside it is unlikely that there are more than 10,000 scientists in Africa and the target of 200 scientists per million inhabitants would call for 55,000 in 1970 and 65,000 in 1980. This he regards as within the capacity of the overall educational plan for Africa, but he is emphatic as to the impossibility of carrying out effectively such a plan for the development of scientific research as was proposed at Lagos without the scientific intellectual and financial co-operation of countries outside Africa.

More especially he refers to the challenge to scientists to assist the newly established universities and institutes in Africa and those now being planned, particularly in their staffing problems, and he fully recognizes the part that bilateral scientific assistance is already playing. Here, indeed, Great Britain has special opportunities and has no reason to be ashamed of her contribution, even if its scale and effectiveness might well be increased. Furthermore, M. Adiseshiah emphasizes the need for African countries to establish their own associations of the scientific disciplines federated in the International Council of Scientific Unions and, as the Plan itself recommends, to participate in the major scientific international programmes.

But the development of a scientific community is not easily achieved, and it is sensitive to many influences which could easily halt its advance or even cause it to wither. M. Adiseshiah's doubts and reservations are expressed in the unanswered question which closes his paper: Can a five-fold increase be achieved in Africa in the next 15 years when the rhythm of the industrialized countries shows merely a doubling of scientific activity during the same life-span? Rightly he looks for the answer outside the domain of science and his paper points fairly enough to the political considerations which Dr. Dedijs has so painstakingly outlined.

Some further light on such questions is forthcoming from the further papers: that on "Priorities in Science and Technology for Developing Countries" which Prof. C. F. Powell gave to the twelfth Pugwash Conference last year and which has since been printed in *Scientific World* (No. 3, 1964); and on nuclear power in developing areas, given originally at the United Nations Conference on the Application of Science and Technology for the Benefit of the Less Developed Areas, and summarized in the same issue of the *Scientific World*, and that by T. Kristensen, "The Western Industrialized Nations and Developing Countries", in the *O.E.C.D. Observer* for December 1964. Prof. Powell, for example, reviews briefly the general nature of the problem of science and technology as an aid to developing countries, and makes some suggestions for promoting such development. He is well aware of the tendency in some countries to reject the idea of external assistance and to rely on their own resources. However, while he admits that it is important to emphasize the mutual advantages of technical assistance, he also recognizes that there are often serious political difficulties in the way of granting aid. Above all, any suggestion or implication of charity on the part of the advanced countries must be avoided, as well as the persistence of old colonial attitudes or relations through the work of technical experts. Instead a growing understanding must be promoted of the politically beneficial role of science among the Governments and peoples of the developing countries, as well as the value of science and technology to the developing countries.

Not all these difficulties are well founded, and Prof. Powell quotes W. A. Lewis's observation that in most of these countries it is the will that is lacking and not the means. What is involved is that political leaders should give priority to economic development, at least to the extent of not using the strains created by development policies to attack each other. Other issues are commonly much more exciting than economic development and also more rewarding as possible sources of political power. Quite apart from the effect of tensions arising out of the cold war hindering the proper planning and development

of aid, while it may be an immediate imperative for a new state to support and extend the work of research stations already established for its specialized crops and to develop research in relation to the improvement of agriculture as a first and essential step towards an increasing standard of living, such measures may not be politically rewarding. Education again presents problems which may be even more difficult for a developing country than for an advanced country, and where the universities are concerned this is linked up not merely with the supply of scientists and technologists but with the choice of projects. In a developing country it may be vital to choose those fields of investigation which it can pursue most advantageously, and to avoid following a pattern which may be appropriate in someone else's situation.

That is one reason for scientists in the developing countries becoming assimilated into the world community of scientists, but the mis-application and mis-direction of aid could well be radically transformed by a different climate of international relations. Here it is not only the African States who have something to contribute. Prof. Powell suggests that scientists might help by promoting frank and friendly discussions on the many political and technical problems that hinder large-scale international collaboration for aid. Certainly they could do this by helping to identify concrete problems, and by suggesting approaches to some of the technical difficulties or ways of overcoming them. Prof. Powell believes that an enthusiastic response could be won from the scientific community if it became clear that the great powers were really determined to see that our great scientific resources were employed for human welfare, and he finds evidence that some of the best leaders of the developing countries are receptive to such ideas.

This plea for creative thinking is supported by the whole trend of the paper on nuclear power in developing areas. While the possibility of producing nuclear power on an industrial scale has been demonstrated for several reactor systems, the question of installing nuclear power reactors in the developing countries with limited resources of conventional energy is now becoming one of suitable timing rather than of principle and, as such, involves exactly those considerations which Dr. Dedijer and Prof. Powell have stressed. The best possible investment of large amounts of capital depends on correct timing, and this involves a careful analysis of existing domestic energy resources, of forecasts of future demand for power, and of the costs and alternative means of meeting this demand. It must take into account the long period required to bring a nuclear power plant into operation and the importance of such a plant for the future nuclear power programme of the country concerned.

All this, and the several concrete steps which the article suggests a less-developed country would need to take before constructing a nuclear power station, demands a degree of sophistication of which the Lagos Conference gave little sign and which is unlikely to be fostered in an atmosphere of intense nationalism. The paper by Mr. Kristensen, secretary-general of the Organization for Economic Co-operation and Development, likewise points to the dispassionate atmosphere which is required if we are to contemplate seriously a *per capita* increase in production of 3 per cent a year in the developing countries. This would mean an overall growth of between 5 and 6 per cent compared with the overall growth of 3 per cent which a 2 per cent capital growth in the industrialized countries means. Even so, it would still take some 200

years for the average income levels in the developing and the industrialized countries to become comparable.

But for this to be achieved Mr. Kristensen insists that the developing countries must meet three requirements: they must have more knowledge, more capital, and wider markets for their products. Of the three, knowledge is much the most important, finding the necessary markets being related to knowledge, just as is capital, and in this last connexion he observes that it would be profitable for Western industrial firms to build a substantial part of their plant in the developing countries and export the goods to industrialized countries where wages are higher. Knowledge, however, is the critical factor since the fundamental difference between the industrialized nations and the poor countries lies in the fact that the former have at their disposal the results of modern science and education and the developing countries have not.

Knowledge is here used in the widest sense of technical skill, commercial, agricultural and industrial experience and craftsmanship, with a well-established social order, stable political leadership and experienced administration. To achieve this the developing countries have to extend the store of knowledge in height, in breadth and in depth. Essentially this is a matter of time as well as of correct choice of priorities and wise and firm policy: it may well require two or three generations to improve the educational level and it is a hard task to plan the development of an educational system so that the right sequence is followed. The Organization for Economic Co-operation and Development has already analysed and planned educational development in six Mediterranean countries for the next 15 years and these studies could well help developing countries elsewhere.

Mr. Kristensen next referred to the factors involved in the problem of helping developing countries to solve this problem during the long period before their fund of knowledge was adequate, capital flowed in of its own accord and production adjusted itself automatically to existing markets. The Organization for Economic Co-operation and Development is at present discussing measures which might be adopted to facilitate the export of private capital and to overcome the obstacles created by elements of insecurity, and Mr. Kristensen added that all the evidence indicated that public financial help would also be required to an increasing extent. On export markets he referred to the export of primary products and of industrial goods. He thought it might be possible to organize markets for primary products better and that the advanced countries could help the developing nations to achieve this. Concerning industrial goods he believed that the developing countries would be well able to compete, because of their lower wages, when they had built up their knowledge. On all counts, in the long run, technical assistance was the most constructive contribution that developed countries could make to the developing countries.

This means helping those countries to analyse their own problems and to plan their own development, and in this large task, Britain's contribution to the building up of education is the most complex. Mr. Kristensen recognizes that there are dangers because some discords are almost inevitable while the process of diffusion proceeds, alike of techniques, of science and of economics. Goodwill, mutual respect and trust are essential and without them the Western nations can scarcely provide the leadership and help that are needed. The whole *tempo* of this paper, however, indicates the opportunities as well

as the responsibilities. Certainly it should help to dispel whatever there may have been in the atmosphere of the Lagos Conference which hinders a constructive approach to the immense tasks confronting the emergent countries of Africa, or the full co-operation of scientists everywhere in assisting those countries to discharge these tasks successfully.

## MATHEMATICAL METHODS IN U.S.S.R. TECHNOLOGY

### A Course of Higher Mathematics

By V. I. Smirnov. Translated by D. E. Brown. Translation edited by I. N. Sneddon. Vol. 3, Part 1: Linear Algebra. Pp. ix+324. 63s. net. Vol. 3, Part 2: Complex Variables/Special Functions. Pp. x+702. 110s. net. Vol. 4: Integral Equations and Partial Differential Equations. Pp. xiii+814. 126s. net. Vol. 5: Integration and Functional Analysis. Pp. xiv+638. 126s. net. (International Series of Monographs in Pure and Applied Mathematics, Vols. 60, 61 and 62.) (London and New York: Pergamon Press, 1964.)

THESE volumes complete the translation of Smirnov's massive treatise, which, it must be remembered, was designed with the needs of physicists and engineers in mind. There is a hint in the author's preface to Volume 5 that a further volume on differential operators might be written some day.

One noteworthy fact is that modern algebraic theories are regarded as being as important as calculus and the theory of functions. Volume 3, Part 1, on linear algebra, is a self-contained text on determinants, linear transformations, matrices, quadratic forms and group theory. While the range is practical enough to include numerical illustrations of the reduction of quadratic forms to canonical form, it is abstract enough to deal with the elements of function spaces and Hilbert space, a pointer to the thorough and recondite work on functional analysis in Volume 5.

The classical theory of functions of a complex variable occupies Part 2 of Volume 3. Much of it will serve the young mathematician as well as the technologist, but the emphasis is, very properly, on elaborating the powerful techniques of this theory. Thus Cauchy's fundamental theorem on the integral of a regular function  $f(z)$  is proved on the assumption that  $f$  has a continuous derivative, so that Green's lemma can be used; the possibility of removing the continuity condition is mentioned, but not pursued. The simple mapping theorem is proved, but Riemann's existence theorem for the mapping of a connected domain on a circle is quoted without proof. This, of course, does not hinder a full development of the technique of conformal mapping, including the Schwarz-Christoffel transformation, and sections on applications to electricity and to hydrodynamics (where we have to recognize Blasius's theorem under Zhukovskij's name). The formalities of the residue calculus, and the theory of special functions (Legendre, Bessel, the elliptic functions) balance elegant formalism neatly against practical applications. One unusual feature is a chapter on functions of several complex variables, while one sad one is the use of  $Q_n$  for the Laguerre polynomials.

The main theme of Volume 4 is that of boundary value problems in mathematical physics. Two hundred pages on integral equations of Fredholm and Volterra type, with singular equations of the Cauchy and Fourier character, and 100 pages on the calculus of variations, with the nature of extrema, strong and weak variations, and an excursus which yields the whole of the fundamental theory of linearized elasticity on a variational basis, form a solid foundation for the study of boundary value

problems. In a long chapter on the theory of partial differential equations, characteristics are made the core of the work, and the culminating chapter deals with boundary value problems in ordinary differential equations, with the use of Green's function, and with elliptic, parabolic and hyperbolic partial differential equations, in great detail and thoroughness. Throughout this section, we are constantly brought back to physical problems in electro-magnetism, heat, elasticity. Nothing much is said about approximative and numerical methods; other well-known Russian texts deal fully with these aspects.

Up to this point, the content of this work resembles, for example, that of Sir Harold and Lady Jeffreys' *Methods of Mathematical Physics*, though the Russian book is much more voluminous. But in the final volume, the abstract disciplines may startle the theoretical physicist, and, *a fortiori*, the engineer. Set theory, the Stieltjes and the Lebesgue-Stieltjes integrals, the theory of completely additive set functions belong to quite advanced mathematics; and when this is followed by a detailed study of metric and normed spaces, and of Hilbert space, some readers are going to ask what all this is in aid of. But the general theory of operators, with the theory of functionals as a special case, can scarcely be understood without the concept of an operator as a transformation between elements in two metric or normed abstract spaces. The author obviously owes much to Sobolev's work on the applications of functional analysis to mathematical physics, but even so, this volume is the least satisfactory of the series when it comes to relating the mathematical content to physical problems. Much has to be taken for granted.

Prof. Sneddon, in his introductions to each volume, rightly indicates the high regard in which this major work is held by applied mathematicians of all kinds in the U.S.S.R. In Britain, the engineer and the technologist have, on the whole, been content to rely on the ingenuity and industry whereby they can extract the maximum amount of value out of a comparatively elementary knowledge of mathematics. How long will this suffice? Recently, a panel of mathematicians in the United States made a survey of Soviet contributions to mathematics, and drew the conclusion that the U.S.S.R. is unequalled in its ability to apply mathematical theories to practical problems. They commented on the large number of Soviet engineers who keep up with the latest mathematical developments and exploit the deepest kind of mathematics. If time spent on learning something of the rather more abstract developments of modern mathematics will mean a significant economy of time and effort at the stage of practical application, can we give the young technologist not only the time but also the taste and incentive to make such a study?

T. A. A. BROADBENT

## ELEMENTARY NUCLEAR PHYSICS

### Nuclear Physics

By Irving Kaplan. (Addison-Wesley Series in Nuclear Science and Engineering.) Pp. xiv+770. (Reading, Massachusetts, Palo Alto and London: Addison-Wesley Publishing Company, Inc., 1963, second edition.) 64s.

THERE seems to be a widespread predilection among the writers of physics books to overestimate the capacities and comprehension of the readers to whom their books are addressed. Thus one finds that many undergraduate texts are appreciated and studied in detail only at graduate level while the presentation of graduate texts often taxes the understanding of the experienced professional physicist, and theoretical treatments aimed at the experimentalist all too frequently fall short of their mark. One result of this must surely be the generation of diffidence and despondency in the average reader of such



oks, a result which one hopes is not intentional. It is a pity, therefore, to pick up a volume which is intended as an introductory text-book for the undergraduate and which looks as though it could indeed be read right through to the end with a high degree of understanding by any undergraduate of average ability willing to devote sufficient time to its 700-odd pages.

In writing his *Nuclear Physics*, Kaplan has tried to make it "elementary", "introductory" and "coherent" and seems to have succeeded to a fairly satisfactory extent on all three points. Perhaps too little mathematics is used, but wherever it occurs it is very clear and is at all times supported by sufficient discussion and description. The book is divided into three sections, and since it is intended for students who are not already familiar with quantum mechanics and atomic theory the first section is devoted to "The Background of Nuclear Physics". This provides very readable and assimilable introduction to what is generally referred to as modern physics. The second section covers the basic material of nuclear physics with emphasis in the first few chapters on the historical development of the subject. By the end of this section the reader could be familiar with much of the language of experimental nuclear physics and with a few of the more essential theoretical ideas. The final section deals with more newsworthy topics such as fission, reactors and particle accelerators.

Throughout, the book reads very smoothly and the style is quite informal. The illustrations are excellent and there are abundant references to the original literature. Quite a number of these have been carefully selected so as to be suitable for the kind of student likely to be reading the book. The problems at the end of each chapter are quite simple, mainly of the arithmetical rather than the analytical variety. Their main function will be to give the reader a feeling for the scale of things and to illustrate the kind of sums which the practising experimental nuclear physicist works out every day in his laboratory.

For those already familiar with the 1955 edition it may be stated that the present edition is not very different. It appears to be rather bulkier but this is partly the result of typographical changes. Apart from one chapter which has been dropped, that on isotope separation, the chapter headings remain the same. But new material has been added to them and all the additions are to be commended. There are two new sections on the neutrino and on the non-conservation of parity, several pages on multipole radiation from nuclei, some small additions to the chapter on nuclear reactions and a general expansion of the chapter on nuclear forces and nuclear structure. Several other all additions have been made, such as a section on controlled thermonuclear reactions, and there is slightly more emphasis on mathematical ideas. For example, in the first part of the book a simple solution of the Schrödinger equation is given and this equation is also discussed a little further in the chapter on nuclear forces. Finally, the value of the problems has been greatly enhanced by the provision of detailed answers.

The main sins of this book are sins of omission. In places it is slightly out of date, despite the revisions. For example, there is no mention of semi-conductor detectors in the section on the detection and measurement of radiation and the description in Chapter 12 of multi-channel differential pulse height selectors is obsolete. It is also regrettable, despite the remarks in the preface, that nuclear moments have not been discussed at all. However, in a book of this length and scope such cutting and trimming are inevitable and the choice is partly a matter of personal preference. It can certainly be strongly recommended for all kinds of students who have an interest in nuclear physics but who are not going to become nuclear physicists. It should be adequate for most students of chemistry, engineering, biology and applied physics and also for the scientific and technical personnel

associated with nuclear research and development. For the student who may eventually pursue research in the subject it can be recommended as a good descriptive introduction, but such a student would very quickly require further material on a more formal level.

W. M. CURRIE

## CONTROL OF NUCLEAR ENERGY

### The Atomic Adventure

*Its Political and Technical Aspects.* By Bertrand Goldschmidt. Translated from the French by Peter Beer. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies.) Pp. xii + 259. (London: Pergamon Press; New York: The Macmillan Company, 1964.) 25s. net.

THIS translation of the second and enlarged edition of Dr. Goldschmidt's book, first published in 1962, is a fitting complement to Mrs. Gowing's subsequent book, *Britain and Atomic Energy, 1939-1945*. He covers a wider canvas, but even the chapter of some 30 pages which covers the same period supplements Mrs. Gowing's account, notably in respect of the French effort. It is interesting to note that Dr. Goldschmidt shows no resentment of the American policy which forced the return home from Montreal and Chalk River of French technicians. It is, in fact, Dr. Goldschmidt's deep understanding of all that was involved, as well as his prescience and wisdom, that give such great value to this discerning account not merely of the development of nuclear energy and its applications but of the wide political and technical implications of these developments.

The first six chapters are chronological: the accounts in the first two of research in radiochemistry, leading to the discovery of fission and the development of the first atomic bombs, are followed by sympathetic accounts of the United States monopoly (1945-49); the race for the hydrogen bomb and the final years of the policy of secrecy (1949-54); the years of optimism (1954-59); and the years of adjustment (1958-62). These chapters are of especial interest for their account of the development of the French Commissariat for Atomic Energy, the industrialization of its programme and the way in which the French Government turned towards the development of nuclear weapons. The account of international collaboration and of efforts at international control, from the early Baruch Plan to the conclusion of the test-ban treaty in 1963, is clear and fair. It provides a balanced picture for the final four chapters, in which Dr. Goldschmidt discusses, successively, the renewal of confidence, nuclear weapons and disarmament, industrial development and the problem of radiation, and the future of nuclear energy.

In the first of these chapters, after describing the renewal in 1962-63 of the nuclear industry, Dr. Goldschmidt gives an interesting account of the French nuclear programme. It is perhaps natural that at this period his references to the position in Britain are not always quite up to date. The discussion of nuclear weapons and disarmament is thoroughly realistic and once again it is emphasized that real international peace is impossible without the creation of a world government or some sort of supra-national authority to which all Powers, without exception, delegate their sovereignty in the field of nuclear energy. The Moscow Test Ban Treaty is set in its proper perspective. The limitations of civil defence are discussed, and the vital importance, at this stage, of adequate nuclear co-operation in the North Atlantic Alliance is well argued.

Nor are all nuclear problems in the armament field. Turning to industrial development, Dr. Goldschmidt dis-

cusses just as realistically the problems presented by radiation and the disposal and treatment of radioactive wastes, which constitute both an additional cost in nuclear power and a physical problem of which the magnitude alone is as yet barely appreciated. To such problems and to the political problems of disarmament Dr. Goldschmidt returns in his last chapter, and here, as throughout the book, it does not need Sir John Cockcroft's preface to indicate that Dr. Goldschmidt writes with the authority of intimate knowledge and experience in the international as well as the national field. His competence is manifest on almost every page and is joined with a felicity of expression, faithfully reproduced in Mr. Peter Beer's translation, that makes for easy and even fascinating reading. The possibilities which nuclear energy has opened up for mankind are soberly and realistically appraised, as are the limitations involved and the price that has to be paid. Nothing short of general disarmament can avert the threat of mass destruction, and to enjoy the benefits of nuclear power and its concomitant advantages of radioactive isotopes man must accept the stern challenge of finding some means to limit national sovereignty and eliminate the threat of war.

R. BRIGHTMAN

## CHEMISTRY AND THE ARTS

### Analysis of Ancient Metals

By Prof. Earle R. Caley. (International Series of Monographs on Analytical Chemistry, Vol. 19.) Pp. xi+176. (London and New York: Pergamon Press, 1964.) 70s. net.

AT first sight there is no reason to believe that analytical techniques applied to archaeology and the arts should differ from the procedures of classical chemistry. However, when the peculiar requirements of the archaeologist and museum curator (such as the fact that no damage may be permitted) are considered, it becomes evident that only certain methods are applicable. Some four years ago Dr. Aitken gave us a book entitled *Physics and Archaeology*, now Prof. Caley fills in the gap admirably and considers what the analytical chemist has to offer to the world of archaeology; unfortunately he limits his book to the consideration of metals alone, and although this is an important aspect, by far the most important conclusions have been reached by the analysis of other archaeological material such as ceramics.

Prof. Caley has had much experience in the analysis of ancient metals, and this has brought him into close contact with archaeologists. In his book he points out the very real problems involved in explaining the significance of results to the non-scientist. We hear much about bridging the gap between the two cultures, but only those actually engaged in the operation can know just how far apart are the two different methods of approaching a problem, let alone the actual ignorance of each other's subject, particularly in the case of the non-scientist knowing the chemist's jargon. Prof. Caley also points out the pitfalls which the scientist must be careful to avoid; in particular the heterogeneity of ancient metals due to corrosion, surface enrichment or previous restoration is discussed in some detail.

The main portion of the book is devoted to describing the methods of analysis best suited to each type of ancient metal object—gold, silver, copper, iron and their alloys are given special attention, while the other metals known in the ancient world, in particular lead and tin, are adequately covered. Both rapid qualitative and accurate quantitative techniques are described on the macro and semi-micro scales. Major, minor and trace elements are covered. The methods described in detail are wet chemical methods, most of which require moderately large samples. It would seem that these are well suited to large metal objects where a small hole into the body of the metal

(to avoid contamination of the sample from corrosion) an unrepresentative sample from surface enrichment would not distress the museum curator. However, to a numismatist in the coin room is quite a different matter; he is particularly fussy, and even the most minute damage to a reasonably well-preserved coin is generally unacceptable. By the number of analyses quoted in his book would seem that Prof. Caley is in touch with an unusually broad-minded museum, and indeed it is entirely rational that limited damage should be permitted to such material if such studies are going to broaden knowledge as a whole.

Perhaps, however, this brings us to a point where Prof. Caley's book is a little weak. Essentially non-destructive methods are important and little space is given to them. Although considerable space is given to the somewhat dubious specific gravity method of assay of gold and silver objects, no detailed description of neutron activation X-ray fluorescence is provided. Recent advances in the techniques obviate some of the objections raised by the author against them. For example, the advent of the milliprobe X-ray spectrometer which uses a 100 fluorescent source and bent crystal geometry spectrometer means that the disadvantages of awkward shape disappear and the errors inherent in surface enrichment may be prevented by scraping a minute area. Also some objection to neutron activation and  $\gamma$ -ray spectrometry has disappeared since the advent of lithium drifted silicon detectors where the resolution of spectrometers has been improved some ten-fold. The use of emission spectrometry has, on the other hand, been given adequate space and short descriptions of the most useful methods are given.

The book ends with an excellent chapter on the preparation of reports, attention being directed again to the special problems involved in writing for non-scientists. Some indication is also given of the type of conclusion which may be drawn from analytical results. Explanations of external appearance, methods of manufacture in the ancient world, evidence of trends in economic conditions and detection of forgeries are all subjects which may be forwarded by this type of investigation.

However, one of the objects of analysis is often to trace the provenance of metals and to try and find evidence for trade between different communities. The evidence quoted in this book for such tie-ups only strengthens the belief held by many people that in the case of metals or very rarely are such archaeological conclusions possible. Because of the crudeness of the smelting operations, and the practice of remelting scrap, the composition of even closely related objects is very variable. E. T. HALL

## ADHESION AND THERMAL DEGRADATION OF HIGH POLYMERS

### Autohesion and Adhesion of High Polymers

By S. S. Voyutskii. Translated from the Russian by S. Kaganoff. Translation Editor: V. L. Vakula. (Polymer Reviews, Vol. 4.) Pp. xiv+272. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1963.) 115s.

### Thermal Degradation of Organic Polymers

By Samuel L. Madorsky. (Polymer Reviews, Vol. 4.) Pp. xiv+315. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1963.) 94s.

THE books by Prof. S. S. Voyutskii and Dr. S. L. Madorsky have certain aspects in common. In other volumes in this series, they are concerned with fields of polymer science which are in process of rapid development. Both subjects have important scientific and technological implications. Application of basic principles

polymer science has led to a better understanding of the nature of adhesion and to the development of synthetic adhesives which are often markedly superior to natural ones and with a widening range of applications. Studies of the thermal degradation of high polymers are important not only theoretically but also in relation to the development of polymers of improved heat stability, in problems of flame-proofing and in the selection of materials designed to protect others from the high temperatures encountered in space-flight. In this series of reviews authors are encouraged to speculate and to present their own views and theories. Both books therefore reflect the personal views and work of their authors to a greater extent than most review monographs.

Almost half Prof. Voyutskii's book is concerned with cohesion—coherence between two contiguous surfaces of the same material. Methods of determining the magnitude of this effect and its interpretation in terms of diffusion precede consideration of the major scientific and technological factors affecting it. Film formation from dispersions of polymers is discussed at some length and consideration of welding of thermoplastic polymers includes the effects of variations in pressure, temperature, time and thermal treatment. The remainder of the book is mainly concerned with what the author terms 'specific adhesion', that between adhesive and bonded surface. Critical consideration of methods of measurement is followed by theoretical aspects including theories of adhesion. Factors affecting adhesion are discussed and the effects of surface treatments. Methods of measurement and theories of polymer-metal adhesion are critically discussed. A final chapter deals with tackiness of polymer emulsions and their ability to give long liquid threads under certain conditions. Emphasis throughout is on interaction between adhesive and substrate and some aspects, such as examination of the forces involved in adhesion and mechanical adhesion, involving penetration of adhesive into the pores of the bonded material, receive little attention. This is deliberate, as is pointed out in an introduction. Interpretation is largely in terms of the author's diffusion theory, permitting a unified approach to the problems of bonding of polymers. Perhaps because of the time required for translation there appear to be no references later than 59.

Dr. Madorsky is largely concerned with experimental investigations of thermal degradation. He points out, at the beginning, that knowledge of molecular weight changes, the products of degradation and of the rates and activation energies of the processes concerned, are essential to full understanding of mechanisms of degradation, and describes the apparatus and methods by which such information is obtained. Thermal degradations, over a wide temperature range, of polystyrene polymers, polyolefins, fluorine- and chlorine-containing polymers, polyvinyl acetate and polyvinyl alcohol, acrylate polymers, acrylic polymers, polymers from butadiene, polyisoprenes and neoprene, polyethers, celluloses, polyamides, polyethylene terephthalate, polybenzyl and poly-*p*-xylylene, and phenolic resins are considered in successive chapters. The effects of impurities and of variations in chemical and stereochemical constitution and in molecular weight on degradation products, kinetics and energetics are early described and illustrated and possible mechanisms are critically discussed. A final summary uses the temperature corresponding to 50 per cent weight loss in 40–45 min as a measure of thermal stability and discusses structural and other factors which affect such stability. Dr. Madorsky admits that he has leaned to a large extent on results obtained by himself and his associates at the S. National Bureau of Standards. These account for roughly half the total references, some of which are to papers published in 1963 and 1964. References to relevant work by other authors seem sometimes to be absent and none of the newer polymers of relatively high thermal stability are not considered.

Omissions and restricted treatments of certain aspects in both books are generally deliberate, reflecting the opinions or inclinations of the authors. They detract little from the value of these books, both of which, within their scope, are valuable reviews of rapidly developing fields. Both contain a wealth of experimental data of great interest to polymer scientists and technologists.

W. R. MOORE

## WHAT IS PARASITOLOGY?

### General Parasitology

By V. A. Dogiel. Revised and enlarged by Yu. I. Polyanski and E. M. Kheisin. Translated by Z. Kabata. Pp. ix + 516. (Edinburgh and London: Oliver and Boyd, 1964.) 105s.

TO librarians and administrators short of time, who merely want to know whether they should purchase this book for a zoological library, my advice is to buy. It is an excellent book of its sort; it provides an introduction to the considerable Russian contribution in this field; it will remain useful for many years; by current standards it is good value.

To those concerned with parasitology, the advice is the same; but more must be said to support it, for several books in this field have appeared recently and neither time nor money nor shelf-space will stretch much further.

There seem in general to be two views about parasitology: that it is the study of parasites; that it is the study of parasitism. The first is the majority view, judging from the literature; but is usually taken to mean the study of some parasites, not of all. In one zoological library, 38 works, plus variant editions and translations, are classed under this heading. Twenty-two of them describe some of the protozoa, the trematodes, the cestodes, and some of the nematodes, as so many types in a zoological text-book. Several go so far as to include blood-sucking flies, lice and ticks, as being vectors of disease. A few others cut across this arrangement and deal with the physiology or ecology of parasites or the pathology of hosts. But thirty-one are virtually restricted to parasites that attack man and domesticated animals. If the contents of these books define parasitology, the conclusion is unavoidable that it is a professional subject related to medical and veterinary practice.

A science is expected to take account of all the evidence, not an arbitrarily selected part. Tracing the distribution of parasitism in the animal kingdom, Dogiel estimates (pp. 43–50) that 65 to 70 thousand animal species are parasites. Of these, 13,500 are protozoa and helminths (including all parasitic forms, not only those on vertebrate hosts); while 45,000 are credited to one order of insects, the Hymenoptera, usually not even mentioned in text-books of parasitology. Of course the ichneumon-wasps are not the concern of medical and veterinary practitioners, but are they not the concern of biological scientists?

Dogiel's book represents the other view of parasitology, and is about the phenomenon of parasitism as a whole. Its four main parts deal with parasitism, its distribution and origin (100 pp.); adaptations of parasites (140 pp.); the parasitic fauna and its environment (160 pp.); host-parasite relations (65 pp.). The first of these surveys the parasitic mode of life throughout the animal kingdom and, by grouping its various manifestations in categories, makes possible some interesting comparisons. This is an excellent review, and could be read with advantage by any zoologist.

In the second part, on adaptations in structure and life-cycles, the author sometimes goes too far in trying to be all-embracing. It is difficult to see what of value can be said in one page on the nervous systems, and less than a page on the excretory systems, of animals belonging to several phyla; and the attempt leads to statements that



are not acceptable. But if a few pages were better out, most of the material is excellent, especially that in Chapter 10 dealing with reproduction, life-span, and adaptations of the life-cycles of parasites to their hosts.

The third part is that for which the book is most useful, and indeed indispensable. Any work on the phenomenon of parasitism must of necessity deal with the relations between the parasite and its host. That these are ecological in nature has long been recognized by parasitologists in several countries, but the Russian workers have especially developed this approach. Dogiel follows Pavlovski in distinguishing two environments of parasites: the host, their immediate habitat, constitutes their micro-environment; but they are also affected by the external environment of their host. Much of the material and some of the ideas discussed in this part of the book will be new and illuminating to many western students.

The final part, on host relations, is less satisfactory, probably because Commonwealth and American investigators have contributed greatly to this subject, and Dogiel's account shows him to be unaware of important work published in English. The division of the bibliography into two parts, Russian and the Rest, affords a convenient survey of Russian publications, but is a nuisance in tracing references from the text.

On the whole, then, this book is strongly recommended. It has failings besides those mentioned—the parasitic insects are scurvily treated and their names are often misspelt—but it is a valiant and generally successful attempt to survey a very large field. If it became widely used in courses on parasitology there would be hope of attracting more and better students to this branch of science.

GEORGE SALT

## ORIGINS OF CRIMINALITY

### Crime and Personality

By Prof. H. J. Eysenck. (The International Series in the Behavioural Sciences.) Pp. xv+204. (London: Routledge and Kegan Paul, 1964.) 25s.

PROFESSOR EYSENCK is already well known for his important contributions to the analysis of neurotic behaviour. A tough-minded, behaviouristic approach, a certain impatience of received doctrine, and a manifest contempt for the *mystique* of psycho-analysis have earned him bitter criticism from the traditionally minded. But it would be idle to deny the fertility of his bold, simple theoretical arguments in generating hypotheses that have made their impact in several areas of psychology. In his new monograph Eysenck turns his attention to criminal behaviour—another field in which dynamic theories have long held great and rather ill-deserved prestige. The basic structure of his theory of criminality, and the style of discourse which he adopts, are familiar; one may confidently look forward to another outburst of protest. But like his earlier work it contains some incisive thinking, and will undoubtedly stimulate some interesting research in an area in which new ideas are something of a rarity.

The core of Eysenck's theory is the proposition that conformity with social rules and proscriptions is normally secured by a system of aversions which have been acquired by a process of associative conditioning during the early years of life. Childish pilfering, for example, is generally visited by punishment or some other anxiety-arousing token of parental disapproval; the association between the action (or the contemplation of it) and this unpleasant state of emotional arousal persists and is generalized to the extent that it will be revived by any situation which involves the property of others. Thus the individual exhibits a strong tendency to avoid any behaviour which he recognizes as belonging to the category

'stealing'. Such aversions are—according to Eysenck—powerful, reflexly automatic, and highly resistant to extinction. In normal people they are organized into functional system which constitutes the mechanism of what is commonly termed 'conscience'.

The conception of socialization has several important implications. It represents a rejection of the assumption long cherished in the courts, that social behaviour governed by the anticipation of satisfaction and the dread of punishment—a belief conspicuously at odds with the experience of penologists. Eysenck argues that what causes ordinary men and women to avoid crime is not fear of being apprehended (which, in view of the low detection rates for many types of offence, would in any case be irrational) but the resurgence of anxiety which accompanied similar actions in the past. The peculiar permanence of such conditioned reactions is attributed to the special circumstances in which they are acquired. Social training normally proceeds by partial reinforcement (that is, by irregular pairing of the deed and the sanction), a state of affairs known to result in most effective learning. Moreover, it has been established by experiments with animals that avoidance responses are highly resistant to extinction, presumably because they have the effect of steering the organism away from any opportunity for testing the appropriateness of the anticipatory fear-principle which has been invoked to account for the persistence of phobias and neurotic anxieties.

Why, in the case of criminality, is this mechanism ineffective? The theory proposes three complementary explanations. First, the individual may be constitutionally unresponsive to conditioning. Eysenck asserts that the speed of acquisition of conditioned responses and the extent to which they resist extinction are functions of introversion—a personality dimension which appears to be determined to a considerable degree by genetic inheritance. (Eysenck has recently defended this controversial 'conditioning postulate' on the ground that it properly applies only to situations, such as the socialization process, in which partial reinforcement obtains: here he argues, it has been amply vindicated by experiment. Persistent offenders, then, may have inherited a natural insusceptibility to conditioning. On the other hand, socialization may fail because the arrangements for training the child are inadequate—he may lack the care of parents, or he may be brought up in a disorganized household. Thirdly, Eysenck suggests (in the teeth of strong sociological evidence to the contrary) that some families actually train their children in criminal values and habits, after the manner of Fagin.

This is but the kernel of the argument; the author ranges, with characteristic panache and persuasiveness over an immense field—here discussing basic questions of scientific method, there touching on the ethics of punishment. The psychologist reader will discover little that he has not encountered in one of Eysenck's many previous books, but this one is written for the layman and for the ordinary student of criminology; they will find it an entertaining (if unrepresentative) sample of what the psychologist has to offer to the study of crime. In one respect, however, the book is a disappointment. Its discussion of penal methods is curiously sterile. The reason is not difficult to identify. Eysenck is critic of our contemporary preoccupation with environmental correlates of criminality, and naturally emphasizes its biological foundations; unfortunately in doing so he virtually ignores such knowledge as we presently possess of the social context in which crimes are committed and penal treatment—and social training itself—takes place. It is an odd omission in a monograph which will certainly be of great interest to sociologists and which, indeed, offers greater promise of integrating our fragmented understanding of the criminal than most of its predecessors in this untidy field of enquiry.

GORDON TRASLER

## MANKIND AND MACHINES\*

By PROF. M. W. THRING

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## The Engineer's Choice

NOBODY can change the past, but the engineer can change the future. The historian, the classicist, the biologist and the archaeologist observe the past, while the biologist, the modern languages scholar and the economist observe the present. The engineer has a choice about the future; he can invent, develop and design machines for the good or for the evil future of mankind. He is not yet fully convinced of this choice and of the moral responsibility that it implies, but he is gradually awakening to his true position.

In order to help him in his choice of machines to invent, it is necessary to call in the aid of those branches of understanding which admit the possibility of there being a purpose of the universe and of man's life in it—philosophy, religion and even politics. I shall take it as axiomatic at a man's most direct knowledge of all is whether he is happy or unhappy. Hence, although human happiness is excluded from the pure sciences because it cannot be measured, it provides the most important criterion of what is good for mankind. Thus the long-term objective of the engineer must be to invent machines which contribute to the increase of human happiness.

To illustrate this we can put some of the machines which have already been invented on a moral spectrum. Teaching machines, machines that help doctors to cure illness, and machines that help the blind and maimed to lead more normal lives, all appear at the top because they increase human potentialities. Machines for torture and for destruction of human life come at the bottom of the spectrum, while in between come machines for travel, communication, agriculture and all the machines that contribute to mankind having a reasonable standard of living without excessive drudgery or working very long hours. Some examples are electric power stations, the machines of the steel industry, cement or brick kilns and plastic-making machines.

These examples are clear enough, but in order to help the engineer to use the small amount of freedom of choice he has, it is necessary to look a little closer into the way in which he can contribute to human happiness in the future.

It is a prerequisite that we must avoid humanity destroying itself in a war. William James, in his *Varieties of Religious Experience*, refers to the need to find a "moral equivalent for war". I believe that the only possible equivalent is to extend the international competition which already occurs in the field of sport into competition in creative activities, especially the creation of objects of real beauty. We could have an arts Olympic every year and the daily newspapers could have two arts pages. The contribution which the engineer can make to the averting of the danger of war is primarily in the field of using his inventive ability to free mankind to spend more of its energies in such creative activities and to solve the food-shortage problems which remain.

Assuming that the averting of a catastrophic war is successful, then the real choice of the engineer lies in the fact that he can concentrate his energies on inventing free different groups of machines which take mankind in three entirely different directions.

The first direction is the logical extension of complete *laissez-faire*—a civilization in which about 75 per cent of the population are working extremely hard and extremely

long hours to give themselves ever more machines to satisfy unnecessary wants (I like to quote the examples of the machine to carry the player around the golf course and a separate car for every member of the family). The remaining 25 per cent are unable to find work and live on a permanent dole, so we can call this direction 'Devil take the ones who cannot keep up with the Joneses, or the affluent society'.

The second future is the planner's dream or the human ant-heap. Everyone is ordered to live in town or country, and towns become vast blocks of identical 'dwelling units'. Freedom to choose the size and shape of your house, the possession of a garden and how you go to work has all disappeared, but everyone is employed (at work for which aptitude tests have 'proved' them best suited) and has a uniformly high standard of living.

The third future is the only one that preserves human dignity and freedom. If mankind decides to ask the engineers to give him this third possible future, the affluent society will change into the creative society. This will be the only genuine democracy in the sense that its basic aim is to give every man and woman the greatest possible freedom to plan and choose their own lives and the education to do this, so that they obtain the greatest possible satisfaction of their creative needs. The only limitation to the freedom of one man will be that he must not unfairly limit the freedom of his neighbour. The profit motive will remain the effective spur to a man to work hard at his career, but its two curbs will be: (1) normal taxation necessitated by the need to give reasonable support to sick and old people and good education to the young; (2) the number of hours of paid work per week has an upper limit decided on a national basis in order to have employment available for everyone of working age. It is this last need which is the key to the advantage of the creative society, since it means that we can harness the routine labour-saving inventions of the engineer to give more leisure to everyone, even the housewife, instead of unemployment. Then education has the immensely exciting duty of restoring the lost satisfaction of creative craftsmanship in the leisure time. This implies a return to the old idea that schools and universities should educate for life as a whole and thus be responsible not only for training people for their careers but also to obtain a real purpose in life out of their chosen leisure activity, and to have a social conscience. Thus they will be taught that they have a duty to the community not only to work for their living but also to serve it with at least part of their leisure time either by teaching their own creative skill to others or by some kind of social service.

Since the beginning of the Industrial Revolution the number of hours in the working week has halved, and engineers can invent machines to halve it again, so that every ten years from now the hours of paid work can be reduced by two or three. Thus machines offer the possibility of replacing the six-day Biblical working week by a four-day week, and the other three days can be filled with the satisfaction of all the skills that are no longer needed for most people's work. Gardening, carpentry, wood carving, silver and copper beating, playing all kinds of musical instruments, painting and sculpture, will all cease to be hobbies of the few, and everyone will have the opportunity to become a skilled amateur at one of them. Fully employed people will have enough leisure and energy to do all the social duties such as magistracy

\* Substance of the Inaugural Lecture delivered on February 19.

and running clubs. The study of the humanities will become a possibility open to all scientists and engineers.

On this third future the maximum possible freedom is left to man, and especially to housewives, to choose the paid work they do and the creative activity for their leisure time satisfaction—the only limitation is that they cannot be paid for more than the statutory maximum number of hours work a week. Since, however, the payment will be at least twice that necessary to support an adequate standard of living for a normal family they can work only half the maximum number of hours if they wish.

I do not need to elaborate this third future here except to say that the engineer must have such a vision if he is to aim to invent those machines which will best serve humanity. Clearly neither complete *laissez-faire* nor complete anti-heap planning offers an acceptable future for mankind and some intermediate solutions along these lines must be found.

One can conclude: (1) The engineer should continue to invent machines which enable human needs to be supplied with fewer man-hours of labour and solve the world food-shortage problem. (2) He should achieve the second industrial revolution and the first domestic revolution by eliminating all routine repetitive work just as in the first Industrial Revolution he replaced human muscular effort by prime movers. (3) The engineer can help those men and women to satisfy their basic creative needs, whose work does not do so, and to satisfy everyone's need for balance between the trained activities of head, feelings and hands in the steadily increasing leisure time which his success with the first two aims provides.

### What is Engineering ?

As my first degree was not in engineering but in physics and mathematics, I dare to attempt my own definition of the work of the university-trained engineer in order to work out the future of the methods of training. This I shall do in the wider context of the engineer's role in shaping the future of mankind which I have sketched in the first section. The engineer is always concerned with inventing, designing and developing new machines that can serve mankind better. He creates the modern equivalent of the slaves and serfs of earlier civilizations. He has to learn to use his emotional brain, that is, his feelings, to enable him to make that creative or inventive leap over the wall of the existing formulation of the problems to be solved, but he has to refuse to be overawed and daunted by them or to admit his personal insufficiency in face of these. He has to use his emotions to bring his mind back over and over again to the fence until at last he finds the weak point in the apparent restrictions and leaps over into a new field.

He must understand with his mind or intellectual brain all the laws of the sciences concerned in his field in such a way that he can use them, not only to show what is impossible, but also to formulate the difficulty so clearly that the solution or way around becomes also clear. This means he must be able to use, for example, Newton's laws of motion with their gyroscopic solid body and their fluid-flow consequences, the laws of thermodynamics, chemical reaction and electrodynamics in a constructive way as tools with which to invent.

Finally, he must be able to use his knowledge of reality through his hands and eyes and body to produce designs which are sufficiently right in regard to size and strength and shape of all those parts which cannot be calculated from the equations of applied science. This the engineer calls by the mystic name 'design', but it is the use of the same body brain that the artist or musician uses as 'technique', the carpenter calls 'craftsmanship', and that we all use to walk or drive a car with when our minds are fully occupied on some other matter, more or less important.

The really creative act in engineering as in all other arts is the sudden coming together of the work of the three brains so that the emotional power generated by the persistent refusal to be defeated, the correct logical formulation of the problem through the intellect and the feeling for the way Nature works through the hand suddenly open up a new field. This experience produces an unforgettable moment of heightened awareness as memorable as those described by Kekulé in organic chemistry (benzene ring) and Hamilton in mathematics (quaternions). Sometimes the field proves fruitful in producing a valid machine of real benefit to humanity at other times snags are found later which are considerable.

This discussion of the human act of creative engineering shows how it is based on an applicable understanding of the laws and differential equations of science. I often visualize useful human knowledge as a tree with the pure sciences as its roots, mathematical and physical understanding of these laws as the trunk and engineering as the branches bearing the fruit in the form of machine useful to man's standard of living and happiness.

An equally useful analogy is to call engineering the bridge that spans the river of ignorance and prejudice that separates the academic pure scientists in their 'Ivory Tower' on the left bank from the practical men of industry with their machinery and products on the right. This provides the idea of a right-to-left traffic of problem from industry to be solved on a more fundamental level and a left-to-right flow of quite new ideas, such as power generation from nuclear fission. One can also regard applied science as the left-hand pier supporting the bridge and technology as the right-hand pier, since the applied scientist solves a wide range of problems approximately from first principles, while the technologist solves a limited range of problems accurately from empirical knowledge.

The briefest definition I can give of the engineer would want to train is "one who uses his logical understanding of the laws of science and his physical experience of solids, liquids and gases to enable him to invent develop and design new machines and products which contribute to human happiness".

### Future of Engineering Teaching

Education is the key to civilization. It is now accepted in all other countries with highly developed industries and in all spheres in Britain, except the educational one that we can only pay for our imports by exporting advanced manufactures in competition with other technologically advanced countries, if the bulk of our brightest young men and women are trained as engineers in the sense I have already defined here, as they already are in these other countries. Britain led the world at the time of the Industrial Revolution because of an upsurge of uninhibited inventive and creative engineers usually with brilliant business partners. Now that the universities have accepted the idea that engineering, and even in some cases business studies, are fit subjects to breach the wall of the 'Ivory Tower', Britain could regain her lead if the schools were enabled to turn out as strong a stream of inventive and creative engineers as that which arose spontaneously 100 years ago. This achievement has three requisites:

(1) Most boys are fully appreciative of the importance and excitement of engineering as a career up until a year or two before they take their General Certificates of Education at Ordinary Level. However, as was shown by the Oxford University Education Department Survey there is undoubtedly a tendency for the schools to steer those who have the highest marks in their science and mathematics papers into the pure science departments of the universities. While it is quite true that many of the most inventive and creative youngsters are already entering engineering schools, we must also have a bigger



proportion of those who obtain the highest marks in their Advanced Level science subjects, if Britain is to avoid the disastrous failure in the competitiveness of its exports that the whole luxury of an educational system will break down. Hence the true needs of Britain for brilliant creative engineers must penetrate to schools very quickly. I have often been accused of exaggerating when I expressed this view in the past, but the recent trend in our balance of payments is beginning to convince me the diehards that they are fiddling while Rome is burning.

(2) An equally dangerous form of snobbishness exists within the 'Ivory Tower' of the university, where engineering is still often looked on as an inferior intellectual discipline. There are various reasons for this; some of them date back to the delegation of manual tasks to slaves in the Greek and Roman civilizations and others to the upsurge of successful engineering business men from outside the universities who achieved the Industrial Revolution. The truth, in fact, is entirely the reverse, cause the engineering education requires more balanced development of all three brains than any other type of academic discipline. The engineer must learn: (a) how to understand and use the logical intellectual concepts of pure science and mathematics; (b) the emotional aspects of human relations including verbal and written communication of difficult ideas to laymen and of inventiveness; (c) the skill with the hands of the craftsman and the experimentalist. The idea that a man who works merely with his head is superior to one who works both with his head and his hands is not only nonsense but it is dangerous nonsense, since it frequently leads to nervous breakdowns and other consequences of lack of balance. I have written of the two cultures, literacy and illiteracy, as if they were isolated cultures. I believe that there are three cultures corresponding to a man's three brains: (1) literacy corresponding to human relations and hence to the emotional brain; (2) numeracy, logic, mathematics and the laws of pure science, that is, the intellectual brain which works with concepts; (3) which can be called craftsmanship, technique, experimental skill, manual dexterity, which corresponds essentially to trained skill with the body and brain, and especially with the hands. A truly educated man is a man who has trained skill in all three brains. The man who says cheerfully, "I cannot knock a nail in", is admitting just as serious a deficiency in his education as the man who says, "I cannot do mental arithmetic", or, "I cannot spell".

(3) It is often said that when the universities do train good creative engineers, industry is not sufficiently definite in its desire for them and does not give large enough rewards to them to justify the effort we have made. There have even been extreme cases of firms deliberately delaying their creative engineers to make sure that their new developments do not upset routine production in the works. This is another example of Britain's habit of not seeing the writing on the wall until her back is up against it. The more backward firms will have to copy the more enlightened ones who are constantly asking their creative engineers when the new process or project will be ready to go into manufacture.

So far as the schools are concerned, I think the most helpful suggestions would be regular lectures on careers in engineering, given to the science sixth-formers of the schools by industrial enthusiasts, university engineering professors and officials of the newly formed Council of Engineering Institutions. The introduction of prizes for essays in applied science and an Advanced Level subject called applied science which would be the logical lead to all branches of engineering when taken alongside mathematics, and thirdly, small final year projects requiring inventiveness, would all help very much.

The change of mind in industry can only be forced on by the problems of direct competition in export markets

against goods manufactured in other countries, since the habit into which many firms have fallen of paying royalties abroad for all their new developments obviously does not put them into a strong competitive position with the firms from which they are buying the developments. Industry can, of course, also help very much in the schools, by giving really interesting vacation projects to younger science masters.

Obviously my main theme in this section must be concerned with what the engineering departments of the universities can do to change this nationally disastrous trend. First and most important, they must become a much stronger bridge between the universities and industry. This implies strong support for the research projects in the engineering faculty by industry. This is particularly valuable both because it makes sure that research is carried out in potentially fruitful directions and because it gives the research students a feeling of the urgency and importance of their work. Secondly, the Continental system of professors extraordinary would help. In this system a senior industrial engineer or research manager is employed one or two days a week as a professor or lecturer in the engineering department. This system is of immense benefit, particularly to the undergraduate teaching, as it ensures a real up-to-dateness in the courses. It also makes it possible for the practical work of the undergraduates to include some really fruitful brief studies in industry.

Thirdly, the university engineering departments have a big part to play in the upgrading of the status occupied by engineering in the schools and the general public. The creation in Great Britain of the Council of Engineering Institutions with its concept of a chartered engineer will help in this direction, but we also need some method of giving greater public recognition of distinguished engineers. The American announcement on December 11, 1964, of the formation of a National Academy of Engineering shows how the United States has met this problem. Its members will be elected in recognition of: (1) important contributions to engineering theory and practice, including significant contributions to the literature of engineering; (2) demonstration of unusual accomplishments in the pioneering of new and developing fields of technology (see *Nature*, 205, 129; 1965).

The most important problem of all for university engineering teaching is how to encourage inventiveness. There is always a danger that the principles of applied science will be taught in such a way that they become 'postulates of impotence', that is, simply statements of what cannot be done instead of being aids to creative thinking. I think in this connexion it is very important to give all engineering students, very early in their university course, a free-roaming speculative talk about some of the machines which may be developed during their 40 years as practising engineers. This applied science prophecy can be based on the needs of humanity to eliminate from life dullness, drudgery, danger, disease, discomfort and fatigue. One can even go so far as to suggest that there should be some kind of Hippocratic oath for engineers that so far as possible they will direct their invention of machines towards these directions. If this introductory lecture is well enough done, much of the theory they learn later in the course can be illustrated not only in terms of existing machines but in its application to the possibility of the proposed machines.

I am sure that a definite encouragement to inventiveness in the third year of the engineering student's course is absolutely essential if we are to turn out really creative engineers. The third-year project can be made completely inventive and to involve the design and construction of the invention. In the practical class of my final year physics course at Cambridge I was given freedom to suggest and design any experiment I liked. This produced a tremendous impression on me, and I can still remember the details of three, an experimental transformer with a

variable iron core, a bench-scale model of a machine to produce a high transitory magnetic field, and an experiment on the torsion of lead strip.

Some very valuable teaching films have already been made in the United States, and my department at Sheffield produced a number of 10-min films illustrating particular fields of research which I have shown all over the world. The use of 10- or 20-min films as part of the lectures and also to give the necessary material at the beginning of a practical class can contribute greatly to the excitement and interest of the engineering students. It is our most important task to arouse this excitement in the many students who are perfectly capable of passing examinations at the present standard if it is aroused. Production of such a film has many advantages since the member of staff who makes it can put more careful production and rehearsal into it if he knows that the same film will be shown over and over again. It also means that there can be close-ups of demonstrations and pictures of industrial plant illustrating the principles so that theory and practice can be brought much closer together. The use of the films also means that the energy of the lecturers can be concentrated on the period after the showing of the film and much more time can be spent on questions and answers if the theory is given in a carefully prepared concise form. A film on an experiment can be shown 20 or 30 times a year to the students who are about to do that experiment, which would be a quite unfair repetition to ask of a lecturer.

Prof. Boris Ford, in an article in the *Sunday Times* (London) last year entitled "Why so Many Failures?", showed that certain universities have consistently higher undergraduate failure rates than others and that it is not correlated with the level of entrance standards at all but that it correlates closely with the amount of individual teaching received by the students. The optimum number in a group of students receiving such teaching is open to discussion, but the number of hours teaching required makes it difficult to have smaller groups than about three. It is my personal view that while larger groups may prove just as fruitful, the smaller grouping has an informality and therefore a freedom for the student to bring up his difficulties which is bound to disappear in the larger groupings. In any case, Prof. Ford's analysis shows that the old-fashioned view that a student was a born pass or failure or honours man is complete nonsense and that improvements to the teaching method can yield a substantially lower failure rate at the same time as the university percentage intake is increased. As a parent of children of university age, I know just what the possibility of failure means in human terms, and undoubtedly the introduction of these personal teaching schemes is a vital necessity in engineering teaching. Incidentally, it provides yet another reason why the teaching of engineering to undergraduates cannot be carried out successfully unless there is a strong postgraduate research school, since the postgraduates are the ideal people to understand the difficulties that the students are finding with their lecture material, and thus the postgraduates are the right people to do this personal teaching.

The diehards on all university senates spoke of 'scraping the barrel' when we increased our university intake from 4 to 5 per cent of an age-group in spite of the fact that all other highly civilized countries have more than 10 per cent. How an increase of intake at the extreme top end Gaussian distribution can be called scraping the barrel I have never been able to see.

#### Machines of the Future

Since I have already stated it is axiomatic that the engineer has some choice in using his talents in various directions, I think it is useful at this stage to expand a little on the kind of machines that he can develop within the next generation once he has really made up his mind

how he can best help humanity. To take two extremes he could spend the whole advanced research facilities (the world on space travel and landing a man on the Moon or the planets and getting him back again alive) or, at the other extreme, with no more engineering effort he could almost completely eliminate drudgery and food shortage from human life in the whole world. Put in this way, the best choice for the benefit of mankind is obvious and yet curiously enough the kind of money being spent in the world as a whole on advanced engineering research on the first of these two objectives is considerably greater than on the second. Let us suppose that mankind come to its senses and decides to concentrate effort on the most beneficial objective of these two. There already exists in the United States a one-armed robot which can do 200 repetitive movements handling objects weighing up to 75 lb. This machine can put its hand into a red-hot furnace and take out fired materials or place materials in special machines for various types of operation and remove them and pack them afterwards. Another machine available in the United States can move across the floor of a large room wherever the obstacles are located and scrub or clean the floor around the obstacles. I have myself made a number of simple robot hands with micro-switches enabling them to locate objects by touching or to close on to an object whatever its size.

The next stage in the development of robots which will take over the routine operations in the home or the factory is the refinement of sense impressions from these simple micro-switches giving tactile sensation into something capable of recognizing shape and position by means of light and even perhaps colour. The Americans are also developing machines in which the skill in movement of man is amplified in power and speed so that a two-legged device with a man inside can walk at 30 miles an hour across the country with legs 30 ft. long. Thus the various component parts necessary for the complete robot are nearly all available when one includes also the ability of computers to take decisions and operate according to the results of various types of signals. Within the next generation it will therefore be possible to make a robot which can have the equivalent of a logical intellect making decisions according to rules built into it and a reasonable degree of dexterity in operating its hand and self-mobility. The robot will, however, never have the emotional brain of a human both because we do not even begin to know how to develop a machine which could make value judgments, and even if we could, we would not do so since the great advantage of robots is that we can build into them logical decisions based on the best value judgments that man can make. These robots will not only eliminate completely all routine operations and drudgery from human life but also will be able to carry out dangerous operations such as putting out oil-well fires, rescuing human beings from buildings or aircraft on fire, or mending machinery at the coal face so that it will no longer be necessary for human beings to enter the mine at all.

A problem in connexion with the elimination of drudgery which is often raised is the way in which people of low average intelligence will be able to contribute in their working time. Such people often can make the best use of their leisure time by creative manual skills such as gardening, but the solution so far as their contribution to the standard of living is concerned is already suggested by the methods developed in the United States Forces for training such people to diagnose faults and repair them in standardized electronic equipment. They could in fact, be the specially trained repairers of the robots.

Another field in which the engineer can make an immense contribution in the next generation is in communication by films and education. We can already see the possibility of putting the whole of the *Encyclopædia Britannica* on to a few ounces of micro-film together with a viewer which can be carried in the pocket. Methods of

toring information even more extensive than that in the *Encyclopædia Britannica* in such a way that it can be ed in and instantaneously retrieved in verbal form can be developed. The language problem can be completely solved in a similar machine which everyone can carry when communicating with foreigners which converts his speech into some machine language and the machine language back into his speech. Thus an Englishman has only to plug his machine to a Chinaman's and they can talk freely, each receiving replies in his own language. The development of a recording tape which can be read by eye as well as being convertible to sound to the ear is also a straightforward engineering problem. I do not think, however, it will be possible to produce teaching machines which really find out what are each student's difficulties, so that it would always be necessary to have a fair amount of teaching in small group discussions with live teachers, but I think the lecture to a large audience as a means of communicating ideas and theory will be entirely replaced by a wide choice of films.

The engineer can make an immense contribution during the next generation to the world food problem by studying how to control the weather and particularly in producing rainfall in arid regions. He is unlikely to be able to do this by brute force as in H. G. Wells's 'wind machines', but he will be able to do it by harnessing the natural forces of solar energy and using the water of the oceans combined with waterproof plastics and earth-moving machinery; to put it in another way, he will learn how to make use of geographical conditions for his own purposes.

### Conclusions

(1) In addition to the two cultures, literacy and numeracy, there is a third: trained skill with the hands.

The happy man is the man who has achieved the highest development possible for him of all three cultures and uses it for real creative work. The necessity of this third culture to every man and woman is frequently underestimated.

(2) The university-trained engineer should acquire all three types of skill—skill in human relations and communication and value judgments; skill in 'physical thinking', that is, using the basic scientific principles in an inventive way; and thirdly, skill in understanding things and materials through his hands.

(3) The engineer can make a positive contribution to changing the future by inventing those machines which make the best and most fruitful changes in man's life. Particularly he can reduce drudgery and the number of hours of work a week that a man or woman has to contribute as their share to the production of all the necessities of life, so that everyone has plenty of energy and free time to satisfy their creative urge by acquiring and using all three kinds of skill.

(4) The universities have the responsibility of teaching a greatly increased flow of engineers not only to be inventive but also how to satisfy their natural altruism by applying their inventiveness in a way genuinely beneficial to mankind.

(5) If British industry is to compete successfully with other technologically advanced countries our universities must train as big a proportion of our brightest youth to be engineers as the universities and Technische Hochschule of the Continent and the United States.

(6) The extra leisure which machines can give mankind will only be profitable if the universities start now to plan for increased leisure by giving positive encouragement to the creative arts and hobbies as non-specialist, non-career subjects for enjoyment.

## MUSKEG IN ARCTIC NORTH AMERICA

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WITH growing interest on the subject of genesis and organization in organic deposits, I thought there could be advantage in examining the relationship between freshly exposed inorganic land surface and the onset of the deposition process of organic overburden. There are no records of any search having been made for muskeg (organic terrain, or peatland, as it is also known) in the Arctic Archipelago of North America where remnants of the ice-cap have long been known to exist. Accordingly, I visited the northernmost island in the Archipelago (Ellesmere) to see if in fact organic terrain did exist that far north and in proximity to retreating faces of the ice-cap. It was hoped that if muskeg did exist, its mode of origin might be used to explain some of the phenomena which characterize well-established organic terrain so prevalent at lower latitudes in North America and elsewhere in the world.

Two surveys were made, the first in 1962 during mid-summer. In this expedition, peat, with living cover, was discovered on the flatlands at the head of Goose Fjord and at Okse Bay. It was also found much more than 100 ft. up the slopes of certain branches of Goose Fjord approximately 100 yards from the face of the permanent ice-cap.

By direct inspection from a small airplane flying at low altitude, patches of organic terrain could be discerned. These were interrupted by large expanses of inorganic terrain which from time to time showed evidence of plant life but not of peat. In both the organic and

inorganic terrain, geomorphic pattern on the local basis was marked.

The second expedition, in the summer of 1963, was partly to examine the constitution of the peat and the causes of geomorphic pattern as it was associated with organic terrain. Another objective in returning was to see whether organic terrain existed as far north as land was known to extend in the Archipelago.

During this journey shallow peatland was discovered less than 100 ft. from stranded pack-ice no more than 1 ft. above sea-level on the northern coast approximately three miles from Alert. Peat deposits covered with actively growing vegetation were also found several hundred feet up the slopes of Crystal Mountain near Alert at the northern extremity of the U.S. Mountain Range. Organic terrain occurred sporadically elsewhere in the vicinity of Alert and sometimes partly covered by snow-drifts that would obviously outlast the summer.

In an aerial traverse made in low-altitude flight between Alert and Camp Hazen on the north-westerly shore of Lake Hazen, sporadic occurrence of organic terrain was again noted. In many cases, it was recognized by an interpretive method close to terminal faces of the ice-cap. It was estimated that shallow peatland occurred less than 100 ft. from the toe of Eugene Glacier. The presence of pattern ground was also detected during this aerial traverse, and pattern was more varied than it was in cases typifying conditions at Goose Fjord, Okse Bay, and elsewhere in the southern portion of Ellesmere Island.



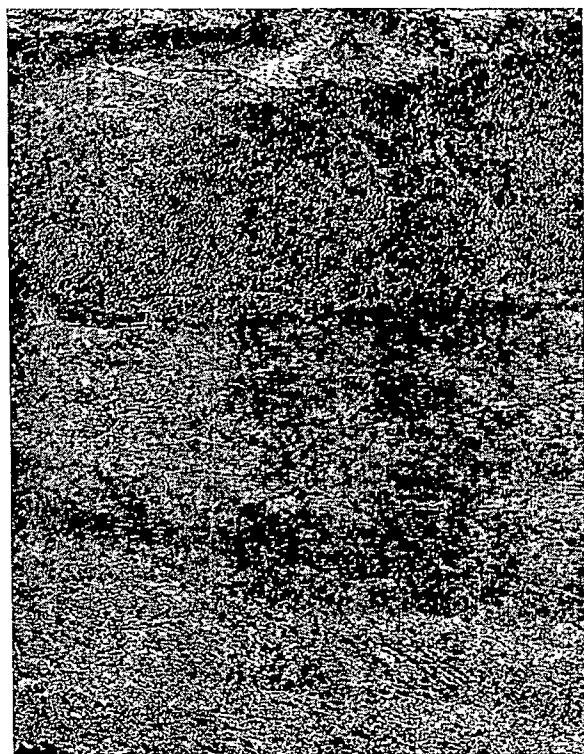


Fig. 1

Examples of organic terrain showing two different patterned conditions are presented in Figs. 1 and 2 on photographs taken in the vicinity of Camp Hazen in the foothills of the U.S. Mountain Range.

From initial examinations made of the peat samples removed from beneath the living cover of the organic terrain, it may now be claimed that pollen, cuticle, and certain plant tissues have been preserved well enough to facilitate botanical identification. The peat, sometimes 1 ft. deep, but more often shallower, differed somewhat as to structural type, but, given the type, constancy as to structure was marked. This phenomenon has already been reported in earlier work on peat types at lower latitudes on the continental mainland<sup>1</sup>.

On Ellesmere Island it was easy to examine distribution of loci of peat initiation through comparative examination on the land where colonization and initiation of peat were contemporaneous. It became clear that usually, once peat type was established, constancy as to type could be expected. Peat in different orders of depth of a single structure type was usually covered by living plants which symbolized and (by reference to habit or 'life-form') characterized peat type beneath them.

From these observations it could be deduced that the colonizers of the new land displayed persistence of biological individuality in the process of becoming established and of spreading to provide total cover for the terrain. Certainly so far as peatland is concerned it could be reasoned that in the process of establishment, succession should be viewed in terms of recurring generations of the same life-form and change from one life-form to another a marked exception. This would account for the fact that significantly large areas of organic terrain do in fact arise independently and retain their cover type even though such constitutionally different areas become contiguous.

When contiguity is in evidence and the cover types of the component areas differ, lines of demarcation appear in what has become continuous or 'unconfined' muskeg. This feature obviously makes its contribution to designation of patterned ground.

Several types of covering vegetation occurred on the peat. A method for differentiating cover types has been expressed elsewhere by me for muskeg at relatively southern latitudes<sup>1</sup>. Types of cover designated by the symbols (cover formulae) *FI*, *EI*, *EFI* and *I* were common on Ellesmere Island.

The environmental inducers of peat formation and the controls which maintain constancy and independence of plant type are still under investigation. Near the ice cap, where peat is originating and the water factor in the environment is such that there is movement of water, the cover type is *EFI*. If the water is motionless the type is *FI*. If there is no free water exposed at the site, the cover type is most commonly *EI*. On slopes and at high altitude the common cover type was *I*.

It would appear therefore that the water factor, on the basis of qualitative examination, is most significant both in the process of initiating peat type and in maintenance of type. Peat appears to form on all types of mineral foundation ranging from silty clay through silt, sand, fine and coarse gravel. Because each of these soils in special ways is often associated with characteristic and perhaps peculiar geomorphic disturbance to the type of mineral matter, the disposition of water varies accordingly. Peat type would appear to follow this dispositional factor and through it one can with reasonable safety predict association of peat type with mineral soil type, but always the latter seems secondary as an agent in controlling peat type.

If the observations and explanations noted are evaluated, one might predict that what the writer has referred to elsewhere as air-form pattern<sup>3</sup> might appear in air photographs of the Ellesmere terrain. The facts confirm that polygoid and vermiculoid air-form patterns are exemplified in Ellesmere organic terrain.

It has also been confirmed near Lake Hazen that both regular and irregular peat plateaux<sup>1</sup> exist. When these topographic features occur at lower latitudes they contribute as main features to the high-altitude air-form pattern designated as marbloid<sup>2</sup>. Local recession and overgrowth in dynamics of peat formation occur where mar-



Fig. 2

loid is characterized. Thus it may be concluded not only that the marbloid condition is generated with other air-form patterns at high latitudes and where colonization of new land surface occurs but also that a factor inherent in the organic material itself takes part in controlling rate and amount of deposition of peat.

Sometimes microtopographic features known to exist in mineral terrain also occur in organic terrain. Polygons<sup>4</sup> are a case in point. On relatively new land surface it is possible to show that given boundaries of polygons may be initiated in mineral terrain and be projected into the adjacent organic overburden (Fig. 1; note that the cleft in the mineral terrain of the hillside on the far slope is projected into the organic terrain covering the valley floor).

The way in which other pattern components arise can also be explained better where colonization of 'new' land surface anticipates muskeg formation. The 'bars' in vermiculoid may be shown to start from sorting processes arising in aggregates of mineral terrain. The sorting, promoted by combined action of ice and water activity, is followed by initiation of peat which maintains and accentuates the identity of the bars. On the other hand, it can be shown now how 'ridging' occurs within organic terrain from coalescing mounds to give 'bars' independent of influence of underlying mineral terrain.

Broader theoretical conclusions are also afforded through the Ellesmere Island investigations. Temperature as a climatic factor controlling peat and cover type is not

necessarily limiting: the constitution symbolized by FI (ref. 1) is now known to occur in a range of latitude at least 4,000 miles wide in North America. This phenomenon, when associated with independence of origin of peat types and predominance of constancy rather than successional change, goes counter to the concept of climax. Finally, lack of a lichenaceous cover type on newly forming muskeg—a condition noted on Ellesmere Island—does not necessarily signify that presence of this type in the south may not be used as an indicator of permafrost (cf. ref. 5, where I claim that lichenaceous cover signified presence of permafrost). When the peat on Ellesmere becomes deep enough, free water at the peat surface will be lacking. This condition will mark the initiation of the lichenaceous cover and EI cover type<sup>1</sup> will become HE. This is indirectly substantiated by observation in aerial traverses to the south from Ellesmere Island. 'H' (cover symbol for lichenaceous habit) comes into view significantly before continental mainland is reached.

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<sup>1</sup> Radforth, N. W., *Eng. J.*, 35, 11 (1952).

<sup>2</sup> Radforth, N. W., *Canad. Min. and Met. Bull.*, 49, 531 (1956).

<sup>3</sup> Radforth, N. W., Rep. No. DR. 124, Defence Research Board, Canada (1958).

<sup>4</sup> Washburn, A. L., *Bull. Geol. Soc. Amer.*, 67 (1956).

<sup>5</sup> Radforth, N. W., *Proc. Intern. Conf. Permafrost, Indiana* (1963).

## EXPERIMENTAL LUNG CANCER: AN ATTEMPT TO PRODUCE EPITHELIAL CHANGES IN THE RESPIRATORY TRACT OF STRAIN A/J MICE USING 3-METHYLCHOLANTHRENE

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THE polycyclic hydrocarbons have long been known to be carcinogenic and, because of their frequent presence as air pollutants, have continued to occupy the attention of investigators in the field of lung cancer. The local application of these chemicals to the respiratory passages of laboratory animals has had variable success in experimental cancer production, and the tumours produced have only rarely resembled those found in human beings. When carcinomas similar to those in human beings have resulted, the methods by which these hydrocarbons were introduced differed frequently from the normal inhalation of these substances in environmental air. The production of squamous metaplasia and epidermoid carcinomas in regions of pulmonary infarction in the lungs of rats with the polycyclic hydrocarbon 3-methylcholanthrene<sup>1</sup> is of considerable interest, since tumours of this cell type occur frequently among human lung cancers, and small pulmonary infarcts may be found incidentally during thoracic surgical procedures or at necropsy. It was the purpose of the work recorded here to determine whether this hydrocarbon might produce similar changes in the lungs of mice genetically predisposed to pulmonary adenomas but in which the factor of local damage was not present.

Forty-eight 6-week-old strain A/J mice and an equal number of control mice of strain C57BL/6J of the same age were injected in sexual pairs with either 0.4 ml. of lard or a mixture of 0.4 ml. of lard and 1.6 mg of 3-

methylcholanthrene. Twelve male and 12 female strain A/J mice and a like number of C57BL/6J mice were given the lard and methylcholanthrene mixture, and 12 males and 12 females of each strain were given the lard alone. Prior to injection, the lard was maintained at mouse body temperature (38° C)<sup>2</sup>, and the needles and syringes were heated before use in order to keep the vehicle liquid. All injections were made subcutaneously into the interscapular region. The animals were housed in standard mouse cages at an environmental temperature of 72°–74° F, fed with Staley's Rockland R4 pellets, and allowed free access to water.

Afterwards, animals were killed with ether anaesthesia at 2-week intervals from 2 to 12 weeks inclusive after the initial injections, at the same hour of the day that the injections had been given. At the time of death, the lungs, bronchi, and trachea were removed in one piece and inflated. They were placed in 10 per cent buffered formalin solution and later were embedded in paraffin wax. Serial horizontal sections were cut at intervals of 8μ through both lungs and major airways to represent a cross-section of the entire pulmonary parenchyma, trachea, bronchi, heart, and mediastinal contents. The sections were stained with haematoxylin and eosin and examined by light microscopy.

Sixteen animals were killed on each occasion, eight from each strain. These numbers were further subdivided in sexual pairs in the control and study groups.

No tumours were encountered in any of the lungs in the first 4 weeks after injection. Between the sixth and twelfth weeks, 32 animals of each strain were killed, and 25 strain *A/J* mice (11 males and 14 females) were found to have lung tumours. Twenty-three had been given 3-methylcholanthrene and lard, and two had been given the lard alone. No tumours were found in the control group of *C57BL/6J* mice. Histologically, all the tumours were adenomas, apparently of alveolar origin. Many were multiple, and all were situated in the lung parenchyma itself; squamous metaplasia or squamous cell carcinomas were not found.

The development of a tumour results from an unknown alteration in the hereditary characteristics of the cells from which it arises. Present knowledge suggests that the carcinogenic process responsible for malignant tumours occurs in two stages<sup>3</sup>. The first is the relatively rapid and irreversible mechanism of initiation during which cellular mutation takes place without recognizable histological change. This is followed by the more gradual and potentially reversible stage of promotion by which the process of malignancy becomes obvious. All known tumour promoters cause epithelial hyperplasia; but although this permits recognition of the temporal sequence of events, this hyperplasia may not be of major importance in tumour production—but only an accompaniment of it.

Although *C57BL/6J* mice have a negligible incidence of spontaneous or induced pulmonary tumours, more than 75 per cent of inbred strain *A/J* mice have been shown to have spontaneous pulmonary adenomas of alveolar origin by the age of 18 months<sup>4,5</sup>. Metastasis is occasionally found in distant organs, and the primary tumours are often multicentric<sup>6</sup>. The subcutaneous injection of polycyclic hydrocarbons has resulted in a shortening of the development time of these adenomas, which then appear in a majority of the susceptible strain within 12 weeks<sup>7</sup>. The numbers of strain *A/J* mice (48 per cent) that developed typical adenomas within 12 weeks of the subcutaneous injections of methylcholanthrene in this experiment is in keeping with these observations. In these circumstances, it seems reasonable to assume that adequate absorption of the chemical occurred in this group and that the lungs of these mice, already genetically conditioned to produce these tumours, were exposed to its action. The intravenous route of administration was avoided in order that pulmonary infarction would not occur. The only known initiating factor in the production

of these tumours was the genetic predisposition of this strain of mice to tumour development, which involved the lung parenchyma diffusely. The tumours themselves do not bear any resemblance to squamous cell epitheliomas or other human bronchogenic carcinomas.

In contrast, Stanton and Blackwell<sup>1</sup> were able to produce squamous metaplasia and squamous cell carcinomas in the lungs of rats after methylcholanthrene was given intravenously in a suitable medium. They observed that malignant lesions did not occur in the absence of pulmonary infarction. The focal damage produced was the most significant factor identified apart from the action of the hydrocarbon itself. The results of the work recorded here substantiate the efficacy of methylcholanthrene in accelerating the development of hereditary lung tumours in strain *A/J* mice. They also show that it failed to produce squamous metaplasia or squamous cell carcinomas in the lungs of these mice, even though genetic predisposition to spontaneous pulmonary adenomas existed. While allowance must be made for difference in species, it seems significant that the same carcinogen produced such changes in regions of focal damage in the lungs of rats without a known tendency to produce lung tumours. This gives indirect support to the suggestion that induced injury of this type to a non-conditioned lung is important in the production of these experimental tumours<sup>1</sup> and appears to be more significant in producing premalignant epithelial change than diffuse congenital susceptibility to tumour formation, within the conditions of the investigation. The influence of genetic factors on the cell type of a subsequent tumour remains unknown. In this work, shortening of the time of development of heritable pulmonary adenomas was not associated with any detectable alteration in the behaviour or morphological appearance of the tumours themselves, which was similar in all respects to those previously described by others<sup>5,6</sup>.

This work was supported in part by research grant CA-06872 from the U.S. National Institutes of Health, Public Health Service.

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<sup>2</sup> Feldman, W. H., Karlson, A. G., and Herrick, J. F., *Amer. J. Path.*, **33**, 1913 (1957).

<sup>3</sup> Walpole, A. L., in *Ciba Found. Symp. Carcinogenesis: Mechanisms of Action*, edit. by Wolstenholme, G. B. W., and O'Connor, Maeve (Little, Brown and Co., Boston, 1959).

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<sup>5</sup> Grady, H. B., and Stewart, H. L., *Amer. J. Path.*, **16**, 417 (1940).

<sup>6</sup> Wells, H. C., Slye, Maud, and Holmes, Harriet, F., *Cancer Res.*, **1**, 259 (1941).

<sup>7</sup> Andervont, H. B., *Pub. Health Rep.*, **54**, 512 (1939).

## OBITUARIES

### Sir George Simpson, K.C.B., F.R.S.

GEORGE CLARKE SIMPSON was born at Derby on September 2, 1878, third of the seven children of Arthur and Alice Simpson. He left school at sixteen and was for two years in his father's business. This he left for Owens College, Manchester, then one of the three colleges of Victoria University. There he sat under Arthur Schuster and Horace Lamb and graduated in 1900 with first-class honours in physics. In 1902 he was awarded an 1851 Exhibition. On Schuster's advice he went first to Göttingen, then a centre of research in atmospheric electricity, and in September 1903 to Karasjok, a village in Lapland, away from the coast, in lat. 69° 17'. He chose this in order to find how the conditions in the absence of daylight in winter and in continuous daylight in summer differed from those revealed in the temperate zone by the relatively new weapons of Ebert, Elster and Geitel, and Benndorf. The results of a year's work were given in a paper in the *Philosophical Transactions of the Royal Society*, 1905. Potential gradient in winter was double, and its diurnal variation treble, the summer values.

Radioactivity was greater than that measured anywhere previously. Both positive and negative ionization at low temperatures of -20° C were less than half their values at 0° C. These and many other results were a rich harvest.

After his return, Simpson worked for a time, without pay, in the Meteorological Office investigating the relation between the Beaufort number, *B*, for wind force and the speed of the wind, *V*, at a height of about 30 ft. above ground in an open situation. The result was, briefly,  $V = 1.87 B^{3/2}$  m.p.h. and led, twenty years later, to the international adoption of *B* for reports of wind.

In 1906 Simpson joined the India Meteorological Office. At Simla, in his spare time, he attacked the problem of the electricity of thunderstorms, a problem which was to exercise his mind for more than thirty years. He designed, and set up, apparatus to record automatically the charge on falling rain and at the same time made experiments to ascertain the charge, if any, on drops of water after splitting up. From the records for the five months, April–September 1908, he found that the charge on rain was predominantly positive, always positive on



rain falling at a rate greater than 0.5 mm/min, contrary to the generally accepted belief that the predominant charge was negative; the laboratory experiments showed that drops of distilled water, broken into spray by a jet of air, became positively charged, the air becoming negatively charged. From these results and the fact that, in thunderstorms, there are vertical currents greater than 8 m/s, that is, enough to carry the largest raindrops upwards until they split, Simpson concluded that a thundercloud must have positive electricity in its central part near its base and negative electricity higher up and away from the centre. This he maintained with firm confidence, in a succession of papers, against the protagonists of the 'influence theory'. Eventually he designed apparatus, carried by free balloons, which recorded the sign of the electric field during the ascent through thunderclouds. Ascents from Kew in 1934-6, and again in 1937-9, showed a positive charge in the upper layers, for which C. T. R. Wilson had contended, a wide distribution of negative in the lower layers with a positive charge near the active centre, as Simpson had pictured it. The upper positive charge and the negative charge beneath it were always at levels where temperature was well below the ice-point. Simpson interpreted this as meaning that the separation of the electricity there was due to collisions of ice crystals.

In 1909 Captain Scott invited Simpson to go as meteorologist with him to the Antarctic. Simpson accepted, came to England to collect the equipment and sailed from London on the *Terra Nova* in June 1910, arriving at McMurdo Sound on January 4, 1911. The ship had nearly foundered when the pumps failed in a very bad storm in December. Observations on the voyage of potential gradient, round the clock, gave for the first time its diurnal variation at sea. At Cape Evans he kept complete meteorological and magnetic records for a year until he was recalled, a year earlier than expected, owing to the illness of Dr. G. T. Walker, his chief in India. In the following seven years, first full of administrative work, then service with the army in Mesopotamia and secondment to the Indian Munitions Board, he wrote a paper demonstrating that coronae seen in the Antarctic could only have been due to liquid-water drops in the atmosphere at temperatures far below the ice-point; and a second paper showing that the spatial distribution of the amplitude and phase of the semi-diurnal variation of atmospheric pressure indicated that it was compounded of two waves, one from east to west and one from equator to pole. In 1920 Simpson was the physicist member of the Egyptian Government Commission appointed to advise on projects for the further regulation of the Nile.

At the end of that work Simpson returned to England to succeed Sir Napier Shaw as director of the Meteorological Office, a post which he held from September 6, 1920, to September 2, 1938. Control of the Office had been transferred to the Air Ministry; and the Meteorological Branches of the Admiralty and Air Ministry, and the British Rainfall Organization, had been officially included in the Office. It fell to Simpson to make the unified organization a good and satisfied scientific service. This he did surprisingly well, in spite of the 'Geddes Axe' and the fact that, like Aphrodite, the Meteorological Office sprang from the sea. The staff was largely drawn from those who had entered meteorology during the War; one of the ablest was most unfortunately lost with the airship *R 101*. By 1926 the Office was running well enough for Simpson to be able to take up again the thunderstorm problem and, after that, the balance between the retained solar radiation and the outward radiation from earth and atmosphere. By taking account of Hettner's confirmation of the 'window' in the infra-red spectrum of water-vapour, indicated in 1898 in the experiments of Rubens and Aschkinass, he was able to make close approximations to the outgoing radiation from the Earth, the troposphere and the stratosphere in different zones of latitude and

thence the horizontal transfer of heat necessary to ensure radiative equilibrium. This led him to the deduction that an increase in solar radiation would cause an increase in cloud and precipitation and possibly have a bearing on the problem of ice ages. He pursued this idea in a number of papers, arriving at the conclusion that an increase of solar radiation would cause first an extension of the polar ice and then a retreat, while the subsequent decrease of the radiation would turn the retreat into an extension followed by a second retreat.

On the outbreak of War in 1939, Simpson offered his services to his successor, Sir Nelson Johnson, who accepted his offer and put him in charge of the four Observatories and the Edinburgh Office, with his headquarters at Kew, where he was able to make further investigations of the electricity carried down by rain. He was president of the Royal Meteorological Society during 1941-42. His increasing deafness in the 1930's made him decline nomination as successor to E. van Everdingen, on the latter's retirement in 1935 from the presidency of the International Meteorological Committee in whose work he had taken and continued to take a very active part.

Simpson was elected Fellow of the Royal Society in 1915 and appointed K.C.B. in 1935. He married Dorothy, daughter of Cecil and Alice Stephen, at Sydney in September 1914. They had three sons and a daughter, all still living. He died on New Year's Day in hospital at Bristol after a short illness. The simple funeral service on January 6, with the first few moving verses of *Wisdom III*, was held at Westbury-on-Trym, where they had lived since they left London in 1961.

E. GOLD

#### Prof. A. F. Barker

PROF. A. F. BARKER died on July 22, 1964, in Portland, Victoria, Australia, at the age of ninety-six. Several obituary notices have already been published, in which tributes were paid to this remarkable man who had devoted the whole of his long life to the textile industry. He was head of the Textile Department at the Bradford Technical College from 1892 until 1914 and professor of textile industries in the University of Leeds from 1914 until his retirement in 1933. At the invitation of the Chinese Government he then took up an appointment as professor of textile industries at the University of Chaio-Tung, Shanghai, but because of the Japanese invasion of China he eventually migrated to Australia, living first in Melbourne and then in Portland, Victoria, where his address was appropriately 'Merino Cottage'.

Prof. Barker was a great enthusiast for textiles. He was very aware of the need for the industry to adopt scientific methods, and to that end he was proud to have introduced into the Department of Textile Industries in Leeds such men as the late Prof. W. T. Astbury and Prof. J. B. Speakman. He realized also the importance of a professional organization for the textile industry and was a Founder Member of the Textile Institute, which now has a membership approaching 8,000. In addition, he was active in promoting more local societies such as the Bradford Textile Society.

His death affords an opportunity to place on record the vast changes which occurred in the wool textile industry during his working life. In his early days textiles was essentially a craft industry, dominated by highly skilled men whose jealously guarded methods of processing had little to do with rational thought. Nevertheless, the products were of surprisingly good quality and British wool textiles were highly regarded throughout the world. Man-made fibres were unknown and the processing machinery had changed little over the years. Although the achievements of science were well appreciated in some industries, the potentialities of science in the wool textile industry were almost completely unrecognized. Within Prof. Barker's life-time, however, this situation changed completely. He was not himself a professional

scientist, but he recognized that the future even of the wool industry would be determined by the extent to which it used the findings of the increasing number of chemists, physicists, engineers and biologists being trained in the universities. Fortunately the wool industry has taken full advantage of the opportunity and is now based on sophisticated applied science and engineering.

Whereas fifty years ago the structure of wool was almost unknown, now, as a result largely of the work pioneered by Astbury and Speakman in Leeds and actively pursued by other groups of investigators not only in Britain but also in France, Sweden, Germany, the United States, Australia and South Africa, it is sufficiently well established to form the basis on which new and commercially significant processes have been developed and which increase the usefulness of wool. In the engineering field, processing has been rationalized and new machines such as the Raper 'Auto-leveller', the Ambler 'Superdraft' system of spinning, and the Sulzer weaving machine have been produced, while advances in biological research and animal husbandry have greatly increased the amount of wool available. The industry is progressive, modern and exciting. In Britain it is well served by an active Research Association and there are ample facilities for training personnel in technical colleges and in universities. The modern industry is based on sound science and ingenious invention and its problems are challenging ones. It offers, and will continue to offer, intellectually rewarding careers for young people with a wide variety of talents, for few industries require the services not only of scientists, technologists, engineers and economists but also of men and women with a true flair for style and design.

In a wider context a note such as this would not be complete without some mention of the fundamental scientific consequences of the researches on the structure of wool, for out of these have emerged important aspects of the new science of molecular biology. Moreover, the complexity of wool—an insoluble protein—necessitated the development of new techniques to obtain information about the sequence and proportions of the constituent amino-acids. One of these procedures, that of partition chromatography, was developed by A. J. P. Martin and R. L. M. Syngé working in the laboratories of the Wool Industries Research Association, and for this work they were awarded the 1952 Nobel Prize in Chemistry.

Until just before his death Prof. Barker retained an active interest in textile education and developments, and he must have been gratified to observe the ever-increasing significance of science in the industry which meant so much to him.

C. S. WHEWELL

#### Dr. Peter Pringsheim

AFTER a long life-time devoted to the subject of luminescence, Dr. Peter Pringsheim died on November 20, 1963.

He was born on March 19, 1881, son of Prof. Alfred Pringsheim, mathematician, of Munich, and took his doctorate there under Röntgen in 1906 with a dissertation on "The Minimum Potential for Spark Discharge". Periods of work at Göttingen, Cambridge (under J. J. Thomson) and Berlin followed, and the summer of 1914 found him in Australia with the meeting of the British Association for the Advancement of Science. Here he was interned for the period of the First World War, after which he returned to Berlin, married his Belgian wife in 1923, and was appointed full professor of the university in 1930. Three years later the Hitler régime caused him to leave Germany for a chair in the University of Brussels, which he retained until 1949. However, he was caught up with again in 1940 by the invasion of Belgium in the Second World War, and sent to internment in the south of France, but obtained liberation the following year on an American visa to carry out research at the University of California, Berkeley. His knowledge of the lumi-

nescence of uranyl compounds was doubtless the reason for his transfer to the University of Chicago, during 1942-44, and again as visiting professor during 1946-47, after an interval as director of research, Ray Control Co., Pasadena.

During 1947-55 he occupied the post of senior scientist, Argonne National Laboratory, Chicago, retiring at the age of seventy-five to Antwerp.

Pringsheim was an active research worker and an assiduous amasser of information from all sources on his chosen subject. Eighty publications, in fourteen journals, describe his investigations; the subjects include the polarization of fluorescence from vapours and dye solutions; general observations on fluorescence and photochemical changes in solutions of hydrocarbons, of dyes and of uranyl compounds and metal complexes; and luminescence and colour centres in irradiated crystals. The introductory chapter in his book *Luminescence* (1943) exhibits his interest in the ancient history of the subject together with his precise yet light touch in description.

His first book, *Fluoreszenz und Phosphoreszenz im Lichte der neueren Atom-theorie* (Springer, Berlin, 1921; third edition, 1928), was written during his time of internment in Australia, and remained for many years the only authoritative book on the subject. Important contributions were made also in Volumes 19, 21 and 23 of the *Handbuch der Physik* (Springer, Berlin). In 1943 he produced, in collaboration with Marcel Vogel, *Luminescence of Liquids and Solids* (Interscience), and finally, in 1949, his massive work, *Fluorescence and Phosphorescence* (Interscience), dedicated to James Franck. This book, containing more than 1,900 references to the literature, has been of immense value to recent workers in this subject of increasing topical interest, not only because of its completeness and reliability, but also because of the clear and critical exposition of the facts in a manner so characteristic of the author.

Pringsheim, during his life-time, was acquainted or associated with all the leading figures carrying out work on luminescence, such as James Franck, Pohl, Hertz, Jablonski, Terenin and Vavilov, and followed its transformation from vague pre-quantum ideas to present-day concepts, constantly both forward and backward looking. Without him the subject would have lacked a great teacher and adviser.

In 1937 he was elected Foreign Member of the Polish Academy. His wife survives him. E. J. BOWEN

#### Dr. A. A. Benedetti-Pichler

DR. ANTON ALEXANDER BENEDETTI-PICHLER, who died suddenly on December 10, 1964, was a pioneer in the establishment of microchemistry in the United States.

Born in Vienna, Austria, in 1894, Dr. Pichler received degrees at the Technische Hochschule in Graz, where he worked for many years with the pioneer in microchemistry, Hofrat Friedrich Emich. It was during his years with Dr. Emich that Pichler learned from him the importance of extreme attention to minute details, which showed up through all his life in his contributions to the field of microchemistry and to general analytical techniques. "During the early years," he once said, "Emich was a very exacting chief, and I did not have an easy time. Later, he relaxed when he found that I really tried to do my best."

After obtaining the *venia legendi* in analytical chemistry, Dr. Pichler decided to take up permanent residence in the United States in 1929, in order to introduce the inorganic microchemical working procedures of Dr. Emich and his co-workers there, and to raise the standards of the analytical chemist.

Mainly through the consistent efforts of Dr. Pichler in the 1930's there have been laid the solid foundations of the Microchemistry Section in the American Chemical

Society, now the Division of Analytical Chemistry. He was also instrumental in the establishment of the Metropolitan Microchemical Society, now the American Microchemical Society. Both organizations guarantee future improvement in the status of analytical chemists and permit a more scientific approach to analytical problems.

With his co-workers, Dr. Pichler laid the foundations of the techniques in handling ultra-micro amounts of samples by strictly logical and mathematical treatment of the principles established for the milligram and microgram ranges by both Emich and Pregl. Based on this fundamental work, it was possible to develop, quickly and efficiently, the chemistry of the transuranium elements which led to the Manhattan Project during the Second World War, and the atomic bomb.

British analytical chemists are familiar with Pichler's work, having listened to his brilliant plenary lecture at the Birmingham Analytical Symposium in 1958. International societies heaped numerous honours on Dr. Pichler; for example, he received the 1932 Fritz Pregl prize in microchemistry from the Academy of Sciences of Vienna; the Emich Plaque of the Austrian Society for Microchemistry in 1955; the Honor Insignia of Arts and Sciences (First Class) from the President of Austria in 1962; and the Anachem Award of the Association of Analytical Chemists in 1963.

His love for beauty in Nature took him on extensive trips to the western parts of the United States on several occasions, and influenced him not only to become a member of the National Parks Association, but also to stay in the United States.

He also decided to develop several acres of farmland near Camden, South Carolina, primarily for his wife, the daughter of an Austrian forester. While there, he became interested in conservation. With the help of the local representative of the Department of Agriculture, he was striving to restore the farm's fertility at the time of his death.

His desire for accuracy can be illustrated by his research on establishing the origin of paintings. He found it necessary to study methods of painting in oil himself so he could interpret correctly his findings of the authorship of paintings and the period of applications.

His dry sense of humour may have caused him to be misunderstood among the people who met him, but students and associates admired him for his brilliant, scholarly mind. They loved his lectures in which he logically developed his points, and the splendid demonstrations, with the simplest equipment proving the essential points of his dissertation.

His final inheritance to the world he left behind was the book *Identification of Materials, via Physical Properties, Chemical Tests and Microscopy*. It represents a summary and splendid combination of experiences during his lifetime in scientific microchemical approaches, in teaching analytical chemistry and in consulting work to large industrial concerns.

Dr. A. A. Benedetti-Pichler must be considered as one of the pioneers in microchemistry, alongside Emich, Feigl and Pregl, especially for introducing it in the United States. To his students and associates he will be remembered as the precise, modest, true scholar and beloved friend.

H. K. AND W. R. ALBER

## NEWS and VIEWS

### Organic Chemistry in the University of Leicester :

Prof. C. W. Rees

DR. C. W. REES, reader in organic chemistry at King's College London, has been appointed professor of organic chemistry in the University of Leicester. Dr. Rees was a student at University College, Southampton, from 1947 until 1953. After working under Dr. (now Professor) N. B. Chapman, he took up an appointment under Prof. A. Albert in the Department of Medical Chemistry of the Australian National University, which was then operating in London. He joined Birkbeck College as an assistant lecturer in chemistry in 1955 and became lecturer in 1956. In the following year he transferred to King's College, London, and was later promoted to a readership. Dr. Rees's research interests have centred largely on the chemistry of heterocyclic compounds with special reference to the mechanisms of their reactions. Certain aspects of his work have been supported by the Medical Research Council and by the British Empire Cancer Campaign. He has numerous publications in the *Journal of the Chemical Society* and in recent years he has been responsible for the section on Organic Reaction Mechanisms in the *Annual Reports on the Progress of Chemistry*.

### The National Institute for Research in Nuclear Science

At the Committee stage of the Science and Technology Bill in the House of Lords on February 23, Lord Bridges again raised the question of the position of the National Institute for Research in Nuclear Science under the Science Research Council. He was concerned that its relations with the Atomic Energy Research Establishment at Harwell might be affected adversely and also that, since from April 1 the laboratories of the National Institute would come under a Nuclear Physics Board, reporting to the Science Research Council which, in turn, reported to the Minister of Education and Science, the

lengthened lines of communication would lead to delay and frustration. He suggested that the Science Research Council should be sufficiently well staffed to be given very wide delegated powers to settle questions of finance and administration, including possibly authority to appoint staff. Lord Bridges was supported by Lord Sheffield and the Earl of Bessborough, and the Parliamentary Secretary to the Ministry of Technology, Lord Snow, readily gave Lord Bridges the specific assurance for which he asked, that absorption of the National Institute for Research in Nuclear Science under the Science Research Council would not be used to impose a more rigid bureaucratic system of control in finance and administration, including the appointment of staff. However, Lord Snow firmly defended the proposed arrangements, and the Minister of State for Education and Science, Lord Bowden, later added that the decision as to whether the Institute and the Atomic Energy Authority should come under the Science Research Council was only taken after very considerable debate. It was decided that the Bill allowed sufficient flexibility, if the proposal proved unworkable, to make a change by administrative action without seeking further legislative powers. On finance, Lord Snow explained that within its annual estimated provision, the National Institute had delegated powers up to £25,000 from capital expenditure and the Authority up to £100,000; above this, authorization by the Secretary of State and the Treasury was required. The Finance Member of the Authority was a member of the Board of the Institute. The Department for Scientific and Industrial Research had delegated powers up to £50,000 for capital schemes and the Government was considering the appropriate level of financial delegation for the Science Research Council. The capital expenditure of the Institute in 1963-64 was about £1.6 million, of which £1 million was on schemes of more than £100,000 and £250,000 on schemes between £50,000 and £100,000.



### Britain's Energy Budget

IN view of present controversy over the output of the coal industry, Mr. R. Bailey's article, "The Energy Perspective", in the *Westminster Bank Review* for February 1965, is of special interest. Mr. Bailey discusses the relative contributions of coal and oil to Britain's energy budget, accepting the view that the needs for energy of the non-Communist world by 1985 would be more than doubled to reach the equivalent of 8,000–9,000 million tons of coal, of which 49 per cent might be provided by oil and 18 per cent by natural gas, compared with 45 per cent and 15 per cent, respectively, to-day. Given a 4 per cent rate of growth for the economy, the total consumption of energy in the United Kingdom would rise from the 1962 level of 191 million tons of coal, 78.5 million tons of oil and natural gas, and 3.5 million tons of nuclear and hydroelectric power (or 273 million tons of coal equivalent in all) to totals of more than 300 million tons in 1966 and about 450 million tons of coal equivalent in 1980. Since it appears unlikely on its present showing that nuclear energy will make a significant addition to energy supplies at an economic price in the next 20 years, the bulk of the increase must come from the coal and oil industries. Under its present financial arrangements, the National Coal Board can break even on sales of 200 million tons a year, and marginal tonnage above this is produced very cheaply; hence the problem still remains as to where else a further 100 million tons of fuel can be obtained at £2 a ton. In searching for a policy to reconcile long-term and short-term considerations, Mr. Bailey points out that the questions remain to be answered whether the coal industry should be encouraged to expand its output to a level nearer its primary capacity and maintain it at that level as an insurance against future needs for energy. If this were accepted, how would oil, nuclear energy and natural gas be co-ordinated within this policy, and what part would the secondary fuel producers—gas and electricity—be expected to play, particularly in their selection of primary fuels? Mr. Bailey emphasizes that there are no immediate or short answers to these questions, and that, since Britain imports a quarter of her energy requirements, high priority must be given to maintaining economic production of her major indigenous fuel—coal. The secondary fuel industries of gas and electricity must not follow policies that endanger long-term supplies of the primary fuels.

### British Overseas Research

THE *Review of Colonial Research, 1940–1960*, is edited by Sir Charles Jeffries for the Department of Technical Co-operation, and Lord Hailey contributes a foreword (Pp. 238. London: H.M.S.O., 1964. 21s.). It covers the developments and achievements in colonial research under the Colonial Research Committee and, later, the Colonial Research Council until the functions of the latter Council were in 1959 transferred to the new Overseas Research Council, dissolved last year. There is also a brief review of earlier developments before the first Colonial Development and Welfare Act of 1940. The Survey is in two parts: a general record occupying about a quarter of the book, and a longer section in which various specialists provide more detailed summaries of progress in ten different broad fields—social science, economics, building and roads; health and medicine; agriculture; animal health; forestry; fisheries; plant and animal products; pesticides; locust research and control; and trypanosomiasis. The general record presents an impressive account, which deserves to be widely read, of a contribution to the welfare and development of the emerging countries, of which Britain can rightly be proud, and the details of which, as Lord Hailey observes, can be studied with advantage by those agencies whose mission it is to-day to improve the standards of life in the many underdeveloped countries.

That applies particularly to the new Ministry of Overseas Development, for the picture presented in this Survey does not indicate the extent to which the results of this expenditure of more than £24 million from 1940 onwards have been endangered by what has taken place since many of the territories achieved independence. It is not merely that interterritorial organizations have been endangered by lack of financial support; the failure of Britain to support the Overseas Research Council and its subsequent dissolution have also contributed, and while the general record and the specialist accounts indicate the opportunities and often illustrate the conditions required for success, they do not specifically refer to the recent shortcomings and failures which lie largely outside the period covered. Essentially, the Survey as a whole summarizes, and presents in most readable form, information already published in the reports of the Colonial Research Council and the specialized advisory bodies which reported with it in *Colonial Research*. There is still needed a survey of the trends and developments since 1959 which the Overseas Research Council did not, or was not allowed to, provide. For all that omission, which the Ministry of Overseas Development might well be wise to rectify in some way later, the book should appeal both to the general reader and to the specialist. It could well stimulate not only a more general appreciation of what Britain has already done in this field but also an understanding and appreciation of the value of her overseas research effort which should ensure that support is forthcoming in future on the scale and in the ways required to make that effort increasingly effective.

### Tropical Pastures Research

THE establishment of an International Tropical Grassland Commission is recommended in a resolution passed by the ninth International Grasslands Congress, held in São Paulo, Brazil, during January 7–21, 1965. In sponsoring the resolution, Prof. G. S. Puri (Kwame Nkrumah University of Science and Technology, Kumasi, Ghana) directed attention to the potential food and fodder production of the tropical grasslands. Their origin, and present status, result from shifting cultivation and fire. Although this type of land use is not ideal, it does represent the simplest means of releasing into the soil-plant system the nutrients that are locked up in plant material. Prof. Puri suggested that tropical pasture research should aim at breeding some drought-resistant perennial grasses and legumes to give material of a high leaf/stem ratio during the dry season. Physiological investigations should be directed towards prolonging the production of leafy material, and delaying flowering, in grasses and legumes, and towards reducing the rest period and activating the quick growth of leafy tissues. He pointed out that this work would involve research of a fundamental nature, to which plant physiologists in temperate parts of the world might be expected to contribute. Prof. Puri went on to advocate research into the utilization in husbanding of the tree pasturage, or woodland savannah, which covers so large an area in the tropics. He further suggested that since a satisfactory herbaceous perennial legume for tropical grasslands is not available, use should be made of some of the leguminous trees and shrubs. Tropical grasslands may be classified as 'reversible' or 'irreversible'. The former category comprises land in which trees and/or shrubs are invariably present, and which must therefore be managed on farm-forestry principles. 'Irreversible' grassland usually bears no trees, owing to shallow or waterlogged soil or other causes. They can and should be mechanized, especially as their management as natural grasslands is uneconomic. These types of grassland overlap the savannah types, and Prof. Puri urged that the savannah research section of the Food and Agriculture Organization should undertake the furtherance of this research and the establishment and maintenance of the Commission.

### Agricultural Research in Northern Nigeria

THE Institute for Agricultural Research now covers the work of the Regional Research Station of the Ministry of Agriculture of Northern Nigeria, at Samaru, together with its sub-stations at Shika, Mokwa and Kano. From October 4, 1962, the Stations became the Institute for Agricultural Research, Ahmadu Bello University, and Prof. H. S. Darling was appointed as its director. The first annual report for the year ending March 31, 1963, is now available (Pp. x + 67. Zaria, N. Nigeria: Institute for Agricultural Research and Special Services, Ahmadu Bello University, 1964. 10s.). Soil fertility and the improvement of crop yields are in the forefront of the Institute's programme, which covers crops important to the area, such as groundnuts and sorghum. Some success has been achieved in the yield of oilseed crops such as sesame, soya bean, groundnuts, sunflower and castor. Work in Bulgaria and Russia was not overlooked, and varieties of sunflower from these countries yielded seed of higher oil content than the older standard varieties. A considerable effort was devoted to the groundnut aflatoxin problem. This included experimentation with methods of drying and a study of the factors affecting contamination of the crop by *Aspergillus flavus*. It was concluded generally that seed from broken or termite-damaged pods was the main factor in contributing highly toxic groundnuts to the crop. Work on *Pennisetum* millets involved the trial of several varieties and the effect of spacing on yield and plant disease. The breeding selection and testing programme of the Cotton Breeding Section follows well-established lines. The maximum yield plot gave a yield in excess of 2,000 lb. per acre for the fifth successive year. The report is a record of excellent progress and a testimony to the good work done by the staff of the Institute.

### Applied Mathematics at the Weizmann Institute of Science, Israel

THE autumn issue of *Rehovoth* (3, No. 3; 1964), a periodical published by the Weizmann Institute of Science and Yad Chaim Weizman, Rehovoth, Israel, is a special number devoted primarily to applied mathematics, particularly to some of the work now being carried out in the Applied Mathematics Department of the Institute under the direction of Prof. Chaim I. Pekeris, who founded the Department in 1948. He contributes a stimulating article entitled "A Brief History of the Department of Applied Mathematics". This is, however, far from being a parochial account of the subject from one centre of learning; it is a fascinating, if brief, discourse of a major revolution in science: the drifting apart, after World War I, of pure mathematics, physics and applied mathematics. Fifty years ago, no distinction was made between an applied mathematician and a physicist; as examples are cited the work of Lord Rayleigh (1842-1919), a leading authority on applied mathematics, a Nobel prize-winner in physics (1904), and at the forefront of physical research all his life; also that "esoteric mathematician" David Hilbert (1862-1943), "... who was not only well versed in physics, but himself contributed to the theory of relativity and to the kinetic theory of gases"; again, Henri Poincaré (1854-1912), ast of the giant "natural philosophers", whose profound contributions to pure mathematics, physics and astronomy are well known in the histories of these disciplines. Among many original and shrewd observations made by Prof. Pekeris in this article are: "Today, pure mathematicians receive little training in physics. In some universities one can get a Ph.D. in mathematics without ever having heard of the Second Law of Thermodynamics". He goes on to say that "The chosen audience of pure mathematicians is, clearly, made up of other pure mathematicians. Similarly, physicists, preoccupied with the atomic nucleus, concern themselves with little else". Remarking that

"... there is no peace in the foreseeable future for applied mathematicians", he lists some of the major problems confronting them "... when they can sit in their offices and, with the aid of electronic computers", proceed to their solution. The schedule includes forecasting weather by numerical analysis; prediction of the tides at every point of the world oceans on the basis of tidal theory alone; explanation on the basis of pure theory of every measured atomic and molecular spectral line; determination of the crystal structure of proteins from X-ray photographs; and interpretation of the significance of every wrinkle in a seismogram in terms of the nature of the explosion (nuclear or earthquake), and the internal constitution of the Earth. "Applied Mathematics, at the Weizmann Institute, ranges from the building and designing of computers, to the study of the internal constitution of the Earth and the prediction of ocean tides". This and other articles in this well-produced and illustrated magazine convey impressively an idea of what has been done in the past, and is now being done, in the realm of applied mathematics in its modern conception.

### Administrative Organization of the Soviet Construction Industry

IT may not be generally known that the Department of Scientific and Industrial Research, through its Overseas Liaison Group at Africa House, Kingsway, London, W.C.2, publishes from time to time, for open distribution, reports from United Kingdom Scientific Attachés and Advisers on matters of primary interest to those concerned with the administrative organization of various industries in foreign countries. One such report by T. Garrett, Scientific Attaché, Moscow (U.S.S.R. 32, August 1964), entitled "Administrative Organization of the Soviet Construction Industry", describes the functions and responsibilities of the various organizations concerned, especially those of the U.S.S.R. State Building Committee. This report is most informative and gives an insight into the problems which have hitherto beset, and still do face, the building industry in the Soviet Union. "Great demands are made on the Soviet construction industry and the co-ordination of this complex and widely dispersed organization which employs one-tenth of all Soviet industrial workers is a mammoth task. Following November 1962 ... a number of administrative changes were put into effect, giving more power to the central bodies concerned with construction and relieving the local economic councils (Sovnarkhozy) of their responsibility for building operations. The aim was to reduce the growing number of unfinished and abandoned projects and the resultant waste of capital investment and also to improve co-ordination, bring about a unified technical policy in the construction industry and to make more effective use of available resources." The subject-matter of this report is dealt with under the following headings: the U.S.S.R. Council of Ministers; U.S.S.R. Supreme Economic Council (VSNKh); the State Building Committee (U.S.S.R. Gosstro); Academics of Construction and Architecture; the Scientific and Technical Council; the Main State Construction Inspectorate; Project Organizations; the Republican Councils of Ministers; Republican Gosstro; Republican Ministries of Construction; Main Construction Directorates; Contract Construction Organizations; the All-Union Capital Investments Bank (U.S.S.R. Stroi bank); Party Committees; and the Plan for Capital Construction. The Soviet construction industry, despite this policy of centralization, is thus involved in a somewhat formidable maze of bureaucratic controls which is perhaps understandable having regard to the size and population of the country; fortunately, the report contains two explanatory appendixes, one giving a schematic diagram of the inter-relationships of the various organizations previously noted as now involved in the construction industry; the other, a diagram of the structure of U.S.S.R.

Gosstroi (State Building Committee) with its Collegium (sub-committee for effective guidance and decisions on present-day problems), Functional Directorates (material-technical supply, economics, finance, mechanization, personnel, management and foreign relations) and Branch Directorates (embracing, among other functions, agricultural, chemical and oil industries construction).

### The Mauritius Institute

THE Mauritius Institute was founded in 1880, "for the purpose of promoting the general study and cultivation of various Branches and Departments of Arts, Science, Literature and Philosophy, and for the instruction and recreation of the people". The foundation stone was laid on November 23, 1880, by the Governor, Sir George Bowen, and on December 3, 1884, Sir John Pope Hennessy, then Governor, formally opened the building to the public on the occasion of the Intercolonial Exhibition. The following year the Natural History Museum, known as the Desjardins Museum, which was then managed by the Royal Society of Arts and Sciences of Mauritius (founded 1829), was transferred from the Royal College of Port Louis to the newly erected Mauritius Institute, together with the library of the Royal Society of Arts and Sciences. The Public Library was opened later, in 1902, when the rich collection of books, consisting of some 9,000 volumes, was bequeathed to the Colony by an eminent Mauritian lawyer and politician, Sir Virgile Naz (1825-1901). The Mauritius Institute is now composed of: a public library which includes lending and reference sections; a public museum, composed of natural history, arts and historical sections; the historical museum near Mahebourg; and five learned societies, which use the premises of the Institute for their meetings and for their library. The work of the Institute during 1963 is described in the annual report (Pp. 15. Port Louis: Government Printer, 1963. Rs. 1).

### Computers in Medicine and Biology

THE July 1964 part of the *Annals of the New York Academy of Sciences* (115, Art. 2. Pp. 543-1140) contains a collection of papers on the general topic: "Computers in Medicine and Biology". The contents are divided into seven principal subjects. The first part discusses digital and analogue computers and their relation to biological and medical application, and deals also with the way in which a medical data-processing centre should be organized; the second is mainly concerned with the technology of the computers themselves and describes a number of general and special purpose devices which are particularly appropriate for medical use. The last four sections of the book contain papers on computer applications in specific branches of medicine and biology; neurophysiology, psychiatry and psychophysiology, general biology and cardiology. The collection is a valuable addition to the relatively sparse literature in this field. It is well written and modern in flavour, and everyone interested either in computers or in their application in biology will wish to read it.

### The National Central Library

THE forty-eighth annual report of the Executive Committee of the National Central Library covers the year ended March 31, 1964 (Pp. 24. London: The National Central Library, 1964). Requests for loans reached 107,742 compared with 98,690 in 1962-63 and of these 78.75 per cent were satisfied; some 78 per cent of requests made by the Library abroad on behalf of British libraries were also satisfied. Total loans made by overseas libraries increased from 3,443 to 3,739—British loans abroad increasing from 2,686 to 3,488, while the number of photographic reproductions supplied increased from 603 to 630 for overseas libraries and from 936 to 1,532 for British libraries. West Germany and France were the chief lenders to British libraries, West Germany being

the principal borrower, followed by Hungary, Italy, Denmark, Poland and the U.S.S.R. University libraries supplied 21,088 loans at the request of the National Central Library and 18,049 loans were made by the Library or through its agency to University libraries. Completion of the new building is not now expected before May 19, 1965, and meanwhile the Library has been reorganized on the basis of two divisions, one dealing with inter-lending, the other with the acquisition of books and periodicals by purchase and donation and dealing with all cataloguing and the completion of the Library's master union catalogue. Work has proceeded on the preparation of the British union catalogue of periodicals on a quarterly basis with annual and quinquennial accumulations as from March 1964, and it has been agreed with the Council of the World List of Scientific Periodicals that the two publications should be merged in future. Each annual accumulation will be published in two parts: a general volume containing all periodicals listed in the previous four quarterly parts, and a scientific volume containing periodicals in science and technology. Representations were made in connexion with the Public Libraries and Museums Bill against the proposal to reduce the Government grant to the Library by 50 per cent, and the Act now makes no mention of the State grant to the Library in relation to its total income. The grant for the year ending March 31, 1964, was £95,000 compared with £82,500 for the previous year. A survey of loan requests during the six months January-June 1964 was commenced, and it is hoped that the results will be of service to the Committee on Library and to the Department of Education and Science.

### Micromechanics

SEVERAL microprocessing methods have been developed for use in microelectronics. These include electron beam and more recently laser beam drilling, both of which are thermal processes, but with different effects. An interesting comparison of the two methods of drilling is described by S. Namba and P. H. Kim in the *Japanese Journal of Applied Physics* (3, 536, September 1964). The electron beam machine consists of two chambers, both at a pressure of  $10^{-5}$  mm Hg. The electron beam chamber contains the electron gun, the electrostatic deflector plates and the focusing magnetic lenses. The beam is emitted from a tungsten hairpin filament, and the location of the beam spot on the workpiece and the intensity of the beam can be controlled either by using a television system to monitor the deflexion and intensity of the beam or by moving the workpiece by means of a digital control system. The laser machine consists of a ruby rod 15 cm long, supported inside two helical xenon flash tube enclosed within an aluminium cylinder, and cooled by compressed air. The workpiece is viewed by means of a built-in microscope at times between the laser flashes. The maximum input energy is 12,500 joules, and the laser beam is focused on to the workpiece by a lens. The workpiece carriage can be moved in three mutually perpendicular directions independently. Photographs of etched cross-sections of holes drilled by both beams in various metals, including Ta, Nb, Ni, Mo, Zr and stainless steel, are shown and discussed. Stainless steel was the easiest to process. Quartz, which is transparent to light cannot be drilled by the laser beam. The minimum spot attainable with the electron beam was about  $40\mu$ , in accordance with the theoretical value of several tens of microns, and from 0.3 to 0.6 mm for the laser beam. In thermal processing, drilled holes are generally tapered if the absorbed energy is not so large as to evaporate the material of the whole spot area at once. The holes are larger for the laser beam than for the electron beam, probably because the laser beam is not perfectly parallel and the melt is carried away over a larger area. The laser beam holes are also rarely round-shaped, usually spiky. Though the laser beam processing is simpler and easier



handle than the electron beam processing, because the former requires no evacuation, neither continuous processing nor continuous output of high energy density is possible. The laser beam is, however, better than the electron beam in power efficiency, as estimated by the amount of material evaporated.

### Rubberized Surfacing Materials for Roads

It has long been known that the rheological properties of tar and bitumen are appreciably altered when small proportions of natural rubber are added. Extensive investigations at the Road Research Laboratory into the properties of rubberized binders have been carried out in co-operation with the Natural Rubber Producers' Research Association and the Natural Rubber Bureau, and a series of full-scale road experiments have been initiated during the past ten years at various places in Britain. The results of these experiments up to May 1963 are now available in *Road Research Laboratory Technical Paper No. 71*, by P. D. Thompson (*Full-Scale Road Experiments Using Rubberized Surfacing Materials, 1953-1963*. Pp. iv+24. London: H.M.S.O., 1964. 3s. net). The types of 'black top' surfacing concerned in these experiments are mastic asphalt, rolled asphalt, bitumen macadam, dense tar surfacing, tar surface dressing, and bitumen surface dressing. A summary of the essential results of these road experiments may be stated as follows: (1) Addition of rubber to mastic asphalt prevents or markedly reduces the cracking which occurs when a bituminous surfacing is laid over joints or cracks in a concrete base; (2) addition of 10 per cent of rubber to the binder of rolled asphalt reduces the number and severity of cracks in this surfacing material when laid over joints and cracks in a concrete base; (3) a substantial increase in life is obtained when 4 per cent of powdered rubber is added to the binder of bitumen macadam; (4) "a limited full-scale experiment has suggested that the life of dense tar surfacing may possibly be increased by the addition of rubber powder to the binder"; (5) "in one surface-dressing experiment, a commercially available rubber-tar showed no improvement over normal tar. Interim results of a second experiment suggest that some advantage may be gained by the use of a graft rubber-tar in the tar surface dressing"; (6) "a considerable reduction in the tendency of bitumen surface dressings to 'fat up' in hot weather under heavy traffic has been shown when rubber has been blended with the binder". In so far as the results of these experiments may be indicative at this stage, it would appear that the greatest advantages of rubberized binders are realized when asphaltic bitumen is the major adhesive. The experiment with dense tar surfacing seems inconclusive. In the case of tar surface dressing, a second full-scale experiment has recently been started further to assess the possibilities when graft-rubber is added to the binder.

### Bibliography of Cold Weather Concreting

THE subject of the many and varied technical problems raised in concrete construction during the winter months has for years past been one of major concern in the building and engineering industries; few indeed will readily forget the disastrous conditions occasioned by the severity and duration of the freezing temperatures in Britain in the winter of 1962-63. The subject, however, is one still receiving considerable attention, not only from cement and concrete technologists, but also in its economic and practical significance; this interest is international, at least in temperate climates. The Cement and Concrete Association, London, has recently produced a bibliography covering articles, papers and books dealing with the subject of cold weather concreting practice, the use of ready-mixed concrete under these conditions, and the rearing of aggregates (*Bibliography of Cold Weather Concreting*. Pp. 29. (Ref. Ch.48. 10/64.) Pp. 29.

London: Cement and Concrete Association, 1964. 5s.). This useful document lists nearly 400 references to this subject in international literature, ranging in time from early 1964 back to 1912. This in itself is sufficient evidence of the undiminished importance of the problem and, perhaps, of the still formidable technical obstacles to be overcome before a final and universally acceptable solution is reached (if ever). To those studying cold weather concreting in all its phases, likewise all concerned with the design and construction of buildings, bridges, roads, runways, involving particularly *in situ* concreting under arctic conditions, this bibliography is bound to prove of great value.

### Respiratory Function and Failure

NORMAL respiratory function requires adequate ventilation of alveoli, proper exchange of oxygen and carbon dioxide between air and blood by diffusion, and adequate transport of blood to and from the lungs via the pulmonary circulation. Respiratory failure can occur through dysfunction of any one of these three processes or of two or more in combination. To understand why such failure has occurred and to treat it correctly, measurements of each physiological process may be required. Accurate and easily performed quantitative tests are available and may be used when necessary to supplement information obtained by radiography, bronchoscopy and routine laboratory procedures. Pulmonary function and the tests for respiratory failure are described in the *Central African Journal of Medicine* (10, No. 10; October 1964) by Dr. A. J. P. Graham, who has also advocated their use at the bedside or in the consulting room. A later article will be devoted to describing the clinical picture, and discussing the management of respiratory failure in the acute and chronic stages.

### The Ornamental Sea-toad

A PHOTOGRAPH showing the carapace of the ornamental sea-toad, *Schizophrys dama*, a spider-crab from Broome, north-western Australia, appears in *Australian Natural History* (14, No. 11). Only one other illustration, now difficult to obtain, of this distinctive crab has appeared since the first hand-coloured plate was published in Berlin in 1804 by the German naturalist, Joann Friedrich Herbst, with his original description of the animal. *S. dama* is found inter-tidally and in shallow water from the Albany area of south-western Australia, through western and northern Australia to northern Queensland, and, during life, the carapace is often partly concealed under a cover of sponge and other marine growths. Two thorn-like spines project laterally from each of the elongate rostral horns at the front of the carapace. This feature serves to distinguish *S. dama* from the closely allied red sea-toad, *Schizophrys aspera*, which has only one lateral spine to each rostral horn. *S. aspera* is found in a similar habitat through southern, western and northern Australia and along the entire Queensland coast and Barrier Reef area. It has not yet been recorded from New South Wales or Victoria.

### University News:

#### Bangor

DR. W. L. WILCOCK, at present reader in instrument technology at the Imperial College of Science and Technology, London, has been appointed to the recently instituted additional chair in the Department of Physics, with effect from October 1.

#### Liverpool

DR. A. YOUNG, at present reader in numerical analysis and director of the Computer Laboratory, has been appointed to the newly established chair of numerical analysis. Dr. N. M. Hancox, at present reader in histology, has been appointed to the newly established chair of histology. The following lecturers have also been appointed: Dr. J. V. Allaby, Dr. K. Bearpark, Mr. L. J.

Carroll, Dr. P. D. Forsyth and Mr. M. F. Thomas (physics); Dr. T. N. Calvey (pharmacology); Mr. T. L. J. Lawrence (animal husbandry).

#### Newcastle upon Tyne

PROF. W. F. K. WYNNE-JONES, head of the School of Chemistry, has been appointed pro-vice-chancellor to hold office until September 30, 1968. Prof. A. G. R. Lowden, Dean of medicine, has been appointed pro-vice-chancellor to hold office so long as he remains Dean of medicine. The following appointments have also been made: *Senior Lectureship*, Dr. D. C. Turk (microbiology). *Lectureships*, Dr. B. E. Johnson and Dr. J. S. Rose (mathematics); Dr. P. Grieseson (metallurgy).

#### Basic Plant Science at Michigan State University

THE new Plant Research Laboratory established at Michigan State University in conjunction with the Atomic Energy Commission has initiated its research programmes and is inviting applications for graduate study leading to a Ph.D. degree and for postdoctoral research associateships in various areas of basic plant science. Graduate degrees will be awarded through the appropriate academic department of the University. The Laboratory, under the direction of Prof. Anton Lang, is at present housed in adequate temporary quarters and will move into its own building in the spring or summer of 1966. By the autumn of 1965, research will be in progress in the following areas: function and mechanism of action of growth hormones, including hormonal control of enzyme activity; growth regulation through regulation of enzyme activity; cell wall metabolism; tissue culture; metabolic foundations of the control of plant growth by environmental factors; ageing of plant cells. Additional research areas will be available as the permanent research faculty is expanded. Further information concerning the programme of the Laboratory can be obtained from Dr. Lloyd G. Wilson, assistant to the director, MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48823. Applications for admission to graduate study should be addressed to the Director of Admissions, Michigan State University, East Lansing, Michigan.

#### The Night Sky in April

NEW MOON occurs on April 2d 00h U.T. and full Moon occurs on April 15d 23h. The following conjunctions with the Moon occur: April 5d 06h, Jupiter  $1^{\circ}$  N.; April 12d 12h, Mars  $2^{\circ}$  S.; April 27d 03h, Saturn  $4^{\circ}$  N. Mercury and Venus are not favourably placed for observation during the month. Mars sets at 5h 00m, 4h 05m, 3h 05m on April 1, 16 and 30, respectively, its stellar magnitude is  $-0.3$ ; its distance from the Earth on April 15 is 72 million miles and is in Leo. Jupiter sets at 22h 40m, 22h 00m, 21h 20m at the beginning, middle and end of the month, respectively; its stellar magnitude is  $-1.6$ ; its distance from the Earth on April 15 is 543 million miles and is in Taurus. Saturn rises at 5h 15m, 4h 10m, 3h 10m at the beginning, middle and end of the month, respectively; its stellar magnitude is  $+1.3$ ; its distance from the Earth on April 15 is 971 million miles and is in Aquarius. The following occultations of stars brighter than magnitude 6 occur, observation being made at Greenwich: April 5d 19h 48.8m, 43 Tau (*D*); April 7d 20h 22.6m, 5 Gem (*D*). *D* refers to disappearance.

#### Announcements

At a meeting of the Royal Microscopical Society, Dr. J. Baker announced that His Royal Highness the Duke of Edinburgh had accepted the office of president for the Royal Charter Centenary Year 1966. Mr. J. Bunyan is chairman of the Centenary Committee.

THE Institution of Chemical Engineers is to institute a medal in honour of George E. Davis, a pioneer in chemical

engineering and author of the first text-book on the subject. Dr. A. J. V. Underwood is to be the first to receive the award, which will be presented in London in the autumn.

PROF. N. N. GREENWOOD, head of the Department of Inorganic Chemistry in the University of Newcastle upon Tyne, has been appointed a member of the Panel on the Application of the Mössbauer Effect in Chemistry and Solid State Physics of the International Atomic Energy Agency (Vienna).

THE annual conference of the Ergonomics Research Society will be held at University College, Dublin, during April 5-9. Further information can be obtained from J. Spencer, 8-10 Berkeley Square, Bristol 8.

AN international symposium on "Scientific Aspects of Snow and Ice Avalanches" will be held in Davos during April 5-10. Further information can be obtained from the Swiss Federal Institute for Snow and Avalanche Research, 7260 Weissfluhjoch/Davos.

A CONFERENCE on "Industrial Diamonds in Metal-working", sponsored by the Haus der Technik, Essen, and De Beers Industrial Diamond Division, will be held in Essen on April 7. Further information can be obtained from the Haus der Technik, P.O. Box 668, Hollestr. 1a, Essen 43.

A MEETING of the Physical Biochemistry Group of the British Biophysical Society on "The Physical Chemistry of Separation Methods on Support Media" will be held in Cambridge on April 7. Further information can be obtained from Dr. P. Johnson, Colloid Science Laboratory, Free School Lane, Cambridge.

A CONFERENCE on "Elementary Particles", arranged by the Institute of Physics and the Physical Society, will be held in the University of Birmingham during April 5-7. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A MEETING of the Basic Science Section of the British Ceramic Society on "Mechanical Properties of Non-metallic Crystals and Polycrystals" will be held in Birmingham during April 5-7. Further information can be obtained from Dr. J. P. Roberts, Houldsworth School of Applied Science, the University, Leeds 2.

A CONFERENCE on "Phenomena in the Neighborhood of Critical Points", sponsored by the U.S. National Bureau of Standards aided by a grant from the U.S. National Science Foundation, will be held at the Bureau during April 5-9. Further information can be obtained from Dr. M. S. Green, Statistical Physics Section, National Bureau of Standards, Washington, D.C.

A SYMPOSIUM on "Vibration in Civil Engineering", arranged by the British National Section of the International Association for Earthquake Engineering, will be held in the Imperial College of Science and Technology, London, during April 5-7. Further information can be obtained from the Secretary, British Section of the International Association of Earthquake Engineering, Institution of Civil Engineering, Great George Street, London, S.W.1.

A DEMONSTRATION meeting concerned with "Methods in Taxonomy" will be held by the Systematics Association at the British Museum (Natural History) on March 31. The meeting will consist of about fifty exhibits of methods and techniques used in taxonomy, arranged under the following headings: collecting; preparation and maintenance; examination and extraction of information; analysis and synthesis; dissemination of results. Further information can be obtained from W. G. Fry, British Museum (Natural History), Cromwell Road, London, S.W.7.

## ORGANIZATION OF RESEARCH AND TRAINING IN AFRICA

THE international conference on the "Organization of Research and Training in Africa in Relation to the Study, Conservation and Utilization of Natural Resources" was convened by the Director-General of the United Nations Educational, Scientific and Cultural Organization in accordance with a resolution adopted by the General Conference of that Organization, and was held at Lagos during July 28–August 6, 1964. The final report of the conference, now issued by Unesco\* in association with the United Nations Economic Commission for Africa, has chapters on national scientific policies from the point of view of research on natural resources; on the preparation and implementation of policies of research on natural resources; on scientific and technical personnel; on finance and research economics; on international co-operation in research on natural resources. It also includes the texts of speeches made at the opening and the closing of the conference. The recommendations adopted are given at the end of each chapter, and the outline of a plan for scientific research and training in Africa, which was agreed in a recommendation, is issued separately and discussed on p. 1141 of this issue of *Nature*.

The factual chapter on national scientific policies is probably the most realistic, giving concise summaries of the position in the 28 countries reporting. Appended to this chapter there is a brief statement of the imperatives of national science policy. These are listed as: realization by African Governments of the need to establish short- and long-term economic development programmes; recognition of the need to establish scientific research and industrial research programmes, both short-term and long-term; of their responsibility for organizing scientific research and encouraging research by creating a favourable atmosphere; recognition by scientists of their responsibilities towards their own countries; respect for

academic freedom and the right to free choice of methods and techniques; recognition of the need to establish a proper balance between fundamental and applied research; scientific collaboration at both regional and continental levels; establishment at the highest level of a body responsible for elaborating the national scientific policy and co-ordinating research activities; recognition of the need for study and research on natural resources and co-ordination of the activities of the bodies responsible; the necessity of training a sufficient number of research personnel as rapidly as possible and of the essential role of the universities in this respect; recognition of the need to include in all national budgets special provision for scientific and technical research.

The chapter on scientific and technical personnel estimates that the present total of research workers in Africa in natural resources may be some 2,280, but even a total of 5,000 would not give a ratio of research workers for the total population of more than 1 : 50,000. For the period 1965–80 a ratio of 200 scientific personnel and university science teachers per million inhabitants is suggested. In this total, experienced postgraduates, university graduates and technicians would be represented in ratios of 1 : 3 : 2.

The chapter on international co-operation recognizes that Africa is already receiving considerable assistance in this field from various Governments as well as from the United Nations, the Economic Commission for Africa and the specialized agencies, and it will be necessary to continue to strengthen and co-ordinate these efforts. It also admits that there are gaps in the present scientific structure, insufficient research workers and teachers, and inadequate financial resources, and that by 1980 some 50,000–70,000 research workers will be required from some source or other. Nevertheless, there is little appreciation of what has been done in the past in this field for Africa by individual Governments, or of the importance of ensuring conditions that will facilitate free international co-operation and interchange in any field of scientific endeavour.

\* Final Report of the Lagos Conference. (International Conference on the Organization of Research and Training in Africa in Relation to the Study, Conservation and Utilization of Natural Resources, Lagos, Nigeria, July 28 to August 6, 1964.) Pp. 102. (Paris: United Nations Educational, Scientific and Cultural Organization, 1964.) 3 francs; 4s.

## THE SCIENCE AND TECHNOLOGY BILL

THE debate in the House of Lords on February 4 on the Science and Technology Bill was of interest chiefly for some further information given by Lord Champion in moving the second reading. The Science Research Council would take over the present responsibilities of the Department of Scientific and Industrial Research for research grants to universities and postgraduate training awards, including grants for support of applied as well as pure science. Lord Champion said that the chairmanship of this Council, unlike that of the Agricultural and Medical Research Councils, would be held by a scientist as a full-time post.

Turning to the Natural Environment Research Council, Lord Champion stated that Sir Graham Sutton had accepted the invitation of the Secretary of State for Education and Science to be chairman of the new Research Council. However, Sir Graham would continue to devote most of his time to his responsibilities as director-general of the Meteorological Office, and a secretary of the new

Research Council would be appointed who would be a scientist holding appropriate qualifications. The Natural Environment Research Council would not be formally constituted until June, but the Science Research Council, for which the nucleus of a staff already exists in the Department of Scientific and Industrial Research, would be formed on April 1. Meanwhile, the activities of the component organizations under the Natural Environment Research Council would continue without interruptions, and on the dissolution of the Department of Scientific and Industrial Research the responsibility for making research grants to universities and postgraduate training awards in the field of interest to the Natural Environment Research Council would be taken over temporarily by the Science Research Council. Responsibility for the Geological Survey and Museum would be taken over temporarily by the Department of Education and Science.

Lord Champion went on to point out that responsibility for the Atomic Energy Authority had already, by Order



in Council, been taken over by the Minister of Technology. Under Clause 4 of the Bill the Authority would be able to extend its research functions outside the field of nuclear energy if the Minister of Technology so desired. This would enable the resources of skill, expertise and facilities in the Authority to be made available to support the Ministry in stimulating technological advance in industry if opportunity offered. Viscount Caldecote later suggested that it would be more appropriate to run down any strength not required in the Authority and transfer such highly qualified staff elsewhere in industry.

Lord Champion announced that an Order in Council was being laid that day bringing the National Research Development Corporation within the responsibility of the Minister of Technology, and it was hoped to introduce a Bill shortly which would increase the effectiveness of the Corporation. He said that a review was under way to establish the feasibility of orientating purchases and contracts in directions which would aid technological progress, including consideration of purchases made by the authorities directly and indirectly financed from public funds. The Minister of Technology was setting up

several technical appraisal teams of scientists, engineers and economists from within and outside the Government service to investigate the performance and products of sectors of industry, to identify any technological weaknesses, and to consider the ways in which these might be remedied. Other methods contemplated were the intensified placing of development contracts with industry, possibly under the supervision of the appropriate research station; steps to improve the supply and status of engineers; action to ensure that in the engineering field the British Standards Institution made the maximum contribution to the objectives of the Ministry. A suggestion from Lord Brown that the universities were themselves mainly responsible for the shortage of technologists and engineers and for failure to expand in this respect was stoutly refuted by Lord Chorley. Lord Snow, agreeing as to the importance of the status of engineers, added that he believed that the use of engineers could be radically improved by constructive action and by attempting to bring into other parts of industry not used to employing professional skill, the kinds of skill which were already used in the advanced industries.

## THE WELLCOME FOUNDATION, LTD.

IT was in 1880 that H. S. Wellcome, an American citizen by birth, came to England to partner S. M. Burroughs, and later to form Burroughs Wellcome and Company. On the death of Burroughs, Wellcome assumed sole control of the Company. His flair for commerce (he pioneered large-scale manufacture of tablets, with scientifically precise dosage, under the famous trade-mark 'Tabloid'); his drive, his profound belief in research, and his philanthropic outlook, these qualities combined to make possible the Wellcome Foundation and, later under the terms of his will, the Wellcome Trust, "... which applies the profits it receives to the advancement of medical and allied sciences and to the establishment or endowment of research museums and libraries throughout the world". The story of this Foundation, unique alike in character and structure, is well told and lavishly illustrated (much in colour) in a new publication\*. It is a document of considerable scientific interest and is well worth reading, especially by those concerned with prevention, control and cure of disease.

Although its headquarters are still in the familiar building in Euston Road, London, the ramifications of the Wellcome Foundation, through its subsidiaries and associated companies, Burroughs Wellcome International, Ltd., and Cooper, McDougall and Robertson, Ltd., are world wide. The chief operating centres are: Canada, Montreal; United States, New York and Chicago; South America, Rio de Janeiro, São Paulo and Buenos Aires; Europe, Paris, Brussels, Rome, Porirua; Africa, Nairobi, Salisbury and Johannesburg; Asia, Karachi and Bombay; Australia, Sydney; and New Zealand, Auckland. Prominence is given in this brochure to the Research Laboratories in the United Kingdom at Beckenham, Kent (Langley Court); to the Chemical Works at Dartford, Kent; and to the Veterinary Research Station at Frant, Kent. Many of the products of Cooper, McDougall and Robertson, Ltd., acquired by the Foundation in 1959, are manufactured in the Kelvindale factory, Glasgow, but the Cooper Technical Bureau is maintained at Berkhamsted, Hertfordshire, wherein is conducted research on insecticides, parasitic and bacterial diseases of animals, animal nutrition, chemical manufacture and pressure packing. Here, too, is the focus of collecting and recording scientific information. Some illustrated details are also

briefly given of the activities of the Company's overseas houses.

It is, however, when we come to examine some of the many outstanding achievements of the Foundation over the years that the impressive service it has rendered to mankind and animals becomes clearly manifest. As long ago as 1894 it was the first British company to produce diphtheria antitoxin. During 1904-14, Sir Henry Dale, then director of research, discovered and investigated histamine, acetylcholine and sympathomimetic amines, and showed how nerve impulses are transmitted. During 1921 the principles forming the basis of all methods of immunization were outlined. The period 1927-31 witnessed the discovery of diphtheria vaccines A.P.T. and T.A.F., also digoxin ('Lanoxin'); this was when the company introduced dog distemper prophylactics. In 1933 it was "... discovered that maternal antibodies in sheep are transferred from ewe to lamb in the first milk. From this have come the present-day methods of protecting lambs against many killing infections". In 1938 came the discovery of the antibacterial effects of sulphones, to-day the chief agents for treating leprosy. Between 1939 and 1949 came the discoveries of globin zinc insulin; the antibiotic polymyxin B ('Aerosporin', 'Thio-sporin'); the pharmacology of suxamethonium (succinylcholine), the short-acting muscle relaxant ('Anectine', 'Midarine'); and the issue of the first pure tubocurarine ('Tubarine'), the muscle relaxant for major surgical operations. In the decade 1952-62 were discovered the anti-cancer drug, mercaptopurine ('Puri-nethol'), the anti-malarial pyrimethamine ('Daraprim'), the anthelmintic bephenium ('Alcopar', 'Frantin'); and in 1962 azathioprine ('Imuran'), making possible transplantation of kidneys. In 1963 the company introduced methisazone ('Marboran'), the first antiviral chemotherapeutic compound, effective against smallpox.

This is a proud record. It is a remarkable tribute to that "blend of science, business and philanthropy" envisaged by the founder, Sir Henry Wellcome, LL.D., D.Sc., F.R.S., as he was when he died in 1936. In the Truman Wood Lecture to the Royal Society of Arts in 1963, Prof. E. B. Chain said: "Rarely can a contribution from a University laboratory have exceeded in theoretical or practical importance the discoveries which originated in the Wellcome Physiological Research Laboratories" (now part of the Wellcome Research Laboratories).

\* *The Wellcome Foundation, Ltd.* Pp. 52. (London: The Wellcome Foundation, Ltd., 1964.)

## TESTING PLASTIC FLOORING MATERIALS

THE testing and examination of plastic flooring materials such as asphalt, vinyl-asbestos, vinyl and linoleum, more particularly performance behaviour, continue to present certain practical difficulties. An objective attempt to clarify the position as it now is, and to prepare the way for further research on test procedures at present considered inconclusive, was recently made by M. J. Vix, chief research officer of the National Building Research Institute, South African Council for Scientific and Industrial Research, Pretoria\*.

The scope of this paper is comprehensive, embracing as it does the four materials quoted, each in respect of the examination of the following properties: flexibility; rittleness; curl; dimensional stability; resistance to abrasion, indentation, tear, scratching, detergents and cleaning solution, to fire and to cigarette burns; discoloration when exposed to solar radiation and to ultra-violet light radiation; acoustical properties; and slipperiness. The Tinius Olsen instrument (A.S.T.M. D747-50) was used to determine stiffness in flexure (lb./in.<sup>2</sup>), and despite their heterogeneous nature, a clear distinction between the different materials was obtained. Brittleness was assessed by the falling ball impact test (interim Federal specification L-T-751), only one sample of asphalt failing, from which is concluded that this standard test is only significant in cases of extreme brittleness. Tests for curl were made in accordance with the same specification; in some samples 'natural curl', regarded as an inherent defect, made testing of this property impossible. In the tests for dimensional stability (again in accordance with L-T-751) it was shown that vinyl-asbestos tiles showed greatest shrinkage; the largest average shrinkage was of the order of 0.1 per cent, in practice negligible. Vinyl flooring showed either shrinkage or expansion; it is suggested that a more suitable routine test should be used to detect excessive dimensional changes of more than 0.1 per cent.

Abrasion resistance was tested on a 'Taber' abrader (A.S.T.M. D1242-56) and also on a newly designed abrasion tester, of which details are given, developed by

the National Building Research Institute; the results indicate "... that the N.B.R.I. abrader shows values that are more comparable with actual wear than those obtained on the Taber abrader". Since the introduction of stiletto heel tips fitted with protruding nail-heads, resistance to indentation of these materials has become an increasingly important property; here again the test procedure accorded with the Federal specification L-T-751, but although vinyl appears to resist indentations better than the others, it is considered that conditions of temperature and time for this test method require further critical investigation. In the resistance to tear (A.S.T.M. D1004-49T), vinyl proved three times as good as other samples, but it is thought that the difference is not of much practical importance, 300-500 lb./in. compared with a minimum tear strength of 100 lb./in., desirable from the point of view of handling a flooring material. In the scratch test (L-T-751) asphalt tiles seem slightly less resistant than linoleum and vinyl-asbestos, while vinyl sheeting varied from very good to poor; some doubt on the efficiency of the scratch tester involved is raised.

Linoleum emerges worst as regards chemical attack. Resistance to fire, cigarette burns and extinguishing cigarettes, by the methods detailed, are somewhat inconclusive, but vinyl sheet showed the best rating on resistance to cigarette burns, linoleum the highest rating on "extinguishing cigarettes". The tests of discoloration when exposed to solar and ultra-violet radiations gave interesting but variable results, some of which are illustrated in two coloured plates; brief details are given. Methods of assessing acoustical properties and slipperiness are discussed.

The conclusions reached are that the following aspects of all this work need further investigation: influence of accelerated ageing on flexibility and abrasion resistance; influence of temperature on flexibility and indentation resistance; influence of chemical agents in relation to indentation testing; and the influence of polish and wax on floor slipperiness in relation to various soling materials. Altogether this paper is a valuable and most welcome contribution to problems of performance testing of these flooring materials.

\* *Rilem Bulletin*, Tome special. Symposium of Liège, 1964. Ref. RD 70. Pretoria: Council for Scientific and Industrial Research, 1964.)

## 'AEROLITE' AND 'ARALDITE' BONDING MEDIA

'AEROLITE' is a synthetic glue manufactured by Ciba (A.R.L.), Ltd., extensively used for bonding timber in building and other constructional work; examples are laminated wood members in building design, glued formwork for concrete emplacement, both recently noted *Nature*, 204, 129, 1139; 1964). The 'Araldite' range of epoxy resins, manufactured by the same firm, constitute the basis of formulations used *inter alia* for bonding metal to wood, as embedding media in biological research, for example, examination of animal and plant tissues in thin sections cut by microtome.

A specialized use of 'Aerolite' is in construction of timber building elements and particularly interior woodwork for churches and similar edifices. In this connection a recent publication, entitled *Church Furniture Bonded With Aerolite\**, is of some arboreal interest quite

apart from the efficacy of the gluing-medium concerned. It is pointed out that use of English oak in church structures and interior timber equipment is traditional; that the scarcity of really big oak trees in Britain to meet what were formerly considered essential dimensional requirements "in one piece" is now very real. "Oak members in the lantern of Ely Cathedral, built in the fourteenth century, are 64 ft. long and 3 ft. square, and it is said that the whole country was searched for trees big enough to supply these timbers. To-day, with many of the old forests gone, one would seek in vain for trees of this size and, even extending one's search much farther afield, one might well continue to be unsuccessful." To-day, large wooden structural members can be built up from relatively small pieces of oak or other timber to make any required unit size, with the aid of modern synthetic resin glue such as 'Aerolite'. The art of joinery as practised now by experts conceals glue-lines and,

\* Ciba (A.R.L.), Ltd., Technical Note No. 262, October 1964. Pp. 8. Oxford: Ciba (A.R.L.), Ltd., 1964.)

although the particular moulded member is composite, the resulting effect is that of solid timber, incidentally possessing extremely durable properties; since the structure of the unit is composite, inherent stresses natural to solid timber are reduced; there is little or no prospect of cracking or splitting. This publication describes and illustrates this type of work as carried out by Ebdons (Beddington), Ltd., of Croydon, who have had long experience of building church furniture and selecting the best possible timber for conditioning by kiln drying for this special purpose. Examples of this skilled workmanship in glued timber structures as shown include churches and chapels in Belham, Bromley, Walworth, Sutton and Cheam, Harting near Petersfield, and Eridge Castle, Sussex.

The use of 'Araldite' in bonding metal to woodwork is exemplified in the case of a processional cross (illustrated) which "... has the shaft reinforced with a steel rod bonded with Araldite epoxy resin. The same

adhesive is used for the bonding of metal fittings on communion rails, chairs, lecterns, etc.". In a later issue of Ciba Technical Notes†, an account is given of the use of "Araldite in Electron Microscopy". In this field it is used as an embedding medium of great stability. In the examination of animal and plant tissues with an ordinary laboratory microscope, paraffin wax is the standard embedding compound from which the requisite thin sections are cut by microtome; but with the electron microscope, exceptionally thin sections are required, and certain synthetic resins have been investigated. According to a report by Miss Audrey M. Glauert, Strangeways Research Laboratory, Cambridge, on which these technical notes are based, "... formulations based on Araldite epoxy resins have been found to be eminently successful, and such formulations have been adopted in research laboratories throughout the world".

H. B. MILNER

† No. 263. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1964.)

## PHOTO-NEURO-ENDOCRINE EFFECTS IN CIRCADIAN SYSTEMS

IT is well established that the exact timing of 24-h rhythms is dependent on environmental influences, and that when all such influences are removed, although the rhythm may persist it is no longer synchronous with solar time but becomes free-running with a periodicity of anything between 20 and 28 h. The importance of the influence of daily changes of light and darkness on many daily and seasonal rhythms in both sickness and health has been recognized, if only dimly, since the times of Hippocrates. A great deal is now known about this influence of light on physiological processes and of the neuro-endocrine pathways involved. The *Proceedings* of a symposium on "Photo-Neuro-Endocrine Effects in Circadian Systems, with Particular Reference to the Eye"\* comprise a comprehensive survey, by the workers concerned, of the more recent additions to this knowledge. While there is some evidence that light waves, when they can penetrate sufficiently deeply, may act directly on hypothalamic structures in both avian and mammalian species, the more general influence of light on the sympathetic system is through a specific nervous pathway from the eye to the hypothalamus. Through the hypothalamic-hypophyseal connexions light can influence the endocrine discharges from the pituitary lobes. It is not surprising, then, to learn that the intensity and duration of light exposure can influence a great number of sympathetically and hormonally controlled physiological functions, particularly those of body temperature, reproduction and metabolism.

Halberg's term 'circadian', to describe the de-synchronized or free-running endogenous rhythmicity of only approximately 24 h, is now generally accepted, at least in North America, and is clearly defined in the course of Dr. Haguë's opening remarks. In some of the subsequent papers, however, no sharp distinction is made between the terms 'diurnal' (when used to describe a rhythm or activity over a period of 24 h) and 'circadian'. In a paper by Halberg and others, the term 'circadian' is extended to include "the special case of an exact 24-h period". This is consistent with Halberg's use, in another paper, of the word 'diurnal' in the more fundamental sense to describe an activity in relation to day-time, as opposed to 'nocturnal' and 'night-time'. Whether the term 'diurnal activity' used elsewhere in the volume describes an activity restricted to the day-time, or

during a 24-h period, is not at all clear. This is most confusing. In view of the stricture made on the use of the word 'circadian' by Williams in 1963<sup>1</sup>, it would be well if agreement could be reached on the use of these terms. We now have three distinct words and three distinct, or distinguishable, meanings which could be used as follows: (1) *diurnal*, that is, pertaining to the day-time, the complement to 'nocturnal'; (2) *nycthemeral* (or *nyc-themeral*, as used by French-speaking biologists), that is, relating to solar time, a period of 24 h, consisting of a night and a day (*Shorter Oxford English Dictionary*); (3) *circadian*, that is, the description of an approximately 24-h free-running endogenous rhythmicity. Perhaps the ambiguity of 'diurnal' is too deeply rooted for any plea for its eradication to be heard, but if the new term 'circadian' is to be wholly acceptable, its meaning must be made perfectly clear.

As is often the case with *Proceedings* of symposia by students of many disciplines and from many countries, this volume is not uniformly easy to assimilate, but it quickly arouses and sustains the interest of the reader. Strictly verbatim reporting of discussions can be irritating, especially when accompanying slides are not reproduced as figures. There is clear evidence, in at least one paper, of considerable alteration to the text subsequent to its original presentation. This would seem to destroy the whole premise for verbatim reporting elsewhere, and careful editing would not have obscured the valuable points which were sometimes raised in discussion. However, the symposium was well conceived, and was well balanced between the fundamental biological significance of the influence of light on physiological processes, and its considerable medical importance. While the subject-matter of some of the papers is not strictly within the terms of reference given in the title of the symposium, they afford a broader understanding of other inter-related factors which should not be ignored in a study of specific physiological processes. Papers on autonomic conditioning and the hypothalamic-hypophyseal neural influence in periodic diseases, for example, give depth to the more general consideration of the nature of biological rhythms, in both normal and abnormal conditions, while the two papers on control theory in relation to biological systems have even wider application and interest. This volume will be a valuable source of information for some long time to come.

JOHN BLIGH

\* *Annals of the New York Academy of Sciences*, 117, Article 1: *Photo-Neuro-Endocrine Effects in Circadian Systems, with Particular Reference to the Eye*. By E. B. Haguë and 64 other authors. (New York: New York Academy of Sciences, 1964.) 9 dollars.

<sup>1</sup> Williams, P. C., editorial comment in *Hormones and the Kidney* (Academic Press, London, 1963).

HYBRIDIZATION OF NORMAL AND NEOPLASTIC CELLS *IN VITRO*

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THE occurrence of hybridization of somatic cells *in vitro* has been established in mixed cultures of several different pairs of permanent mouse cell lines<sup>1</sup>. The purpose of this communication is to describe the hybrids resulting from the mating of cells of a permanent line and freshly explanted diploid cells (the occurrence of these hybrids has been mentioned earlier<sup>1</sup>).

The permanent cell line used in our early experiments is Sanford's high cancer line NCTC 2472 of C3H origin<sup>2</sup>. The modal chromosome number of the stem line cells (1s) of 2472 is 49 (variation range: 46–51). Many 2s cells are always present in the population. All cells carry an extra long telocentric chromosome which is an excellent karyological marker; and some of them contain also a long bi-armed chromosome. In later experiments a clone (No. 6) of line NCTC 2472 was used, characterized by a mode of 50 chromosomes (variation range: 48–53). All cells of this clone contain the two aforementioned markers (Fig. 1). This clone contains very few 2s cells.

The normal cells are fibroblast-like cells derived from secondary cultures of skin of new-born CBA mice carrying the well-known T-6 translocation<sup>3</sup> among its forty telocentric chromosomes (Fig. 2). As can be seen in Fig. 3, the karyotype of the hybrid cells, immediately recognizable by the simultaneous presence of the marker chromosomes of the two parents, is essentially the sum of the two parental karyotypes. The hybrids between cells of clone No. 6 and those of T-6 have a modal chromosome number of 90 (variation: 87–92), as compared with the expected mode of 90 (variation: 88–93).

A low percentage of hybrid mitoses is detected within a few days after the initiation of mixed cultures. However, under the usual culture conditions, the hybrids rapidly disappear from the mixture. During the early stages of a mixed culture, the hybrids can, however, be isolated by cloning. This procedure has permitted the establishment of pure hybrid clones, which appear to be capable of indefinite propagation *in vitro*. Karyological analysis of these clones has shown that hybrids are formed by mating of diploid T-6 cells with both 1s and 2s cells of line NCTC 2472. Inoculation of 10<sup>6</sup> hybrid cells of these two types into C3H mice and F<sub>1</sub> mice from the cross C3H × CBA gave the results presented in Table 1.

Table 1

Cell line	C3H	F <sub>1</sub> (C3H × CBA)
NCTC 2472	19/19*	4/4
Hybrid 2472(2s)/T-6	14/18	6/6
Hybrid 2472 (1s)/T-6	7/11	—
T-6	—	0/5

\* Animals with tumours/animals inoculated.

It is clear that, in this case, the hybrids between cells of a 'permanent' neoplastic line and normal cells are both 'permanent' and neoplastic.

The decline of the percentage of hybrid cells observed at the usual culture temperature (36°–37°) does not occur if the mixed cultures are incubated at 28°–29°. At the lower temperature, the percentage of hybrid cells shows a rapid and continuous increase. Within a month after the beginning of an experiment, up to 99 per cent of the mitoses are of the hybrid type. The mechanism of this



Fig. 1. Metaphase of a cell of line NCTC 2472 (clone No. 6). Note the presence of an extra long telocentric and of a bi-armed chromosome (arrows)

Fig. 2. Metaphase of a diploid cell homozygous for the T-6 translocation (arrows)

Fig. 3. Metaphase of a hybrid formed in a mixed culture of NCTC 2472 (clone No. 6) and T-6. The marker chromosomes are indicated by arrows

effect of low temperature is being investigated on several somatic cell hybrids. If the low-temperature effect proves to be characteristic of other hybrids as well, it could be used as a tool for the selection of hybrids between cells carrying no special selective markers.

This work was supported by a grant from the U.S. National Science Foundation (RG-9916).

\* Ephrussi, B., Scaletta, L. J., Stenchever, M. A., and Yoshida, M. C., *Symp. Intern. Soc. Cell Biol.*, 3, 13 (1964).

<sup>2</sup> Sanford, K. K., Likely, G. D., and Earle, W. R., *J. Nat. Cancer Inst.*, 15, 215 (1954).

<sup>3</sup> Carter, T. C., Lyon, M. F., and Phillips, R. J. R., *J. Genet.*, 53, 154 (1955).

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## A SELECTIVE SYSTEM FOR THE ISOLATION OF HYBRIDS BETWEEN L CELLS AND NORMAL CELLS

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THE occurrence of mating between cultured cells of a permanent line and freshly explanted, normal cells has been recently demonstrated (previous communication). We have developed a system for the selection of such hybrids and for the determination of the rate of their formation. The purpose of this communication is to describe this system.

The permanent line used is A9, a sub-line of the L line, kindly supplied to us by Dr. J. W. Littlefield. A9 is resistant to 3  $\mu\text{g}/\text{ml}$ . of 8-azaguanine. (A similar line was used by Littlefield<sup>1</sup> to establish a selective system involving two biochemically marked L cell lines.) Cells of line A9 are heteroploid and contain 51–58 chromosomes (mode = 55), 18–20 of which are bi-armed. The normal cells are

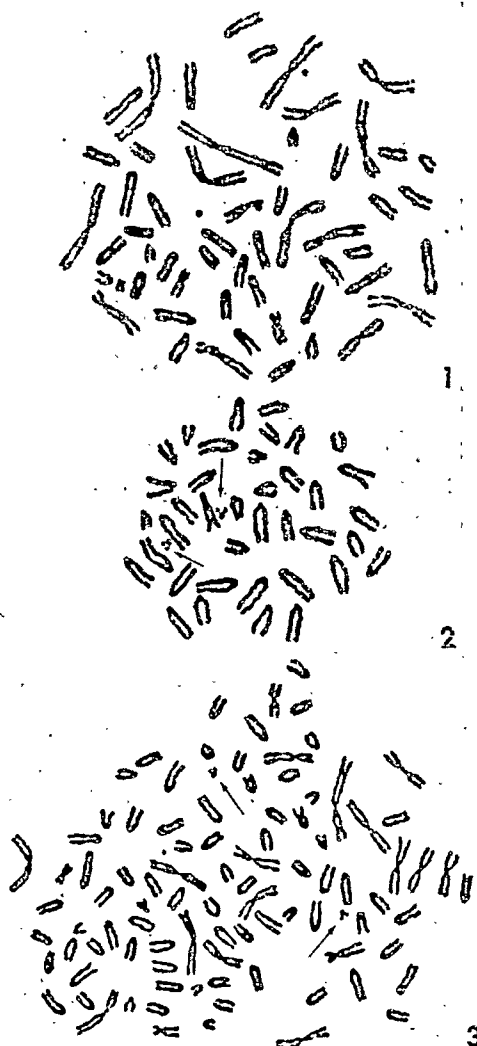


Fig. 1. Metaphase of an A9 cell with 55 large and 2 very small chromosomes. Note the presence of 18 bi-armed chromosomes

Fig. 2. Metaphase of a diploid cell (40 chromosomes) homozygous for the T-6 translocation (arrows)

Fig. 3. Metaphase of a hybrid cell with 94 large and 1 very small chromosomes. Note the presence of 19 bi-armed chromosomes from A9 and of the two T-6 markers (arrows)

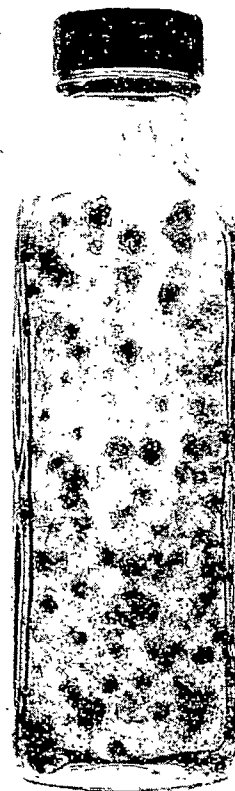


Fig. 4. Colonies of hybrid cells after two weeks in selective medium

fibroblast-like cells from secondary cultures of skin of new-born CBA mice carrying the T-6 translocation<sup>2</sup>. Hybrids between these two cell types are karyologically recognizable by the total number of chromosomes and the simultaneous presence of the parental markers (Figs. 1–3).

A9 and T-6 cells are mixed and either inoculated immediately into culture bottles containing a selective medium, or grown together for one or two days in Eagle's medium with 10 per cent calf serum, which is thereafter replaced by the selective medium. The latter is Eagle's medium with 10 per cent calf serum, supplemented with  $1 \times 10^{-4}$  M hypoxanthine,  $4 \times 10^{-7}$  M aminopterin, and  $1.6 \times 10^{-5}$  M thymidine, as in Littlefield's system<sup>1</sup>.

This selective medium prevents the growth of A9 cells<sup>1</sup>, and no revertants able to grow in this medium have been detected in our experiments. Aminopterin blocks the *de novo* synthesis of inosinic acid and thymidine. Therefore only those cells which can utilize exogenous hypoxanthine and thymidine grow in this medium. Since A9 lacks inosinic acid pyrophosphorylase, it cannot utilize exogenous hypoxanthine. The same medium does not interfere with the growth of T-6 cells, but since they are normal cells, they grow relatively slowly. On the basis of observations recorded in the preceding article,

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we expected the hybrid cells, if formed, to grow rapidly and to produce multilayered colonies on a background of a *T*-6 monolayer. Such colonies are indeed observed after about two weeks in selective medium (Fig. 4). The evidence that all these colonies are produced by hybrid cells is as follows. A bottle containing a large number of colonies was examined cytologically, and almost all of the mitoses were found to be of the hybrid karyotype. The remainder were mitoses of *T*-6 cells. In another experiment, eight colonies were isolated and grown separately. Karyological examination showed that all of them contained hybrid cells.

When the selective medium is applied 1 day after initiation of the mixed culture, the number of colonies formed indicates that as many as one in 7,000 *T*-6 cells

had mated with A9 cells. It should be pointed out that, when the mixture is inoculated directly into the selective medium, more than half as many matings take place. Obviously, the selective medium does not immediately interfere with mating. This fact must be taken into account in calculations of mating rates.

Cultures of hybrid cells recovered from these experiments have been propagated by weekly transfer since October 1964, without decline in growth rate. Hence, the hybrids seem to have inherited from A9 this property of cells of a permanent line.

This work was supported by a grant (RG-9916) of the National Science Foundation.

<sup>1</sup> Littlefield, J. W., *Science*, **145**, 709 (1964).

<sup>2</sup> Carter, T. C., Lyon, M. F., and Phillips, R. J. R., *J. Genet.*, **53**, 154 (1955).

## INTERCHAIN DISULPHIDE BRIDGE IN BENCE-JONES PROTEINS AND IN $\gamma$ -GLOBULIN B CHAINS

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**N**ORMAL  $\gamma$ -globulins seem to consist of two A and two B chains. The B chains are linked to the A chains through an interchain disulphide bridge<sup>1</sup>. Isolated B chains are heterogeneous as shown by starch-gel electrophoresis<sup>2,3</sup>. Single molecular species of B chains have not been isolated from either normal or immunized individuals. The myeloma proteins, on the other hand, do not share this heterogeneity, possibly because they come from a single clone<sup>4</sup>. Bence-Jones urinary proteins are considered to be either very similar to or identical with the B (L) chains of the 7S myeloma proteins of the same individual<sup>5,6</sup>. The relationship between normal B chains and Bence-Jones proteins provides a way of investigating the heterogeneity of normal  $\gamma$ -globulin.

Bence-Jones proteins have been shown to occur as stable monomers, dissociable dimers and stable dimers<sup>7,8</sup>. Disulphide bridges are responsible for the stabilization of the dimers<sup>9</sup>. Investigations of one antigenic Bence-Jones protein of type I led to the suggestion that the cysteine residue involved in the interchain link of the B to the A chains was the same as the one involved in the stable dimerization, but that in dissociable dimers stabilization was prevented by the blocking of the —SH group with free cysteine. After performic acid oxidation the stabilized monomers yielded free cysteic acid<sup>9</sup>. The amount of cysteic acid liberated could be taken as a measure of the presence of dissociable dimers and stabilized monomers in a Bence-Jones protein preparation of type I. The presence of the same type of dimers and stabilized monomers has now been demonstrated in individual Bence-Jones proteins of type II.

Gel filtration of different Bence-Jones proteins on 'Sephadex G-100' in 1 M propionic acid gave rise to patterns in which a monomeric fraction was not always obvious. Three of these proteins (of which two are antigenic type II) gave the results shown in Fig. 1. The difference in the elution patterns of the three samples may possibly arise from differences in the stability of the dissociable aggregates. The fraction MA was not retarded at all by 'Sephadex G-100'; when re-run in the same system 90 per cent of the material appeared in the position of the fraction MB. Fraction MA is therefore thought to consist of aggregates of dimers. The occurrence of this type of aggregate may also explain the asymmetrical elution of XA. On the other hand, different proportions of dimers and monomers in equilibrium could be responsible for the difference in the positions of the more retarded peaks (BJC, MC, XB).

The amount of free cysteic acid obtained after performic acid oxidation of the different fractions is shown in Table 1. The yield in the more retarded peak (presumably stable monomer fractions) of each of the three preparations amounted to nearly 1 mole/mole of protein monomer, as should be expected if all the molecules contained an —SH group blocked with free cysteine. However, the amount of cysteic acid was in one case as low as 0.6 mole/mole of protein. The reason for this discrepancy is not clear; it might be related to the presence of an unidentified

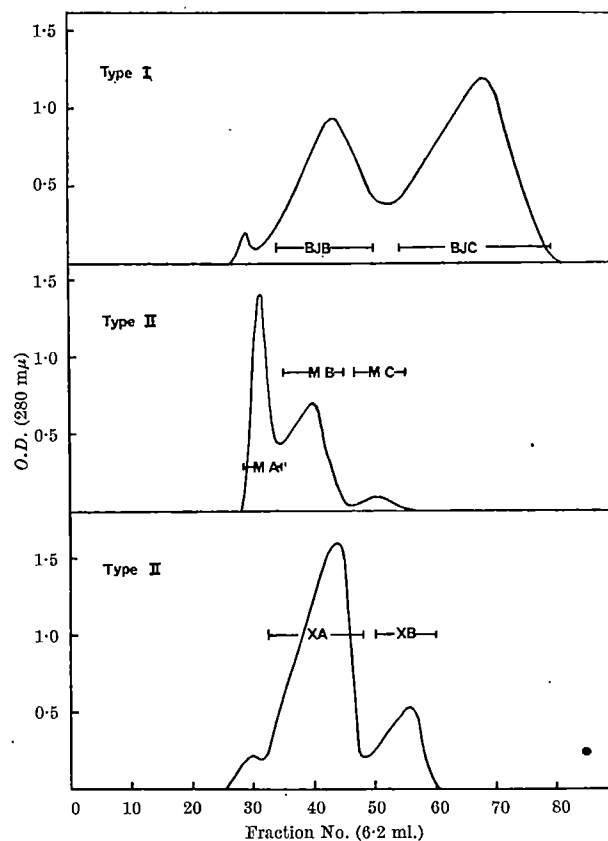


Fig. 1. Fractionation of Bence-Jones proteins on 'Sephadex G-100'. The column was 3.5 cm diameter by 70 cm long and was eluted with 1 M propionic acid.

Table 1. FREE CYSTEIC ACID IN BENCE-JONES PROTEIN FRACTIONS OXIDIZED WITH PERFORMIC ACID

Fraction (Fig. 1)	Fraction eluting as cysteic acid in the amino-acid analyser (mole/mole)
Antigenic type I	
<i>BJB</i>	0.09
<i>BJC</i>	0.83
Antigenic type II	
<i>MA</i>	0.08
<i>MB</i>	0.05
<i>MC</i>	0.63
<i>XA</i>	0.24
<i>XB</i>	1.02

The pooled fractions (Fig. 1) were oxidized with performic acid (6-8 h at 0°). Samples were either analysed for cysteic acid in a Spinco amino-acid analyser or hydrolysed and analysed for basic amino-acids. Calculation of the molar ratios was based on the yield of basic amino-acids assuming an overall recovery of a total of 200 residues after 24 h hydrolysis with 5.7 N hydrochloric acid at 105°.

peak which appears in the chromatogram of the Spinco amino-acid analyser in a position between cysteic acid and carboxymethylcysteine. The substances which were less retarded in the 'Sephadex' column (dimers and higher polymers) released very little cysteic acid on performic acid oxidation, suggesting the presence of aggregated monomers.

**Interchain disulphide bridge in Bence-Jones proteins.** In a type I Bence-Jones protein the monomer and the dimer had been found to be stabilized by a disulphide bridge involving the C-terminal cysteine\*.

Stable monomer (type I):

— Lys.Ser.Phe.Asu.Arg.Gly.Glu.Cys  
|  
Cys

Stable dimer (type I):

— Lys.Ser.Phe.Asu.Arg.Gly.Glu.Cys  
|  
— Lys.Ser.Phe.Asu.Arg.Gly.Glu.Cys

A peptide with the composition (Lys,Ser,Phe,Asp,Arg,-Gly,Glu,CMCys) has now been isolated from normal carboxymethylated *B* chains from pooled human sera. The products of trypsin digestion of this peptide are consistent with the sequence already shown here. This sequence therefore appears to be common to many of the *B* chains of normal  $\gamma$ -globulin.

The type II Bence-Jones proteins (Fig. 1) were digested with pepsin, and the disulphide bridge peptides were located and isolated<sup>9,10</sup>. The peptic digests (enzyme substrate ratio 1/40; 16 h digestion time at 37° in 5 per cent formic acid) were subjected to ionophoresis at pH 6.5. The peptides carrying disulphide bridges were apparent after performic acid oxidation and ionophoresis at pH 6.5 in a second dimension. In both Bence-Jones protein fractions *MB* and *XA* (Fig. 1) three peptides were examined. They appeared to have similar electrophoretic properties and, according to sequence studies, were derived from the following disulphide bridge:

Stable dimers (type II):

— Lys.Thr.Val.Ala.Pro.Thr.Glu.Cys.Ser  
|  
— Lys.Thr.Val.Ala.Pro.Thr.Glu.Cys.Ser

The yield of 'monomer' obtained from one Bence-Jones protein (fraction *MC*, Fig. 1) was too low and no further work was done on it. Fraction *XB* (Fig. 1) was digested with pepsin and the cysteine peptides were located. The differences which could be detected between the 'monomer' and 'dimer' patterns were all related to the peptides described here, and were associated with the presence of a spot running as cysteic acid after performic acid oxidation. This suggests that, as in the type I protein, the monomer fraction of Bence-Jones *X* contains a disulphide bridge peptide involving a single cysteine residue; this replaces the disulphide bridge present in the dimer. A peptide (Thr<sub>2</sub>,Val,Ala,Pro,Glu,CMCys,Ser) has been isolated from normal carboxymethylated *B* chains indicating that

this sequence is also common to many of the normal *B* chains of human  $\gamma$ -globulin.

As in type I Bence-Jones proteins, the interchain disulphide bridge of type II appears to occur at the C-terminus of the protein. None of the peptic peptides examined contained any amino-acids following the serine residue. Tryptic digests of the proteins oxidized with performic acid gave a peptide with the composition (Thr<sub>2</sub>,Val,Ala,-Pro,Glu,CySO<sub>3</sub>H,Ser) (see following). As a confirmation, hydrazinolysis of the protein was performed and serine was obtained as the main C-terminal residue in both type II proteins in yields of about 0.9 mole/mole.

***B* chains and the interchain disulphide bridge of normal  $\gamma$ -globulin.** The following experiments were designed to examine the variation in the amino-acid sequence around the C-terminal cysteine which links the mixed population of *B* chains to the *A* chains to give the normal human  $\gamma$ -globulins.

*B* chains from normal pooled human sera were prepared in two ways. (1) Carboxymethylated chains were prepared as described in ref. 11 except that iodoacetate instead of iodoacetamide was used as the blocking agent and 'Sephadex G-100' instead of 'G-75' was used in the columns. (2) *B* chains oxidized with performic acid were prepared in a similar way, except that no blocking agent was used before separation of the chains in a 'Sephadex G-100', 1 M propionic acid column. Oxidation with performic acid was at 0° for 4-6 h. Trypsin digests were carried out with an enzyme substrate ratio of 1/50 in 1 per cent ammonium bicarbonate for 20-24 h at 37°. Fig. 2 shows an ionogram of the tryptic digests of several Bence-Jones proteins and of both performic acid oxidized and carboxymethylated *B* chains from normal  $\gamma$ -globulin. The band *T*<sub>1</sub> (Fig. 2) of type II proteins (*X* and *M*) contained only free cysteic acid, whereas those of performic acid oxidized and carboxymethylated *B* chains contained a peptide with the composition (Gly,Glu,CySO<sub>3</sub>H) or (Gly,Glu,CMCys), respectively. Type I protein (*BJ*) contained free cysteic acid and the peptide *T*<sub>1</sub> (Gly,Glu,CySO<sub>3</sub>H) in this region. In band *T*<sub>2</sub> (Fig. 2) type II proteins and normal *B* chains contained a peptide (*T*<sub>2</sub>) which was purified by electrophoresis at pH 3.5 and which had the composition (Thr<sub>2</sub>,Val,Ala,Pro,Glu,CySO<sub>3</sub>H or CMCys,Ser). These two peptides are considered to be the C-terminal peptides and also the interchain link in the Bence-Jones proteins under study.

The yield of these peptides was determined, so that an estimate of the minimum amount present in the whole

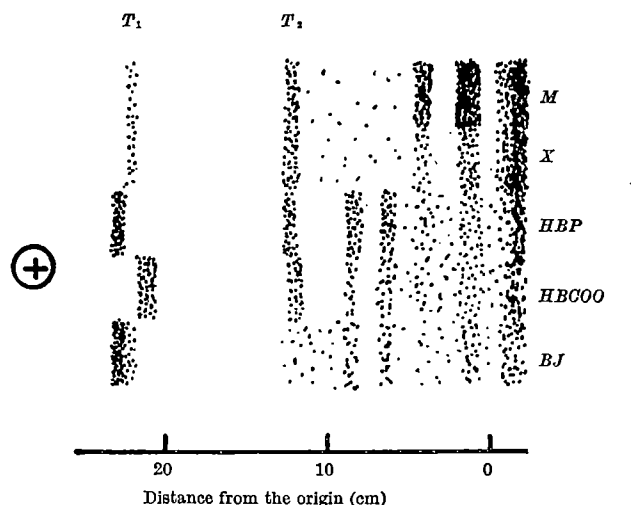


Fig. 2. Acidic components of the tryptic digests of Bence-Jones proteins and normal human  $\gamma$ -globulin *B* chains. The tryptic digest was applied to Whatman 3MM paper and subjected to ionophoresis at pH 6.5 (60 V/cm for 1 h). Bence-Jones proteins (*BJ*, *M*, *X*) were oxidized with performic acid before digestion. *HBP* and *HBCOO* were performic acid oxidized and carboxymethylated normal human *B* chains respectively.

Table 2. YIELD OF TRYPTIC PEPTIDES  $T_1$  AND  $T_2$  (FIG. 2)

Sample	Yield of peptide (mole/mole of starting protein)	
	$T_1$	$T_2$ *
type I (BJ)	—	—
type II (X)	0.7	0.5
normal B chains (oxidized with performic acid)	0.40–0.53†	0.16–0.24†
normal B chains (carboxymethylated)	0.28–0.30†	0.13

\* Peptide  $T_2$  was purified by electrophoresis at pH 3.5 (2 h at 60 V/cm, MM Whatman paper). To minimize losses in the transfer of the peptide, the sewing technique was used (ref. 14).

† Results of two independent determinations.

population of normal B chains could be made. The results are shown in Table 2. For comparison, the yields of the equivalent peptides from two Bence-Jones proteins (one of each type) are also shown. Since no correction for recoveries were made the results should be considered as minimum values. Considering this, the yields obtained with the performic-oxidized B chains are surprisingly high and suggest that all, or nearly all, the B chains in normal  $\gamma$ -globulins have either one or the other of the aforementioned C-terminal sequences. The proportion in which the peptides are found also suggests that their sequences are characteristic of the appropriate antigenic type, since it has been reported that in normal  $\gamma$ -globulin the type I and type II determinants occur in an approximate ratio of 2:1 (refs. 12 and 13).

The low yields of the carboxymethylated peptides compared to those obtained from the performic-oxidized B chains (Table 2) may have three explanations: (a) losses of carboxymethylated peptide occur during handling due to the lability of carboxymethyl-cysteine peptides; (b) all the B chains are linked to the A chains through these peptides, but during reduction and carboxymethylation disulphide interchange occurs, so lowering the yield of the carboxymethylated peptides; (c) not all the B chains are linked to the A chains through the C-terminal peptide. It is considered unlikely that the first mechanism could be responsible for the considerable discrepancies shown in Table 2. Disulphide interchange may have occurred, as preliminary experiments using radioactive iodoacetate led to the isolation of a third sequence, previously found associated with an intrachain disulphide bridge. If an interchain linkage other than that of the C-terminal

Table 3. SUGGESTED STRUCTURE AROUND THE INTERCHAIN DISULPHIDE BRIDGE IN HUMAN BENCE-JONES PROTEINS AND IN  $\gamma$ -GLOBULINS

Type I	
Stable monomer	—Lys.Ser.Phe.AspNH <sub>2</sub> .Arg.Gly.Glu.Cys
	Cys
Stable dimer	—Lys.Ser.Phe.AspNH <sub>2</sub> .Arg.Gly.Glu.Cys
	Cys
$\gamma$ -globulin	—Lys.Ser.Phe.AspNH <sub>2</sub> .Arg.Gly.Glu.Cys
	—Lys.Ser.Phe.AspNH <sub>2</sub> .Arg.Gly.Glu.Cys
	A chains
Type II	
Stable monomer	—Lys.Thr.Val.Ala.Pro.Thr.Glu.Cys.Ser
	Cys
Stable dimer	—Lys.Thr.Val.Ala.Pro.Thr.Glu.Cys.Ser
	Cys
$\gamma$ -globulin	—Lys.Thr.Val.Ala.Pro.Thr.Glu.Cys.Ser
	—Lys.Thr.Val.Ala.Pro.Thr.Glu.Cys.Ser
	A chains

peptide occurred naturally in normal B chains, one might find Bence-Jones proteins in which the disulphide bridge involved in the dimerization differs from the ones in two antigenic types described here.

Table 3 presents a summary of the suggested interchain disulphide links of Bence-Jones proteins and of the interchain link of most or all of the heterogeneous population of normal B chains. The results indicate that the two antigenic types of B chains are well-differentiated proteins. Each type should include a heterogeneity confined to a region or regions away from the C terminus.

I thank Prof. R. R. Porter, Dr. S. Cohen, and Dr. A. Feinstein for their gifts of classified Bence-Jones proteins.

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## TEMPERATURE CONTROL OF THE RESPONSE OF TADPOLES TO TRIIODOTHYRONINE

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THE influence of temperature on the metamorphosis of Amphibia was investigated as early as 1898 (ref. 1). In 1916 Adler<sup>2</sup>, observing histological changes in tadpole thyroids, concluded that environmental temperature determined the length of the larval period of *Rana temporaria*. After the relationship between the thyroid and anuran metamorphosis was discovered by Guderatsch<sup>3</sup>, it remained for Huxley in a Letter to the Editor of *Nature* in 1929 (ref. 4) to suggest the intimate relation between temperature and the response of the tadpole to moderate doses of thyroid powder. Although details of his experiments have never been published, Huxley reported that at temperatures below 5°, *R. temporaria* tadpoles treated with thyroid powder underwent partial metamorphosis and then ceased to change even when returned to room temperature. In urodeles, Hartwig<sup>5</sup> noted that in the 0°–5° temperature range the usual artificial effects of thyroxine could not be obtained in salamander larvae.

It is surprising that so few detailed investigations on the effect of temperature on the response of anura to thyroid hormones have been reported as revealed by several reviews<sup>6–10</sup>. Etkin<sup>8</sup> noted that tadpoles grow larger before undergoing metamorphosis at 16° than they do at 24°. Kollros<sup>11</sup> confirmed this and also mentioned experiments which indicated that the response to temperature is wholly or largely independent of the reactions of the thyroid gland, since hypophysectomized animals also showed a differential sensitivity to low concentration of thyroxine at different temperatures. In the most recent investigation available, Paik and Cohen<sup>12</sup> measured the increase in tadpole liver carbamyl phosphate synthetase during continuous immersion in  $2.6 \times 10^{-6}$  M (0.02  $\mu$ g/ml.) thyroxine at 15° and 25°. After a latent period, the specific activity of this enzyme at 15° increases about half as fast as it does at 25° and reaches a plateau at about half the maximum level attained at 25°. When these animals were brought to 25° the enzyme increase



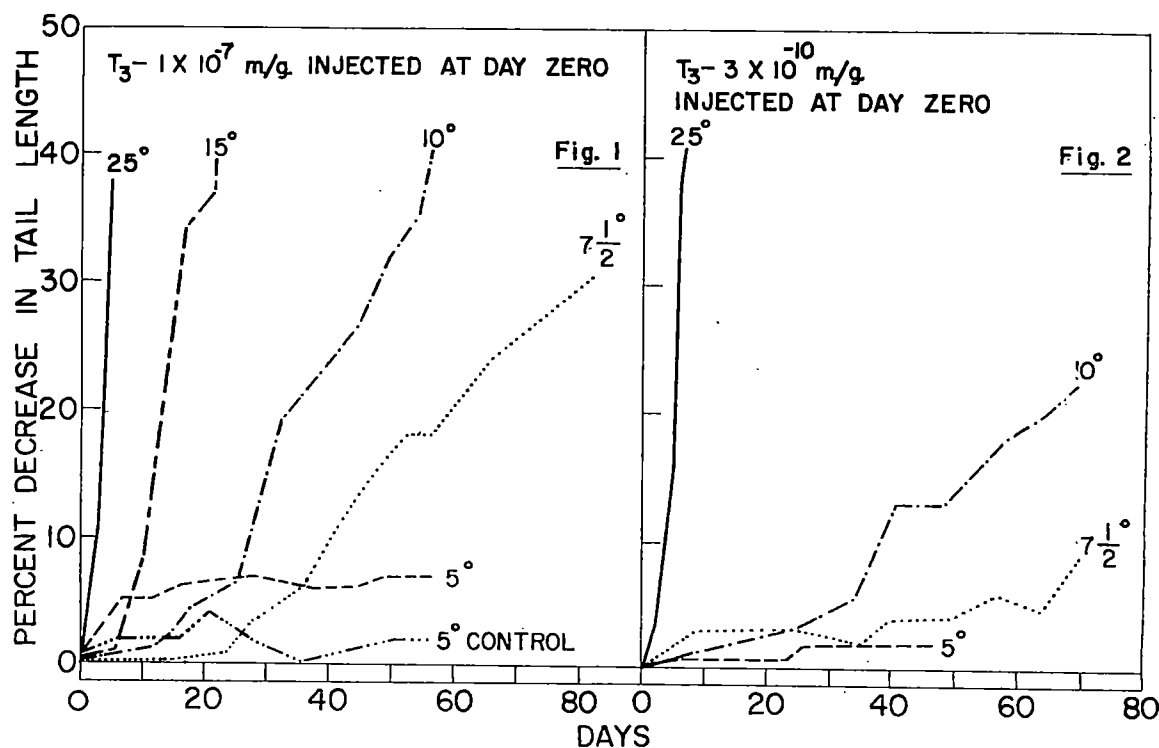


Fig. 1. Effect of various temperatures on the response of tadpoles to a single large dose of  $T_3$ . Groups of eight *Rana grylio* tadpoles were injected with  $1.0 \times 10^{-7}$  moles/g tadpole at day zero. The decrease in tail-length was measured every day during periods of rapid change. Temperatures below 20° were maintained in special thermostatically controlled incubators housed in a cold room. The results in Fig. 1 were obtained from experiments performed simultaneously. Essentially no change was observed at 5° from the 7th to the 56th day. The initial fall in tail-length of the 5° curve appears to be an artifact since it was not observed in several subsequent experiments in which the per cent decrease in tail-length did not exceed 2 per cent in the first 10–20 days. The 5° control line refers to uninjected tadpoles.

Fig. 2. Effect of several temperatures on the response of tadpoles to a single moderate dose of triiodothyronine. Tadpoles were injected with  $3.0 \times 10^{-10}$  moles/g tadpole at day zero. The decrease in tail-length was measured every day during periods of rapid change.

was resumed without delay. In view of the lack of more extensive data, we decided to explore a wide range of temperatures, various periods of time and other conditions in order to determine the full significance of the temperature effect on induced metamorphosis.

In these experiments, we used the percentage decrease in tail-length to measure the metamorphic response of the tadpole to thyroid hormone. Groups of eight pre-metamorphic<sup>10</sup> *Rana grylio* tadpoles were treated with triiodothyronine ( $T_3$ ) and other thyromimetic compounds as described previously<sup>13–15</sup>. In every experiment a single injection of the hormone was administered on day zero. In early experiments, presented in Fig. 1, we attempted to force the tail response with a single massive dose of  $T_3$ ,  $1 \times 10^{-7}$  moles (65  $\mu$ g)/g tadpole. As the temperature was successively decreased from 25° to 7.5°, the rate of tail decrease was greatly reduced. At 5° no significant response to this large dose of  $T_3$  could be discerned even after 80–90 days. Thus, despite the use of at least 300–1,000 times an effective dose of  $T_3$ , the rate of response at 5° was less than 1 per cent of the rate of response at 25°. A similar experiment was initiated at much lower doses of  $T_3$ ,  $1 \times 10^{-10}$  (0.065  $\mu$ g) and  $3 \times 10^{-10}$  moles/g tadpole. As shown in Fig. 2, the response was proportionately reduced at 10° and completely absent at 5°. Similar data were obtained for thyroxine, 3'-isopropyl-3,5-diiodothyronine, and the propionic acid analogue of  $T_3$ . Estimation of the temperature coefficient,  $Q_{10}$ , over several temperature spans from the results in Fig. 2 gives a minimum value of 10. This large  $Q_{10}$  is not unprecedented for an overall biological process; but its size is unexpectedly great for a hormonal effect. The rate of tail response to  $T_3$  at 5° is less than one-hundredth of the rate at 25°. On this basis, assuming no other intervening factors, one might estimate that the larval life-span of a bullfrog might be extended in the cold from a minimum of one year to more than 100 years. However, in one experiment when tad-

poles approaching prometamorphosis<sup>10</sup> were treated with  $10^{-9}$  moles (0.65  $\mu$ g)  $T_3$ /g at 6°, slight but definite body shape changes and tail reduction were observed after several weeks. Thus, as expected, it appears that tadpoles at more advanced stages are more susceptible to  $T_3$  at low temperatures.

When tadpoles were injected on day zero with a low dose of  $T_3$ ,  $1 \times 10^{-10}$  moles (0.065  $\mu$ g)/g, held at 5° for extended periods, and then changed to 25°, a rapid  $T_3$  response was observed. This response curve, as shown in Fig. 3, parallels the curve which is obtained if animals are kept at 25° from the time of the initial injection of  $T_3$ , except that the two-day lag period is almost eliminated. Though not shown in Fig. 3, an identical result is obtained even if the removal to 25° is delayed to 80 days. No increase in the rate of metamorphosis of uninjected control animals has been observed when these animals were transferred to 25° after several months at 5°. This experiment eliminates the possibility that stimulation of the thyroid gland in the cold, as observed in the rat<sup>16</sup>, could account for these results.

In contrast to the foregoing experiments where the  $T_3$  effect proceeds when tadpoles are shifted from 5° to 25°, we have tried the converse experiment. Tadpoles were injected with  $T_3$ , maintained at 25° and then transferred to 5° at the end of the third, fourth and fifth days. As shown in Fig. 4, the tail response was arrested. Because of the relatively poor survival rate of these animals after the tenth day, the logical extension of these experiments—restoring the tadpoles to 25°—has succeeded for only a limited number of animals. With these animals, the tail decrease has always been renewed after restoration to 25°.

On the basis of these results, the remarkable temperature sensitivity and control of the response of tadpoles to exogenous thyroid hormone is now being investigated in our laboratory. The effect of temperature

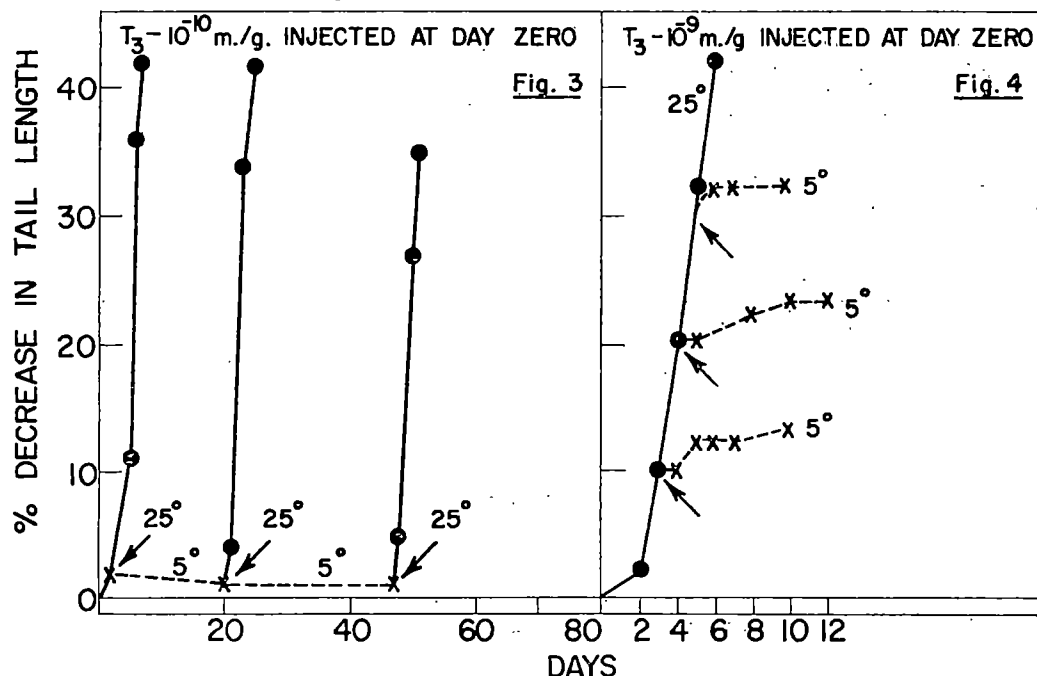


Fig. 3. Restoration of the T<sub>3</sub> response at 25° after tadpoles were held at 5° for extended periods. A single injection of  $1.0 \times 10^{-10}$  moles/g tadpole was administered at day zero, and the decrease in tail-length was measured every day during periods of rapid change.

Fig. 4. Arrest of the T<sub>3</sub> response by lowering the temperature from 25° to 5°. Tadpoles were injected with  $1.0 \times 10^{-9}$  moles/g tadpole at day zero and held at 25°. Different groups were transferred to 5° after three, four, and five days, respectively. Arrows indicate the per cent decrease in tail-length of the group being transferred in addition to the day transferred. The small increase in response after the third day transfer at 10 per cent decrease in tail-length has been observed several times.

Other features of induced and spontaneous metamorphosis is now being investigated. The experiments described here raise two major questions which can be only briefly discussed now. The first question relates to what happens during the prolonged incubation at 5°. The ventral response of the tadpole when it is transferred to 5° could be due to: (a) an appreciable fraction of the T<sub>3</sub> surviving the prolonged incubation and becoming effective at 25°; or (b) T<sub>3</sub> initiating certain subtle preparatory changes at 5°, but a complete response to T<sub>3</sub> not ensuing until the temperature is increased. In order to attempt to answer this question, a preliminary estimate<sup>17</sup> of the biological half-life of the iodine of <sup>125</sup>I-T<sub>3</sub> in the tadpole has been made and found to be  $6 \pm 3$  h at 25° and about 12 days at 5°, when the original dose was  $1.00 \times 10^{-9}$  moles/g tadpole. Thus, after 60–80 days at 5°, less than 1 per cent of the original dose would be expected to remain. This level of hormone probably does not account for the rapid tail decrease observed when the tadpoles were transferred to 25°. Our tentative conclusion is that the hormone has already left its imprint on susceptible cells.

The possibility of subtle metabolic changes being produced by T<sub>3</sub> during the 5° incubation period is particularly intriguing because it would represent a dissociation or separation of metabolic changes during induced metamorphosis. The hormonal signal may have been received, but the low temperature will not permit the proper execution of the message. For example, the appropriate messenger RNA(s) could have been synthesized, but its impact on the protein synthesizing systems could be delayed or prevented at 5°. Thus, this may be a very valuable tool in the investigation of induction of differentiation by the thyroid hormone.

The second significant problem raised by this work is to explain how the metamorphic response can be so very dependent on temperature. It is known that many important biological reactions in homeotherms are extremely temperature sensitive. The thyroid hormone contributes to the ability of homeotherms to maintain a constant body temperature. At low temperatures, thyroid

hormone is secreted at a greater rate in the mammals; the rat, for example, produces three times as much thyroid hormone at 4° compared with 28° (ref. 16). But the temperature sensitivity of many key reactions of poikilotherms has not been widely studied. Where responses have been examined, much smaller temperature effects have been noted. The rate of liver carbamyl phosphate synthetase production decreases only about two-fold after T<sub>4</sub> stimulation over the 10° span of 25°–15° (ref. 12). A recent comparison<sup>18</sup> of the uptake of oxygen of tadpoles to 5° and 25° as described previously<sup>19</sup> showed that the respiration rate of young tadpoles at 5° is 14 per cent of the rate at 25° ( $11 \pm 2$  versus  $81 \pm 20$   $\mu$ l. oxygen/h/g tadpole). This results in a Q<sub>10</sub> for the respiration rate of about 2.7 compared with a Q<sub>10</sub> of about 10 for the tail response. While we are aware that the 'Q<sub>10</sub> Law' (a uniform response to temperature) does not necessarily hold for phenomena related to respiration of poikilotherms, it is clear that the relatively smaller difference in respiration at 5° and 25° cannot quantitatively account for the reduction in response to T<sub>3</sub> at 5°.

We regard the blocking of the metamorphic response by low temperatures as a basic clue to the kind(s) of reaction which might be affected by the thyroid hormones. For example, we might expect characteristic phase changes in certain lipid structures to occur between 0° and 10°. Numerous enzymes undergo marked alterations in temperature coefficients in this range which are not related to phase changes. It is possible that the particular enzymes directly involved in tail resorption could show an unusual temperature sensitivity. It is also known that vital changes in the structure of water (icebergs) occur around 5°. Perhaps certain acute alterations in water–biopolymer, RNA–protein, RNA–DNA or DNA–DNA interactions can also occur in the 5° region. On the other hand, the binding of T<sub>3</sub> by protein would have to be unexpectedly temperature-sensitive to account for the difference in response at 5° and 25°. It is also possible that the large temperature coefficient represents the sum of numerous co-operative effects analogous to protein denaturation. It is hoped that one or more of these

approaches will lead to experiments which will help elucidate the nature of the temperature effect on the response of the tadpole to  $T_3$  or  $T_4$  and perhaps the basic mechanism of action of the thyroid hormone.

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## UPTAKE OF INSULIN LABELLED WITH IODINE-131 BY ELASTIC TISSUE IN VITRO

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RECENT autoradiographic investigations of the distribution of exogenous radioiodinated insulin in the rat have demonstrated an affinity of this hormone, or its breakdown products, for elastic fibres and membranes<sup>1</sup>. However, information concerning quantitative differences in insulin 'binding' between elastic and other tissues is lacking. One purpose of this article is to report quantitative differences in the *in vitro* uptake of insulin-<sup>131</sup>I by slices of the bovine ligamentum nuchae (consisting predominantly of elastic fibres) and the uptake by slices of tendon and striated muscle.

Pieces of ligamentum nuchae, tendon and superficial cervical muscle from freshly killed cattle were immersed in cold mammalian Ringer's solution and used within 3 h. Slices of approximately 1 mm thickness and 1.0 × 0.6 cm were cut perpendicular to the length of the fibres, placed in individual tared vaccine bottles containing 2 ml. of incubation medium with the radiolabelled substrate and reweighed (± 0.1 mg). The tissues were incubated with shaking in an atmosphere of oxygen and carbon dioxide (95:5), pH 7.4, for 10, 30, or 60 min at either 37.5° or 4° C.

After incubation the slices were rinsed for 30 sec in iced buffer and blotted, and the radioactivity was measured immediately in a γ-scintillation well-counter. They were then washed for 60 min in two 20-ml. changes of buffer at 4° C, recounted in the well-counter, dried in an oven at 110° C for 2 h and weighed. The degradation of the labelled insulin in the medium during incubation was measured as the fraction of the radioactive material which was soluble in trichloroacetic acid (TCA)<sup>2</sup>.

Gey and Gey's balanced salt solution<sup>3</sup>, supplemented with glucose (1 mg/ml.) and crystallized bovine serum albumin (Armour Pharmaceutical 6, Kanehake, Illinois) (5 mg/ml.), was used for the incubation medium. The albumin was added to minimize the adsorption of insulin to glass<sup>3</sup>.

All labelled materials were stored at 4° C. Crystalline beef insulin labelled with iodine-131 (Abbott Laboratories, Oak Ridge, Tenn.) was used on the day following its preparation by the manufacturer. The products of spontaneous degradation or radiation damage represented 1.2–2.6 per cent of the radioactivity when evaluated by hydrodynamic-flow paper chromatography<sup>4</sup> and 0.1–2.6 per cent of the radioactive material was soluble in TCA.

In two experiments insulin-<sup>131</sup>I was inactivated by reduced glutathione (GSH) according to Du Vigneaud *et al.*<sup>5</sup>. Following reduction, 21.5 per cent of the radio-

active material was soluble in TCA and 78 per cent migrated with the degradation products of insulin-<sup>131</sup>I during hydrodynamic-flow paper chromatography.

The concentration of radioactivity in the incubation medium was approximately 0.25 μc./ml. medium representing 30–40 mug insulin-<sup>131</sup>I/ml. or 25 μg radioiodinated- (<sup>131</sup>I labelled) human serum albumin (RISA) (Abbott Laboratories, Oak Ridge, Tenn.)/ml. With the equipment used, 0.25 μc. registered approximately 120,000 c.p.m. over a background of 20 c.p.m. Tissue radioactivity was expressed as the *T/M* ratio, where *T* represents counts per min (c.p.m.)/g tissue (wet weight) and *M* is c.p.m./ml. incubation medium. In Figs. 2b and 4b, *T* represents c.p.m./g tissue dry weight.

There was no significant difference in the concentration of radioactivity found in the different tissues following 10-min incubation at 37.5° C in the presence of insulin-<sup>131</sup>I. However, after 30- or 60-min incubation the concentration of radioactivity in elastic ligament was approximately twice that in tendon (*P* < 0.01) and approximately three times greater than in muscle (*P* < 0.01). Elastic ligament

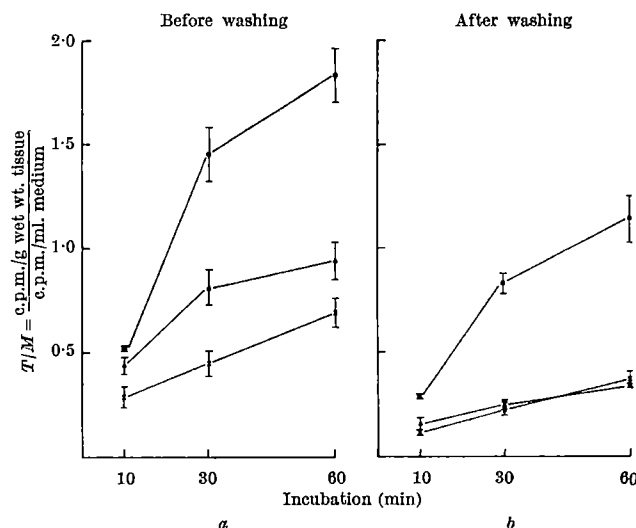


Fig. 1. Radioactive material in slices of elastic ligament, tendon and muscle following incubation at 37° C in a medium containing insulin-<sup>131</sup>I. a, Slices counted immediately after incubation. b, Slices recounted after a 60-min wash in buffer without radioactive substrate at 4° C. Each point is the mean of six tissue slices ± S.E.M. ●—●, Elastic ligament; ▲—▲, tendon; ×—×, muscle.

concentrated the radioactivity against a gradient, since the  $T/M$  ratio exceeded unity after 30- and 60-min incubation (Fig. 1a).

Washing for 1 h at 4° C removed more radioactivity from tendon than from muscle ( $P < 0.01$ ), but proportionally less from elastic ligament than from tendon ( $P < 0.01$ ) or muscle ( $P < 0.05$ ). As a result, the difference between elastic ligament, on one hand, and tendon and muscle on the other, becomes more pronounced after washing. Elastic ligament which was exposed to insulin- $^{125}$ I for only 10 min contained 60 per cent more radioactivity than tendon ( $P < 0.01$ ) and 130 per cent more than muscle ( $P < 0.01$ ). After 30- or 60-min incubation elastic ligament contained approximately four times more radioactivity than either tendon or muscle ( $P < 0.01$ ) (Fig. 1b).

A comparison of the degradation of insulin- $^{125}$ I in the medium by the three types of tissue during incubation at 37.0° C is given in Table 1. Each of the tissues exhibited some 'insulinase' activity, since, during 60-min incubation, the TCA precipitable fraction of the radioactivity decreased in all media. Muscle degraded more insulin- $^{125}$ I than either tendon or elastic ligament ( $P < 0.01$ ), whereas the activities of the latter two were equal and relatively weak. Therefore, the affinity of elastic tissue for insulin- $^{125}$ I is not related to its 'insulinase' activity.

Table 1. DEGRADATION OF INSULIN- $^{125}$ I IN THE MEDIUM DURING INCUBATION WITH SLICES OF ELASTIC LIGAMENT, TENDON OR MUSCLE AT 37.5° C

Incubation (min)	Per cent TCA-precipitable radioactivity in medium*		
	Elastic ligament	Tendon	Muscle
10	98.9 ± 0.58	98.2 ± 0.75	97.2 ± 1.01
30	96.7 ± 0.37	97.1 ± 0.70	92.4 ± 0.88
60	91.0 ± 2.04	90.0 ± 1.95	81.4 ± 2.00

\*  $\frac{\text{TCA precipitable c.p.m./ml. medium}}{\text{Total c.p.m./ml. medium}} \times 100$

Each value represents the mean of 6 tissue slices studied in 3 separate experiments  $\pm$  S.E.M. Before incubation 97.5–99.9 per cent of the radioactivity in the medium was precipitable with TCA.

The greater density of elastic ligament could increase the number of available 'binding sites' per g tissue. The total tissue water of the slices represented in Fig. 1 was  $5.6 \pm 0.51$  per cent of wet weight in elastic ligament,  $0.7 \pm 1.11$  per cent in tendon, and  $81.5 \pm 0.49$  per cent in striated muscle. Thus, although the dry weight/wet weight ratio is greater in elastic ligament than in tendon or muscle, the difference is not large enough to account for the preferential concentration of insulin- $^{125}$ I by elastic ligament. Indeed, when the results illustrated in Fig. 1b are recalculated on the basis of the dry weight of the tissues, the concentration of radioactive material in elastic ligament was still significantly greater ( $P < 0.01$ ) than that in tendon or muscle following 10-, 30- or 60-min incubation with insulin- $^{125}$ I. This fact is further illustrated in Fig. 2b.

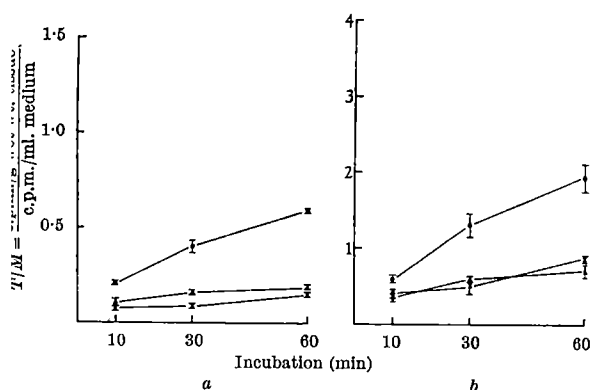


Fig. 2. Radioactive material in slices of elastic ligament, tendon and muscle after incubation at 4° C in a medium containing insulin- $^{125}$ I, followed by washing for 60 min at 4° C in buffer without radiolabelled substrate. The radioactivity present in the tissues after washing is related to the wet weight of the slices in a and to the dry weight in b. Each point is the mean of two tissue slices  $\pm$  S.E.M. Symbols as in Fig. 1

Fig. 2 shows the concentration of radioactivity in each type of tissue after incubation with insulin- $^{125}$ I at 4° C, followed by washing for 1 h at 4° C. More radioactive material was 'bound' to slices of elastic ligament than to tendon or muscle ( $P < 0.02$ ), although the total uptake by each tissue was less than when incubated at 37° C. The results are essentially identical, whether the values are expressed on the basis of the wet weight or the dry weight of the tissues (compare Figs. 2a and 2b). The radioactive material in the medium was 98–99 per cent precipitable with TCA following incubation, indicating that insulin- $^{125}$ I was not degraded by the tissue slices at 4° C and that the material which was preferentially concentrated by elastic tissue was not a product of enzymatic degradation. The affinity of elastic tissues for insulin at 4° C suggests that the reaction can occur under conditions of minimum metabolic activity.

The rate of diffusion of the labelled protein through tissue slices may be a factor which determines its concentration in the slice after incubation. To test this hypothesis, tissue slices were incubated at 37.5° C, with albumin- $^{125}$ I (RISA) (Fig. 3). All tissues contained significantly less radioactive material when RISA was substituted for insulin- $^{125}$ I in the medium ( $P < 0.01$ ) (compare Fig. 3a with Fig. 1a). The greatest concentration of radioactivity in tissue after incubation with RISA (Fig. 3a) was approximately 40 per cent of the concentration in the medium. Tendon contained more radioactivity than elastic ligament although the differences between tissues were not significant. RISA did not penetrate faster into elastic tissue slices than into slices of tendon or muscle. Washing for 1 h removed most of the radioactivity from all tissues (Fig. 3b). The concentration of radioactivity in the tissues after washing was less than 10 per cent of that in the medium, and there was no significant difference between the three types of tissue.

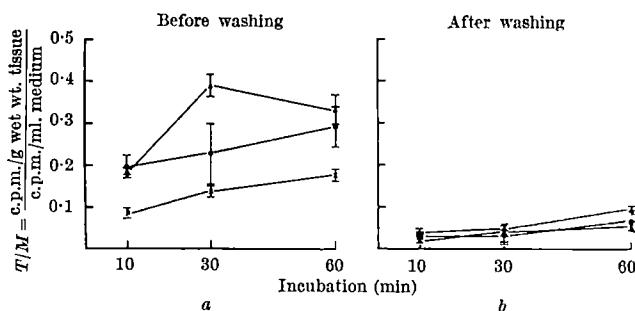


Fig. 3. Radioactive material in slices of elastic ligament, tendon and muscle following incubation at 37° C in a medium containing radioiodinated human serum albumin (RISA). a, Slices counted immediately after incubation. b, Slices recounted after a 60-min wash in buffer without radioactive substrate at 4° C. Each point is the mean of three tissue slices  $\pm$  S.E.M. Symbols as in Fig. 1

Exposure to GSH in a nitrogen atmosphere causes the reduction of the S—S linkages of insulin and inactivation of the hormone<sup>6</sup>. Tissue slices were incubated at 37.5° C with GSH-reduced insulin- $^{125}$ I, then washed for 1 h at 4° C (Fig. 4). The concentration of radioactivity by elastic ligament incubated with 'intact' insulin- $^{125}$ I in other experiments is shown for comparison. Elastic tissue 'bound' significantly less radiolabelled material during 30–60-min incubation with GSH-reduced insulin- $^{125}$ I than with unaltered insulin- $^{125}$ I ( $P < 0.01$ ) (Fig. 4a), while tendon and muscle 'bound' insulin- $^{125}$ I and GSH-reduced insulin- $^{125}$ I equally (compare Fig. 1b with Fig. 4a). Thus, reducing the S—S linkages of insulin- $^{125}$ I decreased the affinity of the elastic tissue for the labelled material. However, after 30 or 60 min incubation with GSH-reduced insulin- $^{125}$ I the concentration of 'bound' labelled material was still greater in elastic ligament than in tendon or muscle ( $P < 0.02$ ), when calculated on the basis of the wet weight of the tissues (Fig. 4a). This



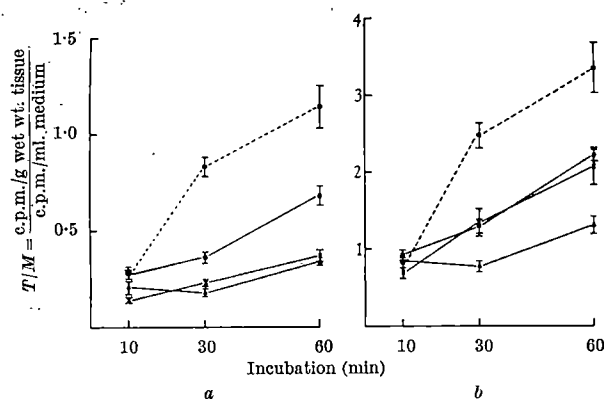


Fig. 4. Radioactive material in slices of elastic ligament, tendon and muscle after incubation at 37° C in a medium containing insulin-<sup>125</sup>I treated with reduced glutathione, and washing for 60 min in cold buffer (4° C) without radioactive substrate. The radioactivity contained in the tissues after washing is related to the wet weight of the slices in *a* and to the dry weight in *b*. Each point is the mean of three tissue slices  $\pm$  S.E.M. ■, Elastic ligament incubated with insulin-<sup>125</sup>I. Other symbols as in Fig. 1 and incubated with GSH-reduced insulin-<sup>125</sup>I.

could indicate that the affinity of elastic tissue for insulin is only partially dependent on intact S—S linkages in the insulin molecule, or that the reduction of insulin-<sup>125</sup>I by GSH was incomplete. All tissues 'bound' some GSH-reduced insulin-<sup>125</sup>I, since the radioactivity remaining in the tissue slices after washing was greater following incubation with GSH-reduced insulin-<sup>125</sup>I than with RISA. The protein content of the tissues may be important. When the concentration of radioactive material in the tissues is calculated on the basis of their dry weight (Fig. 4*b*) there is no difference in the amount of radioactive material bound by elastic ligament or muscle during incubation with GSH-reduced insulin-<sup>125</sup>I.

Although the specificity of insulin binding *in vitro* has recently been questioned<sup>6</sup>, binding of insulin to the target tissue is thought to be a prerequisite for the biological action of insulin *in vivo* and *in vitro*<sup>7-9</sup>. It does

not necessarily follow, however, that the mere fact of binding indicates that biological activity is taking place.

There is at present no evidence that insulin in any way affects elastic tissue or that the elastic tissue affects insulin or that the marked ability of elastic tissue to 'bind' insulin influences the overall metabolism of this hormone. Its mere presence, however, in such relatively large amounts presents a situation which cannot be ignored. Camerini-Davalos and associates have demonstrated by electron microscopy<sup>10</sup> degenerative changes in elastic tissue of prediabetics. There might be some connexion between the selective affinity of insulin for elastic tissue and these degenerative changes. One could speculate that insulin, or the lack of it, may have some effect on the metabolism of elastic tissue or could in some way affect the physico-chemical properties of elastic fibres or membranes. One might also speculate that insulin which binds to elastic tissue may become available to the body economy in certain circumstances. Finally, the unusual affinity of insulin for elastic tissue might give us an insight into the nature of binding sites of insulin in tissues in general.

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## ACTIVITY OF A TUMOUR POLYSACCHARIDE SUBSTANCE ON MICE TRANSPLANTED WITH SARCOMA 180

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IT was predicted by Burnet and Fenner<sup>1</sup>, and consequently demonstrated experimentally by Medawar *et al.*<sup>2,3</sup>, that a state of immunological unresponsiveness or tolerance results from the neonatal presentation of an antigen to an animal. This work was extended by a number of investigators<sup>4-6</sup> who showed that animals can be rendered immunologically tolerant to protein antigens by means of neonatal presentation of the antigen.

Siskind, Patterson and Thomas<sup>7</sup> have shown that both unresponsiveness and immunity can be induced in newborn and adult mice with pneumococcal polysaccharide, high dosage resulting in unresponsiveness while small dosage results in immunity. The basic concept underlying such investigations is that described by Felton and Ottinger<sup>8</sup> and later by Felton<sup>9</sup> and Felton *et al.*<sup>10</sup> and referred to as "Felton's immunological paralysis". Felton *et al.* reported that adult mice which received a relatively large dose of pneumococcal capsular polysaccharide succumbed on subsequent challenge with virulent pneumococci of the same type. In contrast, mice which received a relatively small dose of the same type of polysaccharide survived the challenge.

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Koprowski<sup>11</sup> demonstrated the induction of tolerance to tumour tissues in a host animal following intra-foetal injection of tumour cells. This finding brought to the concept of immunoparalysis nearer to the cancer field. However, in order to understand better the relationship between immunoparalysis and cancer it is necessary to make further simplifications (in terms of chemically identifiable substances prepared from cancer tissue) when attempting to determine the particular antigens responsible for immunity and susceptibility to cancer.

Earlier<sup>12-14</sup> evidence was presented by me for the polysaccharide nature of cancer antigens—referred to as 'tumour polysaccharide substance' (TPS). Prof. J. Smith *et al.*<sup>15</sup> have now demonstrated that TPS has characteristics similar to a glycoprotein. In studies with the Schultz-Dale reaction, I was able to show that TPS can induce immunity in small dosage and immunoparalysis in larger dosage<sup>16</sup>. In other investigations with cutaneous hypersensitivity in man<sup>16</sup>, I was able to show that TPS forms complexes with other macromolecules such as DNA, which are themselves antigenic. Plesci Braun and Paleyuk<sup>17</sup> have recently identified such complex of a polysaccharide antigen associated with

DNA from Ehrlich-Lettre mouse ascites tumour cells. In an immunological unitarian concept of cancer which I described earlier<sup>10,18,19</sup>, TPS was assigned the key role, the 'common denominator', in carcinogenesis. It was postulated that this abnormal polysaccharide is formed in tissues in response to chronic and prolonged irritation by the many carcinogenic agents—chemical, viral and physical—by the alteration of the normal polysaccharide substance which is believed to surround all cells (such as the erythrocyte receptors<sup>20</sup> and the tissue transplantation antigens<sup>21,22</sup>) and which also reside in abundance in the connective tissue of the host. More recently, Defendi and Gasic<sup>23</sup> have demonstrated a thick coat of acid mucopolysaccharides in hamster embryo cells transformed *in vitro* by polyoma virus.

The formation of TPS, according to this concept, triggers off a series of events in the host, starting with immunity to TPS (analogous to the homotransplantation reaction), immunoparalysis to TPS, followed by the development of an embryonic type cell (the result of interaction by TPS of cell-stromal interaction), and leading to the development of an immunologically competent neoplastic cell (the result of alteration of the nucleic acids of the cell by TPS).

These investigations were carried out in an attempt: (1) to test further the validity of the immunological unitarian concept of cancer which I postulated earlier; (2) to understand better the chemical basis for immunity and unresponsiveness in preconditioned mice transplanted with sarcoma 180; and (3) to localize tumours by the use of tritiated TPS (<sup>3</sup>H-TPS). This latter approach was based on two earlier findings: first, that tumour cells are immunologically competent, producing or concentrating both antibodies as well as antigens<sup>24-26</sup>; secondly, that tritiation of TPS does not alter its antigenicity. In other experiments (to be published elsewhere) I was able to induce *in vitro* anaphylaxis in properly sensitized uterine segments with the labelled substance (<sup>3</sup>H-TPS) at the same level of sensitivity (that is, at the 5 µg level) as the unlabelled material. By the use of <sup>3</sup>H-TPS it was hoped to reach selectively the neoplastic cells with the β-emitting isotope without injuring the neighbouring normal cells—a situation which may result in the cure of the tumour-bearing host.

The following macromolecules were used in these experiments:

(1) TPS-sarcoma 180 (TPS-*m*). This tumour polysaccharide substance was prepared from the mitochondrial fraction of sarcoma 180 tumour tissue of mice (that is, at a fraction which sediments between 3,000 and 1,000 r.p.m.). This fraction was then subjected to various chemical procedures as described earlier<sup>12</sup>.

(2) NPS-*m*. This normal polysaccharide substance was prepared from normal mouse lung of the same type AKR/J mice used in the experiments. The procedure of isolation and purification was the same as that used for TPS-*m*.

(3) <sup>3</sup>H-TPS-sarcoma 180 (<sup>3</sup>H-TPS-*m*). This is the tumour polysaccharide substance obtained from sarcoma 180 of mice which has been tritiated by the Wilzbach procedure<sup>27</sup> by the New England Nuclear Corporation. The specific activity of this batch was 0.073 mc. per mg. This material was dialysed against phosphate buffer pH 6.8 for 21 days at 5° C. Each time 500 ml. of buffer is used and changed every 4 days. Only 1.47 per cent of the initial activity was retained.

(4) DNA-sarcoma 180 (DNA-*m*). This deoxyribonucleic acid was prepared from the nuclear fraction of sarcoma 180 tumour tissue (that is, the fraction which sediments at 3,000 r.p.m.). The sediment was treated with freon (2 volumes of freon, 1 volume of sediment, volume of 0.15 M sodium chloride) for 5 min in a Virtis homogenizer at 15,000 r.p.m. with the homogenizing cup cooled in ice. It was then centrifuged at 3,000 r.p.m. for 5 min in a Lourde refrigerated centrifuge. The

aqueous layer was separated and kept for pooling. The sediment-freon mixture was then extracted with 1 M sodium chloride, homogenized, centrifuged, and the aqueous part tested using the Dische reaction<sup>28</sup>. This procedure was repeated until no more DNA was extracted as shown by a negative Dische reaction. All the aqueous fractions giving a positive Dische reaction were then pooled and repeatedly treated with freon (2 volumes aqueous fraction and 1 volume of freon) until deproteinization was complete (that is, until no more precipitate appeared at the intersection). The final aqueous phase was then concentrated by dialysis under pressure in a 'Cellophane' bag against distilled water overnight at 5° C, followed by dialysis against 0.15 M NaCl for 2-3 days until the desired concentration of DNA was obtained (between 100 and 1,000 µg per ml.). The pH was adjusted with sodium hydroxide to 7.2. The final material was standardized by the Dische reaction against a standard from commercial thymus DNA obtained from the Mann Laboratories. This material was passed through a V ultrafilter of fritted glass before use. No attempt was made to separate any contaminating ribonucleic acid (RNA) or any polysaccharide which may be associated with this fraction. Since the starting material was a nuclear fraction, contamination with cytoplasmic RNA was believed to be minimal. Proteins and lipids were removed by freonization.

(5) DNA-sarcoma-*H* (DNA-*h*) was prepared in a similar way to DNA-*m*, except that the starting material was the nuclear fraction of a carcinoma of kidney obtained from a human case (MH-21).

In these experiments 48 new-born litters of AKR Swiss mice, divided into six groups, were used. Of these, five groups were treated with macromolecules, while the remaining group was given sterile 0.15 M sodium chloride. Treatment of the new-born mice (to be referred to as 'preconditioning') was started within the first 24 h of life. Male young were separated from females in each group, while the mother was left with the female offspring as an additional control. Each animal in Groups I-V received daily subcutaneous injections on the first, second and third days of life and weekly intraperitoneal injections for 17 weeks thereafter starting on the seventh day and ending on the 132nd day.

Group I received 20 injections of TPS-*m* (25 µg each), giving a total of 500 µg. Group II received 20 injections of NPS-*m* (25 µg each), giving a total of 500 µg. Group III received 20 injections of <sup>3</sup>H-TPS-*m* (0.83 µg each), giving a total of 17 µg, while Group IV was given 20 injections of a complex formed by <sup>3</sup>H-TPS-*m* and DNA-*h* (0.83 µg of <sup>3</sup>H-TPS-*m* and 2 µg of DNA-*h* per injection) with a total dose of 17 µg for <sup>3</sup>H-TPS-*m* and 40 µg for DNA-*h*. Group V received 20 injections of sterile 0.15 M sodium chloride.

Group VI received daily intraperitoneal injections of TPS-*m* of 100 µg each from the first to ninth day of life (a total of 700 µg). Between 10 and 30 days of age, DNA-*m* was given subcutaneously 100 µg every other day (a total of 900 µg), while between 39 and 80 days of age, both TPS-*m* and DNA-*m* were given every third day subcutaneously—100 µg of TPS-*m* on the left side and 100 µg of DNA-*m* on the right side (a total of 1,300 µg each).

All the litters were challenged with sarcoma 180 on the 135th day of age (3 days after the final injection). The tumour from the donor mouse was removed under sterile precautions into a sterile Petri dish. It was then minced with sterile scissors and transplanted in 2 × 2 mm amounts by the trocar method into the right axillary region. The mothers were also transplanted with sarcoma 180 at the same time. The donor mice were shipped from Jackson Laboratories in Bar Harbor, kept in our laboratories for a week, and then used for transplantation on the eleventh day of tumour age. The mice were observed daily for death, starting with the fifth day of tumour age after

Table 1. THE EFFECT OF PRECONDITIONING IN EARLY LIFE WITH MACROMOLECULES EXTRACTED FROM TUMOUR TISSUE ON SARCOMA 180 TRANSPLANTED MICE

Group	No. mice	Preconditioning		Tumour take				Tumour growth MDG (mm)				Response to therapy (D-84)				
		Material	Dose ( $\mu$ g)	D-5	D-10	D-14	Mean % inhibition	D-5	D-10	D-14	Mean % inhibition	Cure	Death	T/M	Mean tumour wt.	Mean % improvement
I	9	TPS-m	500	7/9 (78)†	8/9 (89)	9/9 (100)	+3	6.5	12.9	16.5	+2	2/9 (22)†	5/9 (56)	0.17	4.26	-73
II	4	NPS-m	500	1/4 (25)	4/4 (100)	4/4 (100)	+23	4.1	12.6	14.7	+17	2/4 (50)	1/4 (25)	0.08	2.06	+25
III	10	<sup>3</sup> H-TPS-m	17	4/10 (40)	6/10 (60)	5/10 (50)	+47	3.9	5.2	5.6	+58	7/10 (70)	0/10 (0)	0.07	1.74	+86*
IV	6	<sup>3</sup> H-TPS-m	17	4/6 (67)	5/6 (83)	6/6 (100)	+10	6.2	11.5	14.8	+11	3/6 (50)	3/6 (50)	0.09	1.89	-13*
		+DNA-h	40													
V	9	NaCl		7/9 (78)	9/9 (100)	9/9 (100)		8.3	11.5	16.0		3/9 (33)	2/9 (22)	0.13	4.10	
VI	10	TPS-m	2,000	6/10 (60)	10/10 (100)	10/10 (100)	+3	6.1	10.9	13.4	+12	9/10 (90)	0/10 (0)	0.02	0.87	+119
		+DNA-m	2,200													
Mothers	5	None	None	4/5 (80)	5/5 (100)	5/5 (100)	-1	9.7	20.3	22.7	-45	1/5 (20)	3/5 (60)	0.14	4.62	-104

Mean percentage inhibition was obtained by observing the difference between the treated groups and the untreated Group V expressed as a percentage of the untreated. A positive value indicates inhibition, while a negative value indicates enhancement.

Mean percentage improvement was calculated for cure rate, death rate and T/M (tumour weight to mouse weight) ratio. Mean tumour weight was excluded.

\* No therapy was given to Groups III and IV. † Figures in parentheses are percentages.

transplantation (that is, D-5). Tumour measurements were recorded every other day in terms of mean diameter of growth (MDG) in mm (the mean of two diameters—the longest diameter and another at right angles to it).

On the fifteenth day of tumour age (D-15), Groups I, II, V, and VI were treated with <sup>3</sup>H-TPS-m—30  $\lambda$  per 30 g mouse with an activity of 200  $\mu$ c. per ml. and a concentration of 2.72 mg per ml. All the males in each group were given one treatment intravenously, while the females were given one treatment orally. Ribonuclease (obtained from Mann Laboratories) was given subcutaneously, 250  $\mu$ g to each mouse, on the day of injection and on the following day. Groups III and IV were left untreated. To be considered a tumour take, the transplanted tumour should have an MDG of 6 mm or more. Similarly, to be considered as a cure it was necessary that a regression of a tumour should be obtained from an MDG of 6 mm or more to an MDG of less than 6 mm. Mice found dead were immediately autopsied. On the 83rd and 84th day of tumour age, the surviving mice were killed. All mice were studied for distribution of radioactivity in the tumour and the various organs. The results of these radioactive studies will be reported elsewhere.

Mean percentage inhibition was obtained for both tumour take and tumour growth by observing on the same day the difference in percentage take and in tumour growth between the treated groups and the untreated control mice in Group V expressed in terms of the percentage of untreated. A positive value indicates inhibition, while a negative value indicates enhancement. The figures reported in Table 1 correspond to the mean values for the three periods referred to in the Table, namely D-5, D-10 and D-14. Mean percentage improvement refers again to the difference in cure rate, death rate, and tumour weight over mouse weight (T/M) ratios between the treated and the control groups expressed in terms of the percentage of the control values. A positive value indicates improvement, while a negative value indicates harm as a result of the treatment used. T/M was used rather than tumour weight *per se* since it is believed to be a better indicator of tumour growth and toxicity.

### Induction of Immunity to Sarcoma 180 by the Use of Small Dosage of <sup>3</sup>H-TPS for the Conditioning of New-born Mice

It is apparent from Table 1 and Fig. 1 that the use of small dosage of <sup>3</sup>H-TPS-m (as in Group III) for the conditioning of new-born mice has induced a definite state of immunity against the transplantation of sarcoma 180 later in life. This increased immunity was apparent by D-10 where the percentage tumour take was 60 per cent for this group as compared with 83 per cent for Group IV and 89–100 per cent in the remaining groups. By D-14 all the other groups had 100 per cent take, with the exception of Group III, which maintained its initial

inhibition level at D-10 and which, in addition, showed a cure of the tumour in one of the mice. Thus only 5/10 (50 per cent) of the animals had tumours at this time.

The mean percentage inhibition of tumour take for D-5, D-10 and D-14 as shown in Table 1 was +47. Similarly, the mean percentage inhibition for D-5, D-10 and D-14 showed a high value of +58. By D-21 two other animals in this group became cured, leaving only 3/10 of the animals with tumours. This situation remained until D-83, the day of killing. This meant that 7/10 (70 per cent) of the mice in whom small dosage of <sup>3</sup>H-TPS-m was used in early life have achieved protection against tumour challenge—with either no tumour developing at all or with subsequent regression of their tumours. There were no deaths in this group and the T/M ratio was 0.07 (the second lowest), while the mean percentage inhibition was +86 (the second highest).

It is to be emphasized that this group did not receive any therapy after tumour transplantation. It is also to be emphasized that the designation of the dose used in this group as 'small' is relative to the larger dose used in other groups. The work of Siskind *et al.* points to the ease with which immunity can be induced in new-born mice with pneumococcal polysaccharide antigens. It is likely that immunity in new-born mice could be induced with much smaller doses of TPS given only once on the day of birth.

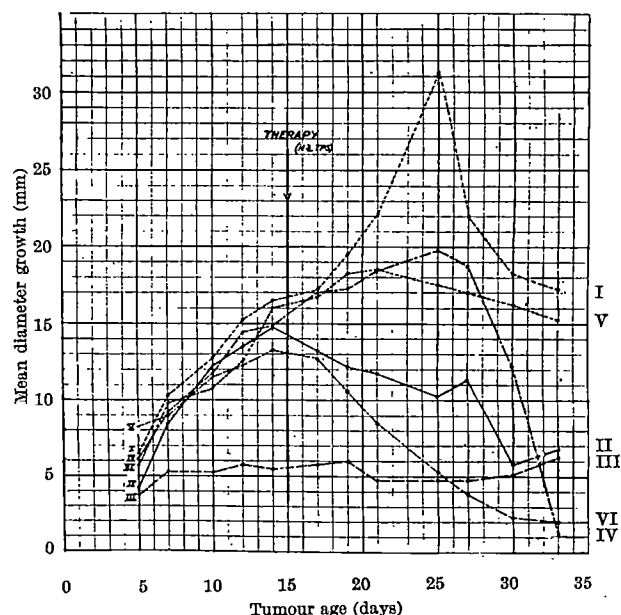


Fig. 1. The role of tritiated tumour polysaccharide (<sup>3</sup>H-TPS) in the prevention and treatment of sarcoma 180 of mice. Groups I (TPS-m), II (NPS-m), V (NaCl) and VI (DNA-m and TPS-m) were treated with <sup>3</sup>H-TPS-m. Groups III (<sup>3</sup>H-TPS-m) and IV (<sup>3</sup>H-TPS-m + DNA-h) were immunized but not treated.

It was not possible to determine from these experiments the exact role of the tritium labelling of TPS in this beneficial host response. Tritiation does not affect the antigenicity of TPS<sup>16</sup>. Although it may be possible to explain the improved host response in this group on a purely immunological basis, it appears that tritium labelling is of additional benefit to the host. The results of therapy with <sup>3</sup>H-TPS-*m* and radioactive isotope determinations (to be published elsewhere) suggest that both factors, the immunological and the radiological, are responsible for this phenomenon.

The finding that <sup>3</sup>H-TPS-*m* in small doses is capable of inducing immunity in mice which later received sarcoma 180 indicates that TPS is the antigen concerned with immunity to tumour transplantation. It is, therefore, the key antigen which is expected to bear a causal relationship to tumours and tumour induction, and justifies the basic role assigned to it in the immunological unitarian concept referred to earlier. Moreover, the protection of 70 per cent of the mice in this group represents one way for the successful immunization against the transplantation of sarcoma 180 in mice.

#### Interference with the Immunizing Capacity of Small Dosage of TPS-*m* by DNA-*h*

Of great interest is the behaviour of Group IV, which indicates that DNA-*h* can interfere with the immunizing capacity of small doses of TPS-*m* (as demonstrated earlier in Group III). The mice in Group IV, preconditioned with a complex formed *in vitro* by TPS-*m* and DNA-*h*, manifested a 67 per cent tumour take on D-5 (compared with 40 per cent for Group III); 83 per cent on D-10 (compared with 60 per cent for Group III); and a 100 per cent on D-14 (compared with 50 per cent for Group III). By D-21, while Group III had a 70 per cent cure rate, this group had only one-sixth (that is, 17 per cent) cure rate. On D-33 only three-sixths (50 per cent) were alive as compared with 10/10 (100 per cent) in Group III.

The mean percentage inhibition of tumour take was 4.7 times less, and that for tumour growth 5.3 times less, than the comparable values for Group III. The cure rate was decreased from 70 per cent in Group III to 50 per cent in Group IV, while the death rate rose from 0 in Group III to 50 per cent in Group IV.

Earlier it was shown that a common antigenicity exists between TPS obtained from sarcoma 180 and that obtained from human tumours<sup>16</sup>. It is, therefore, not surprising to find that an interaction has taken place between TPS-*m* from the mouse and human DNA-*h*. This further indicates the specific interaction between TPS and DNA as reported earlier<sup>16</sup>. It appears that as a result of the complexing of TPS and DNA, the immunologically reactive sites on the TPS molecule are only partially available for the stimulation of the immune response. In addition, antibodies to DNA and to DNA-TPS complexes may be formed which may compete with anti-TPS antibodies and interfere with their beneficial effect. The result is a state of only partial immunity associated with a higher death rate.

This interaction between TPS and DNA is believed to be of basic significance in host-tumour relationships. In this connexion it is of interest to refer to my earlier finding<sup>29</sup> that the uptake of <sup>3</sup>H-TPS by cancer tissue was found to be blocked by commercial DNA, RNA and by NPS but increased with pretreatment with TPS. Recently, Mannick<sup>30</sup> reported an inhibition of the homograft immune response by incubation of immune lymph node cells with RNA extracted from lymph nodes of the homograft donor or from lymph nodes of an indifferent rabbit. Since transplantation antigens are believed also to be polysaccharide in nature<sup>21,22</sup>, this interaction between nucleic acids and polysaccharides is further emphasized. It is pertinent to refer again to the investigations of Paleczuk *et al.*<sup>31</sup> and of Plescia *et al.*<sup>17</sup> concerning DNA-

associated antigens. These workers found complexes between nucleic acid and polysaccharide revealed by heat in *Brucella abortus* and also in the Ehrlich-Lettre mouse tumour cells. These antigens, which were found to be polysaccharide in nature, were only revealed after heating DNA-rich preparations to 100° for 10 min. It is of interest to note that in our original preparation of TPS<sup>12</sup> heat at 100° was used for 30 min. It is not unlikely that this interference by DNA in the antigenicity of TPS may explain in part some negative results in which investigators were unable to detect antigens in whole homogenates of tumour tissue.

#### Induction of Susceptibility to Sarcoma 180 by the Use of Large Dosage of TPS-*m*

The mice in Group I receiving 500 µg of TPS-*m* in early life did not manifest an increased immunity, but rather an increased susceptibility to transplantation of sarcoma 180 in later life when compared with the control group receiving 0.15 M sodium chloride (that is, Group V). This trend was less apparent in the early part of the experiment before treatment was started, but became well established after therapy with <sup>3</sup>H-TPS-*m*. Thus by D-5 the percentage tumour take was similar in the two groups, while the MDG was greater for Group V. By D-10, the percentage tumour take was 89 per cent for Group I as compared with 100 per cent for Group V, while the MDG was now slightly greater for Group I than for Group V. The mean percentage inhibition before therapy was +3 and +2 for tumour take and tumour growth respectively, while the mean tumour inhibition became -73 after therapy. The cure rate was 22 per cent (compared with 33 per cent in Group V), the death rate was 56 per cent (compared with 22 per cent in Group V) and the *T/M* ratio was 0.17 (compared with 0.13 in Group V).

This unresponsiveness to sarcoma 180 induced by the use of large dosage of TPS-*m* seems to be a specific phenomenon, since NPS-*m* given at the same dose-level (that is, 500 µg as in Group II) did not give rise to it. In this latter group (Group II), there was a mean percentage inhibition of +23 for tumour take and +17 for tumour growth, while the mean percentage improvement was +25. It was of interest to note that the cure rate was 50 per cent as compared with 22 per cent for Group I and 33 per cent for Group V. The death rate for Group II was 25 per cent as compared with 56 per cent for Group I and 22 per cent for Group V.

The finding that small doses of TPS-*m* can induce immunity, while large doses can induce susceptibility to sarcoma 180, may explain the paradoxical phenomenon of increased immunity as described by Barrett<sup>32</sup> and of enhancement as described by Kaliss<sup>33</sup> in response to immunization with tumour tissue. It is of interest to note the fate of one animal in this group, which was not included in this investigation since it died before tumour transplantation but which manifested a unique phenomenon. This was a male injected with large doses of TPS-*m* (25 µg per injection with a total dose of 475 µg). Three days after the nineteenth injection and two days before receiving the final injection, the mouse experienced breathing difficulty and paralysis of the hind legs, while lumps became apparent under the jaw, at the back of the neck and in the thigh regions. The mouse died the next day. At autopsy, 11 bean-shaped glands were found, the largest measuring 13 × 8 × 8 mm and the smallest measuring 2 × 1 × 1 mm—four in the cervical region, five in the axillary region and two in the inguinal region. Microscopic examination as reported by Dr. C. F. Varga<sup>34</sup> of the Pathology Department, Muhlenberg Hospital, revealed that "the tissue is composed of lymphocytes heavily infiltrated by plasma cells and having very little underlying stroma. There was no evidence of malignant tumour and the picture was believed to be compatible with lymphocytic and plasmocytic hyperplasia". Baruah<sup>34,35</sup>



had shown that the host animal (rat or mouse) reacts to a progressively growing transplanted tumour by developing a characteristic plasma-cellular reaction in the draining lymph node and spleen and that plasma cells are involved in the production of antibodies against the tumour. This plasma response was believed to be the basis for the heightened 'adoptive immunity' described by Mitchison<sup>36</sup> for mouse lymphosarcoma and confirmed by Billingham *et al.*<sup>37</sup> using skin homografts. The marked plasma cellular reaction in a mouse receiving TPS provides another link in the chain of evidence pointing to TPS as the antigen concerned with the host response against tumours.

#### Response of Mice with Sarcoma 180 to Therapy with <sup>3</sup>H-TPS-*m*

The response of mice in Groups I, II, V, VI and that of the mothers to the one treatment given on the D-15 tumour age with 30  $\lambda$  per 30 g mouse (containing 0.82  $\mu$ g of <sup>3</sup>H-TPS with an activity of 60  $\mu$ c.) is recorded in Table 1 and Fig. 1. The most successful results were shown by Group VI, in which unresponsiveness to both TPS-*m* and DNA-*m* was induced. In this group 90 per cent were cured, none died by D-83 and the mean tumour weight was 0.87 g, with a *T/M* ratio of 0.02—the lowest among all groups. The mean percentage improvement was +119.

The next best response was that of Group II, in which unresponsiveness was induced to NPS-*m*. In this group 50 per cent became cured and 25 per cent died. The mean tumour weight was 2.06 g and the *T/M* ratio was 0.08. The mean percentage improvement was +25 per cent. The control group (Group V) receiving only 0.15 M sodium chloride in early life had a 33 per cent cure in response to treatment with <sup>3</sup>H-TPS-*m*; 22 per cent death rate; a mean tumour weight of 4.20 g, and a *T/M* ratio of 0.13.

Among the litters, the group with the poorest clinical response to therapy was Group I, in which unresponsiveness to TPS-*m* was induced in early life. In this group the cure rate was only 22 per cent, the death rate 56 per cent, the mean tumour weight 4.26 g, the *T/M* ratio 0.17, and the mean percentage improvement, -73. The response of the mothers to therapy was very poor—a 20 per cent cure rate, 80 per cent death rate, a mean tumour weight of 4.62 g, a *T/M* ratio of 0.14 and a mean percentage improvement of -104. Of great interest is the finding that the only cure in this group was the mother of Group III—the group which received a small dose of <sup>3</sup>H-TPS-*m* in early life and in which a 70 per cent cure rate was observed without any treatment. This may represent a form of repeated immunization of the mother by minute doses of <sup>3</sup>H-TPS-*m*, the result of licking offspring, or of contamination from the urine of the offspring. The other mothers died as follows: *M*-VI on D-37; *M*-I on D-27; *M*-IV on D-23; and *M*-V on D-20.

The remarkable response of Group VI to therapy with <sup>3</sup>H-TPS-*m* will be analysed more fully elsewhere when the distribution of radioactivity in various tissues will be reported. In this article two possible explanations of this phenomenon of cure will be briefly referred to. First, it may be explained on the basis of increased and selective uptake by tumour cells of <sup>3</sup>H-TPS-*m* as compared with normal cells. This could destroy the tumour cells—the result of damage by  $\beta$ -rays of tritium—without affecting the neighbouring normal cells. This was the original aim of these investigations. This possibility, however, seems to be less important, since the uptake of <sup>3</sup>H-TPS-*m* by tumour cells does not seem to correlate with cure, while the uptake of <sup>3</sup>H-TPS-*m* by lymph glands has a more meaningful correlation.

Secondly, this phenomenon may be explained on the basis of what may be called 'reverse tolerance', where the state of immunological unresponsiveness becomes reversed

to a state of responsiveness and immunity in the host as a result of a direct effect on the reticulo-endothelial lymphocyte system. On the macromolecular level, this could be explained as follows.

While a poor response to therapy was obtained in Group I made unresponsive to TPS-*m* in early life, a remarkable response to therapy was observed in Group VI made unresponsive to both TPS-*m* and DNA-*m*. The difference therefore seems related to the host immune response to DNA. In the presence of such an immune response to DNA (as in Group I), antibodies to DNA would be expected to form and to combine with any reactive sites on the abnormal DNA molecules—a situation which would interfere with the uptake of <sup>3</sup>H-TPS-*m* by DNA-*m*. In this connexion it is interesting to note my earlier finding of antibodies to DNA in cancer homogenates<sup>38</sup>. In the absence of such an immune response, and in the presence of a state of unresponsiveness to DNA (as occurs in Group VI), the reactive sites on the DNA molecules will remain free and available for interaction with <sup>3</sup>H-TPS-*m*. The result of such an interaction between <sup>3</sup>H-TPS-*m* and DNA-*m* may well transform an abnormal DNA into a normal DNA as the result of the energy liberated from tritium. Should this happen in a lymph gland (made unresponsive to TPS and therefore unable to form protective antibodies against TPS), such a reverse transformation of abnormal DNA to normal DNA may well mean a reactivation of protective antibody formation and may explain the cure observed in this group.

It has been shown earlier using the Schultz-Dale technique and cutaneous hypersensitivity<sup>16</sup> that such an interaction between TPS and DNA can occur. It is in fact evident from this investigation, as shown by Group III, in which interference with the immunizing effect of small dosage of TPS was manifested by DNA. It is to be emphasized that in Group VI, TPS-*m* and DNA-*m* were not complexed before injection as was done in Group III. In fact, TPS-*m* injections were made on the left side of the animal, while DNA-*m* injections were made on the right side. It is also worth noting the finding by Siskind *et al.*<sup>7</sup> that between the day of birth and one month of age mice do not appear to differ with respect to ease of induction of unresponsiveness. The fact that the mice in Group VI received TPS for the first nine days of life and DNA from day 10 to day 30 of age should not make much difference so far as the induction of unresponsiveness is concerned.

In conclusion, it should be emphasized that the results reported in this investigation are of theoretical and practical significance in cancer immunology. Theoretically, these results provide confirming evidence for the immunological unitarian concept of cancer as postulated earlier<sup>16,18</sup>, in which TPS has been assigned a key role in the explanation of immunity and susceptibility to cancer. This concept is to be published in book form shortly<sup>19</sup>. Also this novel interaction between TPS and DNA, which has been described earlier<sup>16</sup> and which we believe to be of basic significance in biology, is further confirmed.

From a practical point of view, these results indicate clearly the significance of TPS in relation to immunity and susceptibility to cancer. Thus, while mice given small doses of <sup>3</sup>H-TPS-*m* in early life manifested a definite immune response against transplanted sarcoma 180, mice given large doses of TPS-*m* became immunoparalysed and exhibited an increased susceptibility to the disease as well as an increased death rate. The utilization of small doses of TPS-*m* as a vaccine to stimulate the immune response against tumours in man thus becomes a possibility. Furthermore, the successful treatment of tumour-bearing mice with <sup>3</sup>H-TPS-*m* when the host is properly conditioned may afford a new and promising approach to the therapy of cancer in man.

This work was done while I was director of research at the Muhlenberg Hospital, Plainfield, New Jersey. ]

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## FINE STRUCTURE OF A VESICULATED RETICULUM IN THE LIGHT ORGAN OF THE GLOW-WORM, *Lampyrus noctiluca*

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RECENTLY a histochemical and electron microscopical study of the light organ of the glow-worm (*Lampyrus noctiluca*) was undertaken<sup>1,2</sup>. A vesiculated reticulum, not previously described, was found to be present in the cells of the photocyte layer.

Previous electron microscopical studies of the light organ of fire-flies have given much morphological information<sup>3,4</sup>. Smith<sup>5</sup> described details of nervous innervation and attempted to correlate the fine structure with known biochemical reactions in light production.

Specimens for electron microscopy were prepared in the following manner. Female animals were killed by decapitation and appropriate pieces of the light organs of abdominal segments 5 and 6 were dissected out. Light organs also occur in the male but are considerably smaller and were considered inconvenient for use in this investigation. The pieces were fixed in 1 per cent potassium permanganate<sup>6</sup> or 1 per cent osmium tetroxide solution (both buffered with veronal acetate at pH 7.4) for 3 h. They were dehydrated in graded ethanols and embedded in 'Araldite'<sup>7</sup>. Sections were cut on a Porter-Blum ultra-microtome and mounted on carbon-coated grids. Sections were stained with 1 per cent uranyl acetate in absolute ethanol for 30 min at 50° C, or with lead citrate for 30 min at room temperature<sup>8</sup>, and examined with a Siemens Elmiskop electron microscope. The original magnifications used were from c. × 4,000 to 20,000, usually about 10,000. Osmium-fixation was not very successful for this particular tissue.

The light organs consist of four main parts: a transparent cuticle, a hypodermis, a photocyte cell layer several cells thick, and a single layer of cells comprising the reflector layer. Photocyte cells contain photocyte granules and a vesiculated reticulum (Fig. 1).

The reticulum joins the nuclear membrane (Fig. 2), and in every section of a nucleus the reticulum can be clearly seen to join as many as ten times (see Fig. 1).

It is not certain that it also joins the outer cell membrane, but it appears that it may do so (Fig. 3). The reticulum is invaginated, forming vesicles that vary considerably in size and shape, some being as large as average-sized photocyte granules (0.6 μ diameter). Some of these vesicles contain structures that have the appearance of photocyte granules in the course of formation (Fig. 4). In addition to these invaginations, it is likely that some of these vesicles are discrete structures and are not in con-

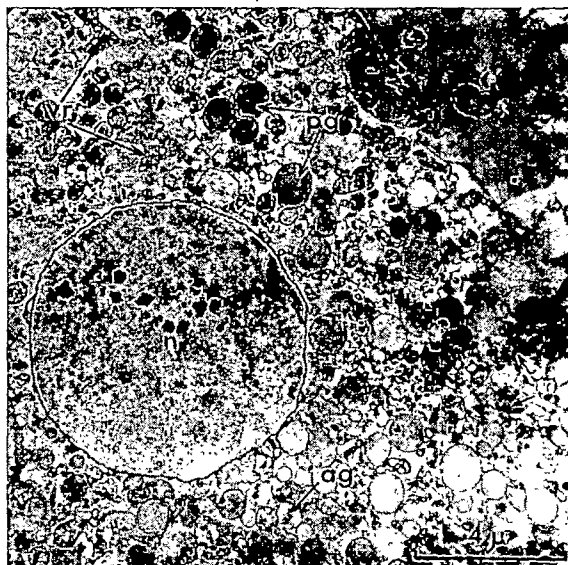


Fig. 1. General view of photocyte cells (permanganate fixed, uranyl acetate stained) showing amorphous granules (ag), mitochondria (m), nucleus (n), photocyte granules (pg) and the vesiculated reticulum (vr).

tinuity with the general cell cytoplasm. In osmium-fixed material the reticular vesicles can be seen to contain granules that are sited on the membranes and are probably ribosomes (Fig. 5).

Apart from the photocyte granules and mitochondria, the photocyte cells also contain amorphous 'granules' which may be the same as the "differentiated zone granules" of Smith<sup>6</sup> and the "granules containing dense material" of Kluss<sup>4</sup>.

A hypothesis is that the photocyte granules are produced by the reticulum and then released into the general cell cytoplasm. The granules may then release their contents in some way and light production occurs. Following light production, the granules may revert to "differentiated zone granules". Alternatively it could be that following light production the photocyte granules



Fig. 2. The vesiculated reticulum joining the nuclear membrane (nm) in a complex fashion (permanganate fixed, uranyl acetate stained)



Fig. 3. The vesiculated reticulum showing it apparently joining the nuclear membrane and outer cell membrane (om) (permanganate fixed, lead citrate stained)

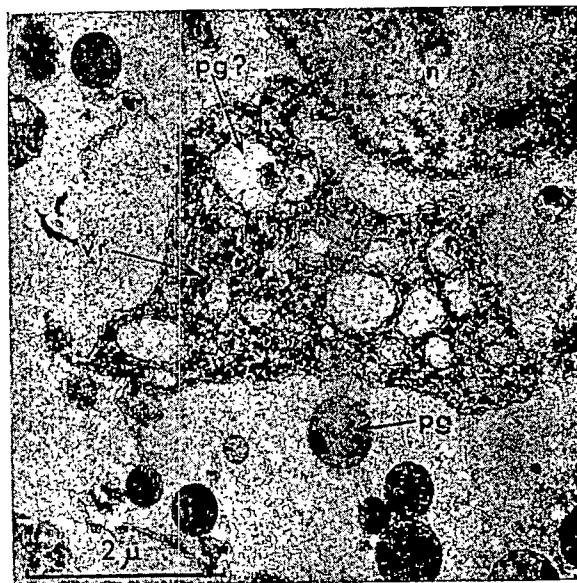


Fig. 4. The vesiculated reticulum possibly containing a photocyte granule *pg* (permanganate fixed, uranyl acetate stained)

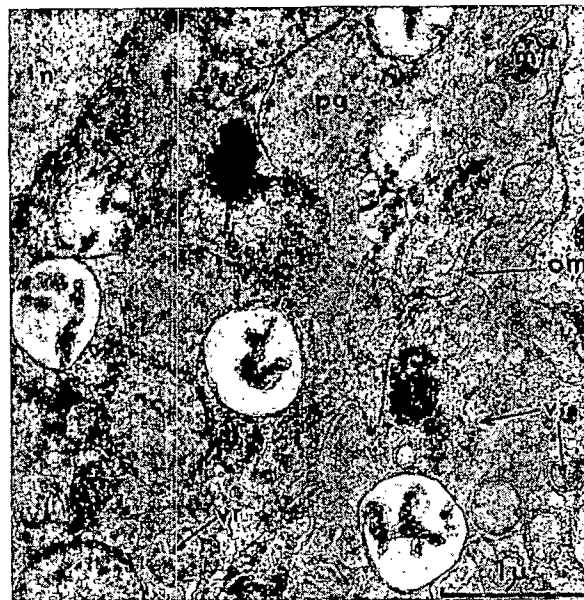


Fig. 5. The vesiculated reticulum showing the contained granules, probably ribosomes (osmium tetroxide fixed, lead citrate stained)

are taken into the vesiculated reticulum and broken down there. The circumstantial evidence for the association of the reticulum with granule production and hence light production is that neither the granules nor the vesiculated reticulum are present in the so-called reflector layer ("dorsal cell layer" of Smith<sup>5</sup>) although there is normal endoplasmic reticulum. What the photocyte granules contain is purely conjectural; for example, it could be a luciferin-luciferase complex. Previous investigations of other tissues have at least established the granule producing function of the Golgi apparatus<sup>9</sup>. Approximate calculations show that the photocyte granules take up about 1 per cent of the total cell volume (not including the nucleus) so the reticulum would seem quite capable of producing this quantity.

It is possible that this unusual reticulum is peculiar to glow-worm light organs. However, permanganate fixation, which fixes mainly membranes<sup>6</sup>, does not appear

have previously been used for fire-fly light organs, and this could explain the apparent absence of the vesiculated reticulum in previous investigations. With osmium tetroxide the reticulum is, to a certain extent, masked by the subsequent staining of additional structures.

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## SELENO-CYSTATHIONINE, A PHARMACOLOGICALLY ACTIVE FACTOR IN THE SEEDS OF *Lecythis ollaria*

### Cytotoxic and Depilatory Effects of Extracts of *Lecythis ollaria*

COCO de mono (*Lecythis ollaria*), or 'monkey nut', is a large deciduous tree widely distributed in Central and South America. In certain parts of Venezuela, ingestion of the nuts of this tree can produce a toxic syndrome in man<sup>1</sup>. The acute symptoms include abdominal discomfort, nausea, vomiting and diarrhoea. A striking feature of the syndrome is the loss of scalp and body hair a week or two after ingesting the nuts, after the initial acute phase has terminated. The alopecia is not permanent, and new hair grows.

It seemed possible that this alopecia could be due to the presence of a cytotoxic factor affecting the hair follicle itself, since most of the cytotoxic agents used in cancer chemotherapy can also produce hair loss<sup>2</sup>. Consequently, extracts of these nuts were tested for cytotoxic activity using mammalian cells (mouse fibroblasts) growing *in vitro* as the test system. The methods employed have been described previously<sup>3</sup>; briefly, replicate cultures of mouse fibroblasts (Earle's 'L' cells<sup>4</sup>, in 20 ml. Eagle's<sup>5</sup> medium) are established in 8-oz. prescription bottles. The number of cells in each of a representative group of bottles (3 bottles per group) is determined the following day, and sterile test solutions (volumes no greater than 2 ml.) are added directly to the culture medium of the experimental groups. The cultures are then returned to the incubator (37° C) for a period of five days. Cell counts are obtained with a Coulter electronic cell counter. Controls are always included; these will generally reveal 10- to 30-fold multiplication of the 'day 1' cell count ( $2 \times 10^6$  cells/bottle) after five days of incubation. The experimental bottles will have a variable number of cells; this is conveniently expressed as a percentage of the control growth.

Nuts were obtained from a tree in the western plains of Venezuela (state of Portuguesa) which was known to produce a nut toxic to man. The shelled, crushed nuts were extracted first with benzene, then sequentially with ethanol, water, and finally 10 per cent sodium chloride. These various fractions were tested for cytotoxic activity in the tissue culture system. Benzene and ethanol were removed by evaporation before testing; the oily residues were suspended in water. A marked inhibition of growth was found with water extracts of these nuts; the other actions were inactive. Using the tissue culture system as a bioassay, it was readily shown that the water-soluble, active material was dialysable and heat stable (boiling water bath, 5 min) in both acid and alkaline solutions. It was insoluble in ethanol, and could not be extracted into chloroform from acid or alkaline aqueous solutions. Incineration of the samples was done by a dry method (direct incineration in an ignition tube) and the appearance of a light red deposit (now realized to be red selenium) as noted at the upper end of the tube. The residual ash was dissolved in water and tested for cytotoxicity; it was inactive. Wet ignition was done with concentrated

sulphuric and nitric acid, boiling off the nitric acid. Water was then added, the sample neutralized, and tested for activity. All cytotoxic activity was lost in this procedure also, demonstrating the organic nature of the active agent. The biologically active material was well absorbed from neutral solution on 'Dowex-50' (H<sup>+</sup>), and (partially) on 'Dowex-1' (Cl<sup>-</sup>) from an alkaline solution.

A concentrated extract of coco de mono was prepared as follows: 68 g shelled crushed nuts were extracted with benzene and the residue suspended in 200 ml. water. This was placed in dialysis tubing and dialysed for 2 days against 5 l. distilled water at 3° C. The dialysis bath was changed once, after 24 h. The combined dialysates were reduced in volume to 70 ml. on a rotary evaporator, and shaken vigorously with chloroform to remove traces of proteins or lipids. The resulting clear yellow solution was assayed for cytotoxicity. Further purification was effected by ion exchange chromatography on a 'Dowex-50' (H<sup>+</sup>) column (Fig. 1). Biologically active material was obtained when the concentrations of KCl reached 1 M. Optical density at 260 mμ was also determined since it seemed at first to be associated with biological activity. However, purer samples of the biologically active material had little ultra-violet absorbing property. The two chromatographic fractions (numbers 24 and 25) showing biological activity were combined, and a yellowish hygroscopic material (30.5 mg) obtained by the addition of ethanol. Bioassay (Fig. 2) of this material revealed a 50 per cent inhibition of growth of mouse fibroblasts at a concentration of about 4 μg/ml., and about 80 per cent of the biological activity present in the original aqueous extract of coco de mono was accounted for in these two chromatographic fractions.

At about this time a group of chemists at Wyeth Laboratories<sup>6</sup> succeeded in isolating a larger quantity of pure crystalline material from these nuts which they identified as seleno-cystathionine, the selenium-containing analogue of the sulphur amino-acid, cystathionine. A comparison of the chemical and biological properties of the material obtained in the two laboratories showed them to be similar in every respect.

Attempts were made to reverse the cytotoxic effect of seleno-cystathionine by the addition of a variety of sulphur-containing compounds. It was observed (Table 1) that *L*-cystine (but not *D*-cystine) was effective in blocking the cytotoxic effect of seleno-cystathionine, and *D,L*-allo-cystathionine and *L*-cystathionine were also effective, although not as active in this respect as *L*-cystine. Other sulphur-containing compounds such as reduced glutathione, homocystine and mercaptoethanol could not be shown to have any effect. *L*-Cysteine was toxic to the cells in concentrations greater than 10 μg/ml. (possibly by chelating trace metals in the culture medium), but a slight protective effect could be demonstrated with *L*-cysteine if 200 μg (that is, 10 μg/ml. final concentration in the culture medium) was added daily during five days incubation with 10 μg/ml. seleno-cystathionine. Cystine was equally effective in blocking the cytotoxic effects of



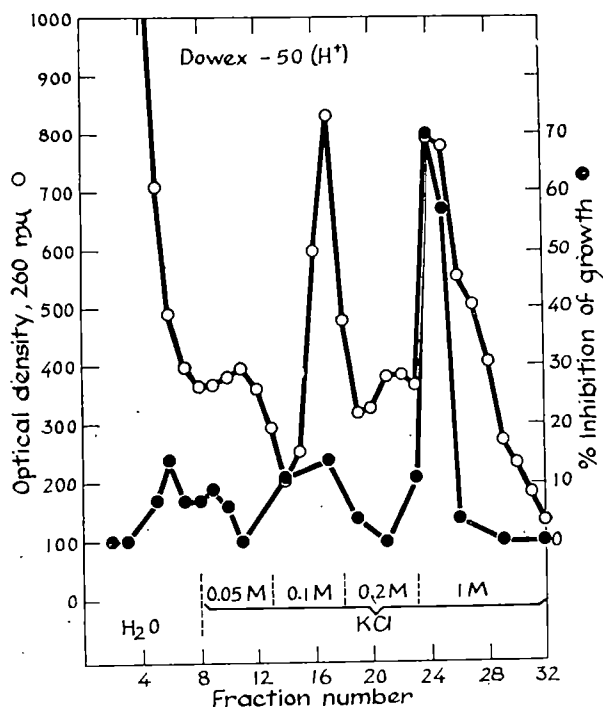


Fig. 1. Chromatographic purification of cytotoxic material from an aqueous extract of coco de mono. An aqueous extract was prepared as described in the text, and a portion representing 30 g of shelled nuts was applied to a 'Dowex-50'  $\times$  4(H<sup>+</sup>) column, 11  $\times$  120 mm. It was washed through with water, and elution was begun with increasing concentrations of KCl. Fractions of 25 ml. each were collected. Optical density at 260 m $\mu$  was determined directly, and biological activity was determined by testing the effect of 0.1 ml. of each fraction on mouse fibroblasts growing *in vitro*, as described in the text

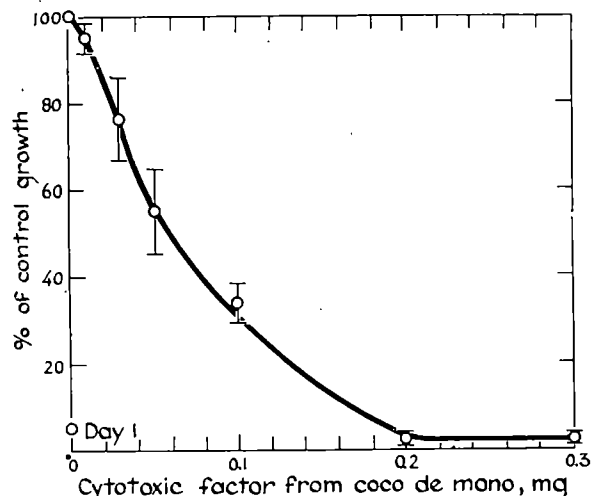


Fig. 2. Dose-response curve to an aqueous solution of purified material from coco de mono. Material obtained from fractions 24 and 25 (Fig. 1) was dissolved in 0.01 N HCl at a concentration of 1 mg/ml. Various amounts of this solution were added to mouse fibroblasts growing *in vitro*, as described in the text. The volume of culture medium used was 15 ml.; hence, the IC<sub>50</sub> (concentration required for 50 per cent inhibition of growth) is about 4  $\mu$ g/ml. in this experiment. The mean and standard errors of groups of three bottles are indicated on the figure

seleno-cystathionine, or of crude water extracts of coco de mono. This fact, plus the fact that virtually all the cytotoxic activity present in water extracts of coco de mono was accounted for by the seleno-cystathionine isolated, leads us to suggest that while there may be other selenium-containing compounds present in these nuts, cytotoxic activity seems to be due solely to seleno-cystathionine.

Seleno-cystathionine was first isolated, as a 2 : 1 mixture with cystathionine, from *Astragalus pectinatus* by Horn and Jones<sup>7</sup> in 1941. More recently, it has been detected

Table 1. REVERSAL OF SELENO-CYSTATHIONINE TOXICITY

Various compounds were added to the culture medium, in the presence and absence of a toxic concentration of seleno-cystathionine, in an effort to reverse the growth-inhibitory effect of the selenium-containing amino-acid. All agents and the seleno-cystathionine were added to the culture medium or the day after inoculation of the bottles. The results are given as a percentage of the control growth, after 5 days of incubation, expressed as the mean of a group of three bottles.

Culture medium supplemented with:	Without seleno-cystathionine (%)	With seleno-cystathionine, 10 $\gamma$ /ml (%)
Nothing	100	7
<i>l</i> -cystine, 10 $\gamma$ /ml.	111	11
<i>l</i> -cystine, 100	108	89
<i>l</i> -cystine, 200	121	111
<i>d</i> -cystine, 100	83	6
<i>d</i> -cystine, 300	68	7
<i>d,l</i> +allo-cystathionine, 10	96	6
<i>d,l</i> +allo-cystathionine, 100	86	38
<i>d,l</i> +allo-cystathionine, 300	97	76
<i>d,l</i> -homocystine, 100	89	3
<i>l</i> -glutathione, red., 10	94	6
<i>l</i> -glutathione, red., 100	10	4

in *Stanleya pinnata* by Virupaksha and Shrift<sup>8</sup>. However the larger part of organic selenium in the selenium accumulating plants *Astragalus bisulcatus*, *A. crotalaria*, and *Oenopsis condensata* seems to be Se-methylseleno-cysteine<sup>9,10</sup>, and it seems likely that this is the organic form of selenium present in several plants which is responsible for the livestock diseases called 'blind-staggers' and 'alkali disease'.

Human intoxication with seleniferous plant material has not been extensively described, although it is known to occur in parts of Colombia, South America<sup>11</sup>. Report of hair loss associated with toxic foods go back as far as 1560 in that part of the world. In the United States hair loss associated with selenium poisoning has been reported among certain Indians<sup>12</sup>. Weisberger and Suhrlund<sup>1</sup> administered synthetic *d,l*-seleno-cystine to a few patient with leukaemia and reported severe toxicity with symptoms of persistent and severe nausea, vomiting, anorexia and hair loss, a syndrome strongly resembling the toxic effects seen in man after ingesting coco de mono.

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### Structure of the Pharmacologically Active Factor in the Seeds of *Lecythis ollaria*

It has been known for many years that the Brazil nut like seeds of *Lecythis ollaria*, or coco de mono, a large deciduous tree indigenous to Venezuela, contain a factor which will produce alopecia and a variety of other pharmacological manifestations<sup>1</sup>. It was therefore of interest to determine the chemical structure of the agent or agents responsible for these effects.

Paper chromatography of crude aqueous extracts showed the presence of one intense, three moderate, and at least three very weak ninhydrin-positive spots. The major component was well differentiated from most common amino-acids and mimosine in standard paper chromatographic systems: *n*-butanol/propionic acid/water 10 : 5 : 7, *n*-butanol/pyridine/water (1 : 1 : 1), and phenol/water (5 : 2). Since the major component had ion exchange properties similar to those found in preliminary isolation experiments based on tissue culture cytotoxicity, subsequent purification steps were guided by paper chromatography.

A satisfactory isolation scheme was developed as illustrated in the following example. Unshelled nuts (2.6 kg) were ground in a Waring blender and the solids defatted first for 1 h with 4 l. of benzene at room temperature and then with 3 l. of ethanol at 60° C. The residue was extracted with 6 l. of water at 80° C for 1.5 h. The filtrate was concentrated to 200 ml. and dialysed against 18 l. of cold distilled water. The dialysate was concentrated to 1.1 l., adjusted to pH 11.0 with sodium hydroxide, and centrifuged. The supernatant solution was passed through a 40 × 1.8 cm column of 'Dowex-1' × 10 (Cl<sup>-</sup>). The adsorbed active compound was then eluted with 1 M pyridine-acetic acid buffer, pH 6.7, and the fraction with the highest amino-acid content, as determined by ninhydrin assay<sup>3</sup>, was concentrated 10-fold and then chilled, with the resulting deposition of 250 mg of needle-shaped crystals. Gradual addition of ethanol in the cold yielded an additional 650 mg of crystals which were structurally and chromatographically identical with the original crystals.

Infra-red analysis of the crystals strongly pointed to an amino-acid and showed no hydroxyl, amide, or phenyl groups, while ultra-violet spectra showed only end-absorption. Nuclear magnetic resonance spectra in deuteronium oxide suggested an asymmetrical compound with no methyl groups. Qualitative assays showed a high selenium content, which, together with the other properties observed, suggested that this was one of the selenium analogues of the natural sulphur amino-acids previously observed in grasses and legumes grown in seleniferous areas<sup>3-6</sup>. Paper chromatography, elemental analysis, and various colour tests readily eliminated the selenium analogues of numerous —S—S—, —SH, —S—CH<sub>2</sub>—S— and —S—R— compounds, and led to the possibility of a *bis* compound with a single selenium atom, such as a selenium ether: HOOC—CH(NH<sub>2</sub>)—(CH<sub>2</sub>)<sub>n</sub>—Se—(CH<sub>2</sub>)<sub>m</sub>—CH(NH<sub>2</sub>)—COOH. The compound was therefore subjected to Raney nickel hydrogenolysis by the procedure of Mozingo *et al.*<sup>7</sup> to remove the selenium. The products were chromatographed, using the three paper chromatographic procedures described here, and the only components found were alanine and α-amino butyric acid. These results indicated that the compound was the selenium analogue of either cystathionine or β-methylanthionine: HOOC—CH(NH<sub>2</sub>)—CH<sub>2</sub>—Se—CH<sub>2</sub>—CH<sub>2</sub>—

CH(NH<sub>2</sub>)—COOH or HOOC—CH(NH<sub>2</sub>)—CH<sub>2</sub>—Se—CH(CH<sub>3</sub>)—CH(NH<sub>2</sub>)—COOH, respectively.

The infra-red spectrum of the isolated compound was practically identical with a published spectrum for cystathionine<sup>8</sup>, and the nuclear magnetic resonance data eliminated β-methylanthionine. Throughout the foregoing work some sulphur was present, presumably due to the crystallization of cystathionine with the selenium analogue, as had been observed by Horn and Jones<sup>4</sup>. Subsequent recrystallization, however, yielded an essentially sulphur-free product with the following elemental analysis: calculated for C<sub>7</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>Se: C, 31.3; H, 5.2; N, 10.4; Se, 29.4. Found: C, 31.3; H, 5.2; N, 10.0; Se, 29.2. The optical rotation was +36.5° (1 per cent in 1 N hydrochloric acid).

Incubation with D-amino-acid oxidase and subsequent chromatography showed no loss of the starting material and thus the compound would seem to be L-2-amino-4-[(L-2-amino-2-carboxyethyl)selenyl]butyric acid or cysta-selenonine.

Recent investigations by Aronow<sup>10</sup> have shown the crystalline product to produce the same type of toxicity in rats as crude aqueous extracts of the *Lecythis ollaria* seeds.

The previously described principal source of the selenium analogue of cystathionine is *Astragalus pectinatus* ('loco weed') of the western United States<sup>4</sup>, but Virupaksha and Shrift<sup>9</sup> have recently shown by methods similar to those reported here that the compound is also produced by *Stanleya pinnata*. The present results add a new genus, *Lecythis*, to those known to produce selenium amino-acids<sup>11</sup>. To our knowledge, plants containing more than trace amounts of selenium have not been previously observed on the South American continent.

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## EFFECT ON MYXOVIRUS OF MITOMYCIN C, ACTINOMYCIN D, AND PRETREATMENT OF THE HOST CELL WITH ULTRA-VIOLET LIGHT\*

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THE results previously obtained concerning the multiplication of influenza viruses suggest that host-cell DNA plays an important part<sup>1</sup>. This conclusion has been drawn from the following evidence: Although

\* The experiments were performed in part at the Max-Planck-Institut für Virusforschung, Tübingen.

influenza viruses contain single-stranded ribonucleic acid (RNA)<sup>2</sup>, their multiplication can be inhibited by actinomycin D<sup>3,4</sup>, which is known to interfere with the template function of DNA<sup>5-8</sup>. Furthermore, if the host cell is pretreated with ultra-violet light and then infected with an influenza A virus (fowl-plague virus), there is no multiplica-

tion of the virus as measured by haemagglutination<sup>1</sup>. On the other hand, synthesis of new DNA is not necessary for multiplication of fowl-plague virus, since this is not influenced by the DNA-inhibitor aminopterin<sup>2</sup>. Thus, while agents which render the host-cell genome non-functional, or which inactivate it, prevent the formation of influenza viruses, inhibitors of DNA synthesis do not.

By way of contrast, another myxovirus, Newcastle disease virus (NDV), multiplies normally in actinomycin-treated cells<sup>3,4</sup> or in ultra-violet-irradiated cells<sup>1</sup>.

This article is concerned with the action of some agents which react with, or inactivate, host-cell DNA, and reports experiments carried out to determine whether or not the host DNA does influence the multiplication of influenza viruses.

The 'Rostock' strain of fowl-plague virus and the 'Italien' strain of NDV were used throughout. Methods concerning the preparation of tissue cultures and their infection and the assays and chemicals used have already been described<sup>5</sup>. Ultra-violet irradiation of the tissue cultures was performed in the absence of medium with an ultra-violet lamp (NK 20/40 V, Quarzlampengesellschaft, Hanau, Germany) at a distance of 32 cm. The actinomycin D used was a gift of Merck, Sharp and Dohme, New York. Mitomycin C was obtained from the Sigma Chemical Co., St. Louis, Missouri.

(1) *Influence of pretreatment of host cells with mitomycin C or ultra-violet light on the multiplication of myxoviruses.* Mitomycin C and ultra-violet light are known to destroy the cell genome<sup>10-12</sup>. If cell DNA were necessary for the multiplication of fowl-plague virus, it is conceivable that pretreatment with this antibiotic or ultra-violet light would have an inhibitory effect on the formation of this virus but not on NDV.

Mitomycin C does not influence the isolated virus particles so far as infectivity and haemagglutination are concerned. The effect of mitomycin C on the multiplication of fowl-plague virus and NDV was tested by addition of different doses of the antibiotic to the cultures immediately after infection. Seven or eight hours later, respectively, the infectivity, haemagglutination, neuraminidase, and complement-fixing (CF) activity were tested in the disrupted cells. The results are shown in Table 1. The antibiotic inhibits the formation of all components of fowl-plague virus but not of NDV. However, the synthesis of the various components of fowl-plague virus shows different sensitivities to the action of mitomycin.

Table 1. INFLUENCE OF DIFFERENT DOSES OF MITOMYCIN C ON THE MULTIPLICATION OF FOWL-PLAGUE AND ND VIRUSES

	Fowl-plague virus				NDV			
	$\mu\text{g/ml.}$	HA-units	Neuraminidase $\mu\text{g}$	CF-titre	HA-units	Neuraminidase $\mu\text{g}$	CF-titre	
	mitomycin	P.F.U.	NANA/ml.	Q	P.F.U.	NANA/ml.	Q	
0	1,024	$3.7 \times 10^7$	226	4.8	128	$2.3 \times 10^7$	40.5	3.7
10	1,024	$4.1 \times 10^7$	251	3.7	128	$8.0 \times 10^7$		3.7
20	64	$2.7 \times 10^7$	110	3.7	128	$9.2 \times 10^7$		3.7
30	16	$3.2 \times 10^6$	28.5	3.3	128	$1.0 \times 10^8$		3.7
40	2	$1.6 \times 10^6$	6.2	2.9	64	$3.0 \times 10^7$	32.5	3.7
50	2	$1.0 \times 10^6$		2.2	64	$1.5 \times 10^7$		3.3
60	2	$1.2 \times 10^6$			64	$2.3 \times 10^7$		

Mitomycin was added immediately after infection. The virus activities were determined in the cell layers 7 h post infection (fowl plague) or 8 h post infection (NDV), respectively, after freezing/thawing the cells three times and after removal of the cell debris by centrifugation. The yields in the incubation medium were negligible. The residual virus not removed by the washing procedures from the infected cells was about  $10^6$  P.F.U.

In another experiment 40  $\mu\text{g/ml.}$  of the antibiotic was added to the cultures several hours before infection and was removed either immediately after, or 2 h before, the cultures were infected. Even when the time of mitomycin action on the host cell was greater than 1 h, inhibition of the production of fowl-plague virus could still be detected in the absence of the antibiotic (Table 2). Addition of mitomycin 1 h or more after infection had no significant

Table 2. INFLUENCE OF PRETREATMENT OF THE HOST CELLS WITH MITOMYCIN C ON THE MULTIPLICATION OF FOWL-PLAGUE AND ND VIRUSES

Time of addition of mitomycin C (h)	HA-units	Fowl-plague virus		NDV	
		Neuraminidase $\mu\text{g}$	CF-titre	Neuraminidase $\mu\text{g}$	P.F.U.
		NANA/ml.	Q		
5 (a.i.)	4	10.8	4.8		$1.2 \times 10^8$
3 (a.i.)	128	48.5	4.8		$1.5 \times 10^8$
1 (a.i.)	512	142	4.8		$2.1 \times 10^8$
1 (p.i.)	512	220	4.8		$1.4 \times 10^8$
3 (p.i.)	1,024	195	4.8		$8.5 \times 10^8$
5 (p.i.)	1,024	270	4.8		$8.0 \times 10^8$
No mitomycin	1,024	212	4.8		$1.2 \times 10^8$

40  $\mu\text{g/ml.}$  mitomycin C was added at different times before infection (a.i.). Mitomycin was removed by several washings immediately after the infection, and the cells were further incubated without the antibiotic. In those cases when mitomycin was added at different times after the infection (p.i.) the antibiotic was not removed. For further details see Table 1. Essentially the same results have been obtained when the mitomycin was added 7, 5, and 3 h a.i. and removed 2 h before infection by change of the medium.

effect on the multiplication of fowl-plague virus. Formation of NDV is not affected by this antibiotic.

The results of the pretreatment of the host cells with ultra-violet light are summarized in Table 3. After a certain length of time of ultra-violet irradiation of the host cells prior to infection in the absence of medium the production of the fowl-plague components tested is inhibited. But, here again, the synthesis of the RNP-antigen is less sensitive to the pretreatment of the cells with ultra-violet light, as has been found with mitomycin. NDV multiplies normally in such cells pretreated with ultra-violet light. If the host cell genome is necessary for fowl-plague virus production, it should influence one of the very early events following infection, since pretreatment of the cells with ultra-violet light or mitomycin affects the synthesis of all virus components tested.

Table 3. INFLUENCE OF PRETREATMENT OF HOST CELLS WITH ULTRA-VIOLET LIGHT ON THE MULTIPLICATION OF FOWL-PLAGUE AND ND VIRUSES

Time of ultra-violet irradiation (sec)	HA-units	Fowl-plague virus		NDV	
		P.F.U.	CF-titre	HA-units	P.F.U.
			Q		
0	1,024	$5.3 \times 10^7$	3.3	128	$3.4 \times 10^7$
5	1,024	$9.8 \times 10^7$	3.3	256	$1.2 \times 10^8$
10	32	$8.5 \times 10^6$	3.3	256	$1.4 \times 10^8$
15	2	$1.8 \times 10^6$	2.5	256	$1.3 \times 10^8$
20	2	$1.2 \times 10^6$	2.2	256	$7.5 \times 10^7$
25	2	$1.8 \times 10^6$	2.2	256	$4.0 \times 10^7$
30	2	$1.3 \times 10^6$	1.9	256	$5.1 \times 10^7$
80	2	$8.0 \times 10^5$	1.14	128	$2.7 \times 10^7$

The cells were irradiated with different doses of ultra-violet light immediately before infection. For further details see Table 1.

(2) *Synthesis of fowl-plague RNA in the presence of actinomycin.* When actinomycin was added to cultures immediately following infection with fowl-plague virus the incorporation of  $^{14}\text{C}$ -uridine into RNA was only about 0.5 per cent of that found in non-infected cells without actinomycin. If, however, the antibiotic was added 2 or 4 h post infection, the residual incorporation during a 1-h pulse went up to 4 per cent of the controls without actinomycin. Therefore, we characterized this RNA by determining its base composition and comparing it with fowl-plague RNA. The base composition of the viral RNA following digestion with alkali can be seen in Table 4.

Since there is still relatively high labelling in the 2', 3'-CMP-fraction of the soluble RNA in non-infected cells (following alkali digestion) in the presence of actinomycin<sup>15</sup>, this fraction was separated in a sucrose gradient from the  $^{32}\text{P}$ -labelled high-mol. wt. RNA of fowl-plague-infected cells. The results concerning newly synthesized RNA-fractions in actinomycin-treated and fowl-plague-infected cells are summarized in Table 4 (experiments 5 and 6). The radioactivity was never found in a single peak, which might be due to the inability to isolate an unbroken fowl-plague RNA<sup>16</sup> or to its destruction by actinomycin (see later). Up to 80 per cent of the total radioactivity in experiment 5 is found in the 23S and 16S fractions. Since the base composition of this RNA is

Table 4. BASE COMPOSITION OF FOWL-PLAGUE VIRUS-RNA, RNA-FRACTIONS OF ACTINOMYCIN-INHIBITED AND FOWL-PLAGUE-INFECTED CELLS, AND OF CELLULAR RNA

Exp.	Sample	CMP	AMP	UMP	GMP	Total counts
1	Virus-RNA	25.4	23.0	32.0	19.6	
2	Virus-RNA	25.2	23.2	31.9	19.7	
3	Virus-RNA	25.1	23.0	32.1	19.8	
4	Virus-RNA	25.2	23.2	31.8	19.8	
5	23 S fraction	25.3	20.8	34.2	19.7	49,838
	16 S fraction	25.2	21.8	33.4	19.6	110,851
	8-RNA	45.5	20.9	19.4	14.2	27,050
6	23 S + 16 S fractions	25.1	22.6	32.8	19.5	18,820
	8-RNA	35.5	24.0	24.1	16.7	7,880
	Cellular RNA*	29.0	20.1	20.0	30.9	

For the labelling of viral RNA in each experiment 5 infected cultures each received 1 mc. of  $^{32}\text{P}$  immediately after infection. 8 h p.i. the virus particles were isolated from the supernatant medium without disrupting the cells<sup>12</sup>. In experiments 5 and 6, 10 cultures each received 1 mc. or 0.2 mc.  $^{32}\text{P}$ , respectively, 2 h p.i. together with 5  $\mu\text{g}/\text{ml}$ . actinomycin. The RNA was extracted 5 h p.i. and fractionated in a sucrose gradient<sup>14</sup>.

\* Taken from Scholtissek and Rott (ref. 23).

close to that of fowl-plague RNA, it is concluded that about 80 per cent of the RNA newly synthesized under the conditions used is viral RNA.

These results suggest that actinomycin is able to reduce the synthesis of fowl-plague RNA, but does not inhibit it completely, if the antibiotic is added after the beginning of viral RNA synthesis. Therefore, experiments were performed to determine how far incubation of the host cells with actinomycin influences this viral RNA synthesis. For this purpose the antibiotic was added to different cultures 2, 4 or 6 h post infection. The cultures were then treated with  $^{14}\text{C}$ -uridine between 6 and 7 h post infection. As shown in Fig. 1, the incorporation of  $^{14}\text{C}$ -uridine increased as the interval between infection and addition of actinomycin increased. The inverse experiment was carried out by adding the antibiotic in all cases 2 h post infection, but the  $^{14}\text{C}$ -uridine was given at different times thereafter. The later the  $^{14}\text{C}$ -uridine was added the less was the incorporation. These results are shown together with corresponding controls in Fig. 1.

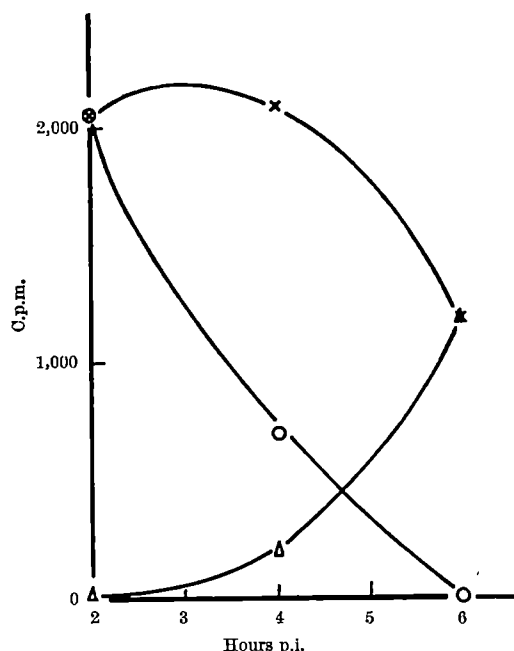


Fig. 1. Action of actinomycin on the incorporation of  $^{14}\text{C}$ -uridine into the RNA of cells infected with fowl-plague virus: x, 5  $\mu\text{g}/\text{ml}$ . actinomycin plus 1  $\mu\text{C}$ .  $^{14}\text{C}$ -uridine were added at the time p.i. as indicated on the abscissa; Δ, the actinomycin was added at the times indicated on the abscissa, and the  $^{14}\text{C}$ -uridine was added between 6 and 7 h p.i.; O, the actinomycin was added 2 h p.i., the  $^{14}\text{C}$ -uridine at the times as indicated on the abscissa. One hour later the radioactivity was determined in the RNA. All values were corrected for the residual incorporation of  $^{14}\text{C}$ -uridine into non-infected cells in the presence of actinomycin.

The results show that viral RNA synthesis—as soon as it has started—can be suppressed completely by actinomycin only when the infected cells are incubated with this antibiotic for at least 4 h. This is in contrast to the inhibition of cellular RNA synthesis, which occurs almost immediately<sup>4</sup>.

Pre-incubation of NDV-infected cells with actinomycin had only little effect on the synthesis of NDV-RNA. The greatest incorporation of  $^{14}\text{C}$ -uridine (given 6.5–8.5 h post infection) occurred when the antibiotic was added 4 h post infection (about 30 per cent of non-infected cells without actinomycin).

The multiplication of fowl-plague virus can be inhibited by actinomycin *D*<sup>4</sup>, by mitomycin *C*, and by pretreatment of the host cells with ultra-violet light<sup>1</sup> or mitomycin *C*. Since these treatments had no effect on the formation of NDV, it was conceivable that the host cell genome played an important part in the multiplication of influenza viruses, of which fowl plague is a member<sup>1,3</sup>. It is most unlikely that the total viral genome is coded by the host cell; but it is possible that one step during the multiplication common to all influenza strains is controlled by the host DNA. If this were the case, then this step would have to occur very early in the multiplication cycle, because the addition of actinomycin *D* immediately after infection completely prevents the formation of the first-appearing viral component—namely the viral RNA. Once viral RNA synthesis has started (2 h post infection), the addition of actinomycin does not inhibit the formation of viral RNA immediately. It requires about 4 h incubation with the antibiotic before its synthesis is blocked completely (Fig. 1). This result could be interpreted in the following way: For viral RNA synthesis the presence of a protein is necessary, which is coded by the cell genome, and the synthesis of which is induced by the infection. Furthermore, it should be relatively unstable and must be continually newly synthesized in order to keep the viral RNA synthesis going.

This interpretation is contradicted by the following fact: An 'early protein' necessary to start the synthesis of fowl-plague RNA has been demonstrated by using the amino-acid analogue *p*-fluorophenylalanine<sup>2,17</sup>. This 'early protein' is completely stable for at least 4 h. The design of the experiment excludes a second unstable protein necessary for the start of viral RNA synthesis. Therefore, a direct participation of the host-cell DNA during the multiplication of fowl-plague virus is rather unlikely.

We must therefore consider another possibility in order to explain the results. There exist several reports concerning breakdown of RNA by actinomycin<sup>18,19</sup> or mitomycin<sup>11</sup> which might not be explained by a normal turnover. It is conceivable that in a similar way fowl-plague RNA or its template is affected during the multiplication cycle by the agents mentioned. During the first hour following infection the few viral RNA molecules have no chance to establish an RNA-synthesizing centre in the presence of actinomycin. Later, when viral RNA synthesis has already started and many viral RNA molecules are present, it takes some time to inactivate all the fowl-plague RNA (or their templates for further RNA synthesis; see Fig. 1).

There are two points which are in favour of the latter interpretation: (1) If actinomycin is added 1.5–2 h post infection, the infectivity titre, when tested 6 h later, is just beginning to rise. At this time the plague test showed an effect which has been interpreted as multiplicity reactivation<sup>4</sup>. The same effect has been found during the inactivation of isolated fowl-plague virus with Bayer A 139 (ref. 20) or hydroxylamine<sup>21</sup>. Both compounds inactivate the viral RNA. (2) There was a different response to the synthesis of the distinct fowl-plague components using increasing doses of ultra-violet light or mitomycin, respectively (Tables 1–3). A corresponding effect has been observed during the stepwise degradation of the fowl-plague genome with Bayer A 139 or hydroxylamine<sup>22,23</sup>.



Nevertheless, the difference in response to these agents between the influenza viruses and NDV remains to be explained. It is known that the RNP-antigen and viral RNA of fowl plague are formed in the nucleus<sup>24,25</sup>. In NDV-infected cells, however, all virus material is synthesized in the cytoplasm<sup>26,27</sup>. We have some evidence that the RNA-degrading effect of actinomycin might be confined to the cell nucleus. Actinomycin does not influence protein synthesis for about 3 h after its addition to fibroblasts. This means that the templates (RNA) for protein synthesis, which occur in the cytoplasm, are not degraded directly by actinomycin. The late inhibition of protein synthesis by this antibiotic is probably due to an exhaustion in the cell of messenger RNA, which is no longer produced in the presence of actinomycin<sup>5-8</sup>.

According to our investigations a specific contribution of the cell genome to one of the early steps during the multiplication of influenza viruses is unlikely. There is no need for such an assumption, since the inhibitory effect of agents reacting with the cell genome might be explained by a destruction of the influenza genome in the cell nucleus without influencing the NDV multiplication in the cytoplasm.

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## LOW-TEMPERATURE FLUOROGRAPHY INDUCED BY TRITIUM-LABELLED COMPOUNDS ON THIN-LAYER CHROMATOGRAMS

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**A**UTORADIOGRAPHIC methods have not yet found application to thin-layer chromatography of <sup>3</sup>H-labelled compounds because of the low tritium energy ( $E_{\max} = 18.5$  keV;  $E_{\text{mean}} = 5.7$  keV). The tritium range in a layer of sodium-*o*-(carboxymethyl) cellulose and in sodium alginate has been determined by Isbell, Frush and Peterson<sup>1</sup> to be 0.28 mg/cm<sup>2</sup>. The surface density of a 250- $\mu$  thin layer of silica-gel *G* or cellulose is 5.5 mg/cm<sup>2</sup> or 7.5 mg/cm<sup>2</sup>, respectively. This is about 20 times the tritium range, which means a self-absorption loss of radiation of more than 95 per cent.

If the thin-layer material is mixed with an adequate scintillator the tritium radiation can be made visible by the fluorescence induced in the scintillator, provided the scintillator molecules are within the range of the tritium  $\beta$ -particles and the thin layer is transparent for the fluorescence radiation. We therefore prefer the term 'fluorography' to the misleading name 'autoradiography'.

The maximum range of a tritium  $\beta$ -particle expressed in microns in a silica-gel layer of 250 $\mu$  can be calculated from its surface density and its thickness:

$$250 \times 0.28/5.5 = 12.7\mu$$

Two methods of using scintillators for fluorograms of tritium-labelled compounds have been described. Wilson<sup>2</sup> puts the paper chromatogram into a solution of scintillator (*p*-terphenyl in toluene), covers it with an X-ray film and leaves both chromatogram and film in the scintillator solution during exposure time. This method cannot be used for thin-layer plates, their surface being easily damaged by this procedure. Furthermore, the method is limited to compounds which are insoluble in the solvent of the scintillator.

Parups, Hoffmann and Jackson<sup>3</sup> dipped their paper chromatograms into a saturated benzene solution of anthracene and applied the X-ray film after evaporation of the solvent. The sensitivity of their method depends entirely on the amount of anthracene introduced into the chromatogram. They obtained a fluorograph of a minimal radioactivity of 0.38  $\mu\text{C}/\text{cm}^2$  after 7 days exposure.

The technique for thin-layer chromatography described here uses solid anthracene as a scintillator which is finely ground in a ball mill with an equal amount of silica-gel *G* (content of gypsum 13 per cent) from Merck, Darmstadt, to a crystal size of 1–5 $\mu$ . The diameters of the anthracene and silica gel crystals are thus about one-third of the maximum  $\beta$ -range of tritium. The low solubility of anthracene in most solvents under mild conditions makes this microcrystalline powder suitable as thin-layer material. 30 g of this mixture is added to 80 ml. of 96 per cent ethanol. After thorough shaking the suspension is spread over glass plates with a special distribution apparatus (furnished by Desaga, Heidelberg) and dried at room temperature. 30 g is sufficient for ten thin-layer plates (20  $\times$  10 cm<sup>2</sup>) 250 $\mu$  thick, their surface density being 5.5 mg/cm<sup>2</sup>. They can be used for most common solvent mixtures. Colour reagents which do not react with anthracene may be used for detection of the compounds. Fig. 1 shows the separation of a mixture of tertiary and quaternary compounds. Their chromatographic properties are the same as on pure silica gel except for slightly prolonged travelling times. Chromatograms of tritium-labelled compounds are covered with X-ray films ('Kodirex', Kodak) and exposed over dry ice at  $-70^\circ\text{C}$ . 0.08  $\mu\text{C}$  of tritium can be detected after exposure for one day (Fig. 2). Control experiments have to be made with the

unlabelled compound. When no fluorescence picture develops after the same exposure time at  $-70^{\circ}\text{C}$ , the blackening of the film is only due to tritium-induced fluorescence.

Low temperature during exposure is essential. A tritium-induced fluorograph is twice as intense when exposed at  $-30^{\circ}\text{C}$  than at  $+4^{\circ}\text{C}$  and 30 times as intense at  $-70^{\circ}\text{C}$ . No increase of film blackening is obtained by further lowering the temperature to  $-200^{\circ}\text{C}$  (Fig. 3). These amplification values were obtained by densitometric measurements of the films. A similar, but much slighter, effect was produced with a mixture of silver-activated zinc sulphide and  $\text{SiO}_2$ . A similar dependence on temperature was observed with red-light-induced fluorescence of anthracene by Singh and Lipsett<sup>4</sup>. The temperature variation of the scintillator response seems to be correlated with the vibrational freedom of the molecules. At low temperature vibration is 'frozen in' and less energy is lost for radiationless transitions<sup>5</sup>.

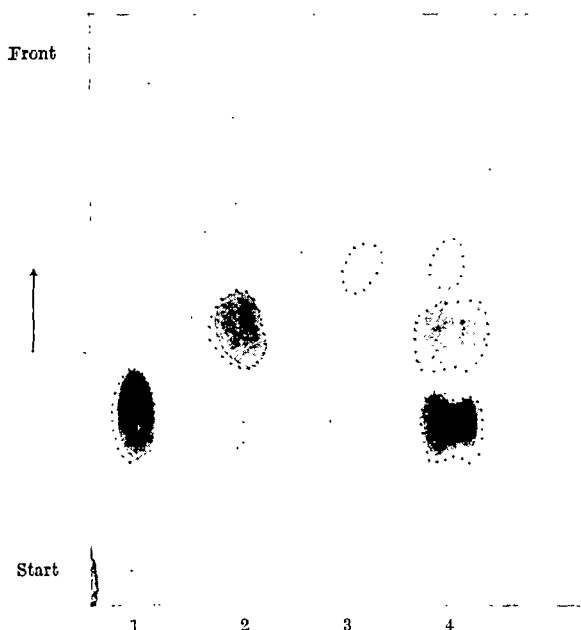


Fig. 1. Chromatogram of an amine mixture on an anthracene/silica gel 1/1 thin layer ( $250\mu$  thick). 1, Hexamethonium; 2, *Bis-N*-dimethyl-hexamethylenediamine; 3, Trimethylamine; 4, Mixture of 1, 2, and 3. Solvent: acetonitril/ $\text{HCl}/\text{H}_2\text{O}$  (7/2/1). Colour reagent: Dragendorff-reagent

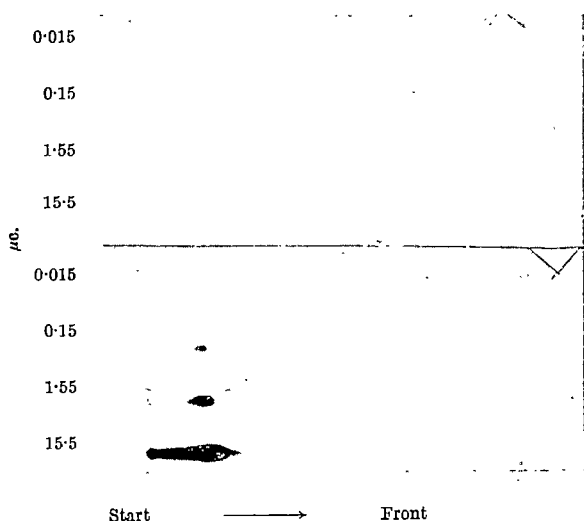


Fig. 2. Fluorogram of tritium labelled diallyl-nor-toxiferine ('AlloferinR'). a, On silica-gel G; b, on anthracene/silica-gel G (1/1). Exposure time: 18 h at  $-70^{\circ}\text{C}$ . Solvent: acetonitril/hexane/diethylamine (75.5/17.5/5)

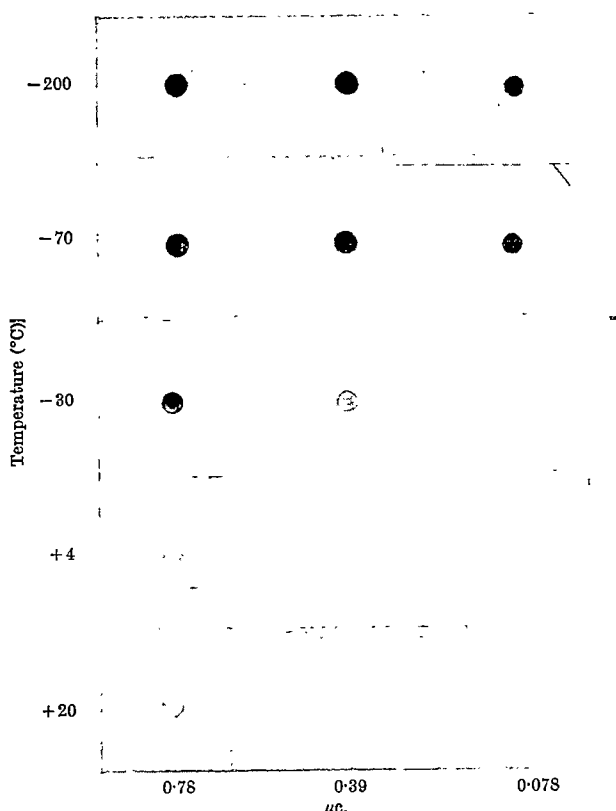


Fig. 3. Fluorograph of tritium ( $^3\text{H}$ -'AlloferinR') on anthracene/silica gel (1/1) exposed at various temperatures. Exposure time: 18 h

The application of the technique described here to  $^{14}\text{C}$ -labelled compounds shows no advantage over the ordinary contact autoradiography. No increase of film blackening was obtained by a  $^{14}\text{C}$  compound on anthracene/silica gel when compared with the blackening on silica gel without the scintillator. The  $\beta$ -energy of  $^{14}\text{C}$  is about ten times that of  $^3\text{H}$ . Therefore, a  $^{14}\text{C}$   $\beta$ -particle is more likely to react with the photographic emulsion than with the microcrystals of the thin layer.

The method described is an application of fluorography to thin-layer chromatography of tritium-labelled compounds. It takes advantage of four properties of anthracene: its high fluorescence efficiency; the temperature dependence of its fluorescence intensity; its low solubility in most of the common solvents; its low chemical reactivity.

This method could be further improved by raising the ratio anthracene/silica gel. The amplification of tritium-induced fluorescence on pure anthracene is about 15 times higher than on mixtures containing 50 per cent anthracene. Anthracene thin layers are easily prepared, with 10 per cent gypsum or with small amounts of detergents such as sodium-lauryl-sulphonate. However, some substances investigated in our laboratory (tertiary and quaternary ammonium compounds) did not separate on anthracene thin layers. The purity of the anthracene is not of primary importance. Both the scintillation and the purum grade gave equal results. Shifting of the anthracene fluorescence spectrum produced by trace impurities has no influence on X-ray films.

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## POSSIBILITY OF NON-THERMAL IONIZATION COMBUSTION PRODUCT MAGNETOHYDRODYNAMIC GENERATORS

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**W**HEREAS it is widely accepted that some form of non-thermal ionization is likely to be practical with closed-cycle magnetohydrodynamic generators, the possibility of obtaining significant reductions in working temperature for a given electrical conductivity in open-cycle combustion product magnetohydrodynamic generators is normally considered to be remote. In this article the various methods of obtaining such enhanced conductivity are reviewed and it is shown that magnetically induced ionization may possibly give a significant increase in conductivity.

The possibility of making use of the relatively long recombination times at low temperatures has been briefly discussed elsewhere<sup>1</sup> and is not included here, since it will only give the ionization appropriate to the temperature somewhat earlier in the magnetohydrodynamic generator. The methods discussed here are: (1) by causing excessive ionization from an external source, for example, ultra-violet lamp, radio-frequency breakdown, electron beam injection, etc.; (2) by using the currents flowing in the gases to cause an increase in ionization by raising the electron temperature; (3) by using the non-equilibrium effects that occur when chemical reactions occur in flames to raise the electron temperature.

Before discussing these in detail, it is desirable to discuss the orders of magnitude of various important parameters governing the departure from equilibrium. In a magnetohydrodynamic generator of the type that might be used on a power station, the temperature falls from about 2,500° K at the entrance to the duct to about 2,000° K at the exit, the pressure falling from perhaps 5 to 1 atm. and the electron density from perhaps  $10^{14}$  to  $10^{13}$  electrons/c.c. The length of time a volume of gas takes to pass along a full-scale magnetohydrodynamic generator is typically  $10^{-2}$  sec.

Suppose energy has been given to the electrons in some manner. The electrons interact with each other in a time of order  $6,000/n$  sec if the electron temperature is of the order 2,000° K. The Maxwell tail capable of ionizing potassium will take rather longer to form—a time of order  $5 \times 10^6/n$  sec ( $n$  is the electron density).

The time taken for ionization of the potassium may now be calculated. The ionization process may be described by an equation of the form:

$$\frac{dn_e}{dt} = 6 \times 10^{-24} (n^2 n_e - n^3_e)$$

The constant  $6 \times 10^{-24}$  refers to 2,000° K; it varies inversely as the sixth power of the temperature. Hence, near equilibrium:

$$\frac{dn_e}{dt} = 1.2 \times 10^{-23} n^2 (n_e - n)$$

and an ionization time constant,  $8 \times 10^{22} n^{-2}$  sec.

Electrons can lose energy by inelastic collisions with molecules. In combustion products, the probability of energy loss is greatest with  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . The time constant for this process has been calculated from the data of Healey and Reed<sup>2</sup> and would be  $10^{-10}$  and  $5 \times 10^{-10}$  sec at the duct entrance and exit respectively.

If the electron temperature were maintained at a given level, the vibrational energy-levels of the molecules (assumed decoupled from the translational states for the moment), would gradually attain the electron temperature.

The time constant would be equal to the electron cooling time constant multiplied by the ratio of the number of molecules to electrons. The time constants are therefore  $5 \times 10^{-6}$  and  $5 \times 10^{-5}$  sec at the duct entrance and exit respectively.

However, the vibrational relaxation times are very short in  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  mixtures, being of the order of  $10^{-7}$  and  $6 \times 10^{-7}$  sec at the duct entrance and exit respectively.<sup>3</sup>

The results of these calculations are given in Table 1. Some conclusions can be drawn immediately. All the times are short compared with the time for a gas element to flow through the duct. Hence a steady state is reached in all phenomena.

Table 1  
Times (sec)

	Duct entrance	Duct exit
Flow through duct	$10^{-2}$	$10^{-2}$
Electron-electron energy exchange	$6 \times 10^{-11}$	$6 \times 10^{-10}$
Maxwell tail formation	$5 \times 10^{-6}$	$5 \times 10^{-5}$
Ionization time constant	$2 \times 10^{-6}$	$8 \times 10^{-1}$
Electron energy transfer to molecules	$10^{-10}$	$5 \times 10^{-10}$
Electron heating of vibrational levels in molecules	$5 \times 10^{-6}$	$5 \times 10^{-5}$
Translational-vibrational relaxation	$10^{-7}$	$6 \times 10^{-7}$

### Chemi-ionization

Two types of mechanism have been suggested. In one type, electrons are produced as an end-product of a chain of chemical reactions. This mechanism is attractive since the conductivity depends directly on the electron density. The difficulty lies with the efficiency of the mechanism—electron densities in excess of  $10^{13}$ /c.c. are not normally produced by this process, and even at the exit of the duct one expects  $10^{13}$  electrons/c.c. by thermal ionization. The electron temperatures are kept close to the molecular temperature by the rapid transfer of energy to the molecular vibrational levels and under these conditions the recombination time constant (equal to the ionization time constant) will entail the process having to be repeated many times along the length of the duct. Moreover, the choice of fuel and oxidant will be determined by economic considerations, so there is not much room for using reactions particularly favourable to this process. If the process were used at all, it is clear that it would only be used to boost the ionization at the very end of the duct.

The other possible mechanism of chemi-ionization<sup>3</sup> is that the combustion process leaves the vibrational levels of the molecules in excited states. Because of the short time constant for energy transfer between the electrons and the molecular vibrational levels, the electron temperature is close to that of the molecular vibrations. With the resulting high electron temperature, ionization will begin to build up with the appropriate ionization time constant, but because of the short translational-vibrational relaxation time the process will have to be repeated many times along the duct to maintain the ionization, that is, there will have to be continuous combustion.

An upper estimate of the additional electron density can be obtained from the times in Table 1. Because of the short translational-vibrational relaxation time, the process will have to be repeated at least  $10^{-2}/6 \times 10^{-7}$  times or  $2 \times 10^4$  times. On each occasion only a fraction equal to the translational-vibrational relaxation time divided by the time for electron heating of the molecular vibrational states can go to the electrons, that is, a number less

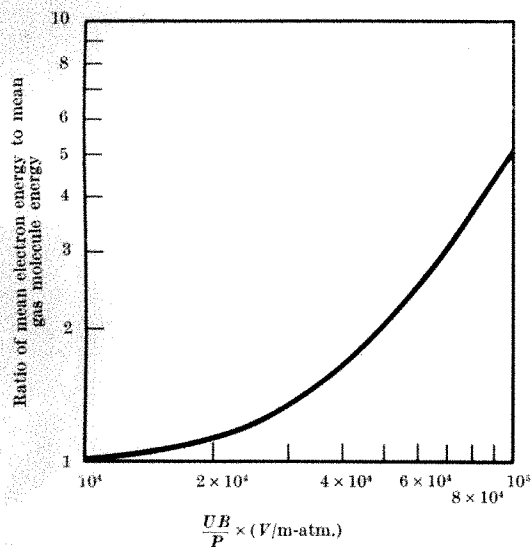


Fig. 1

than 1/50. Hence, even assuming that all the combustion energy went into exciting molecular vibrations, less than  $10^{-6}$  of this would go into electron heating. Since each free electron takes 4 eV to ionize, the maximum electron density is  $10^{12}$  and the process is unimportant for magnetohydrodynamics.

#### External Source Ionization

The use of an external source (other than a radioactive one) to supply additional ionization implies the use of external power (for example, electricity) to cause ionization. It is therefore less efficient than the use of magnetohydrodynamic currents themselves to raise the electron temperature.

#### Magnetically Induced Ionization

The currents flowing in a magnetohydrodynamic generator are carried almost entirely by the most mobile particles—the electrons, and afterwards by energy transfer from the electrons to the gas molecules. The mean electron thermal energy can, therefore, in certain circum-

stances, be significantly greater than the gas temperature. The enhanced mean electron energy can be calculated as a function of Mach number of the gas flow and magnetic field strength<sup>4</sup>. Because of the strong energy coupling between electrons and molecules this process has been assumed to be inapplicable for molecular gases. However, Fig. 1 shows the results of detailed calculations using this method. The values of the fractional energy loss of an electron per collision as a function of electron energy have been taken from ref. 2. The values given in ref. 2 were obtained from experiments where the electron energy was many times greater than the gas molecules energy and the gas was at room temperature. In the portion of Fig. 1 where the electron energy is close to the gas temperature the amount of enhancement may, therefore, be considerably in error but will, however, cause little error in the electron energy. It is seen that a 40 per cent increase in electron energy may be obtained by working at velocities ( $U$ ), pressures ( $P$ ) and magnetic field strengths ( $B$ ):  $UB/P = 3 \times 10^4$ . This condition would be achieved by running the generator supersonically.

However, there are some doubts in interpreting the increased electron energy as an enhanced electron temperature and assuming the ionization and conductivity appropriate to equilibrium at the electron temperature. For example, it is not clear that the electrons will form a Maxwell distribution. Moreover, many of the ionizing collisions are caused by electron collision with excited seed particles, and the population of these excited states is likely to be determined by the molecular vibrational temperature rather than electron temperature.

Thus, although magnetically induced ionization would seem to be the most favourable of the enhanced conductivity possibilities, a realistic assessment of its potentialities must await the results of more detailed investigations. Both theoretical and experimental investigations are at present in progress.

The work was carried out at the Central Electricity Research Laboratories, and is published by permission of the Central Electricity Generating Board.

<sup>1</sup> Carter, C., Freck, D. V., Harrowell, R. V., and Wright, J. K., Paper 85, *Intern. Conf. on Magnetohydrodynamics* (Organization for Economic Co-operation and Development, Paris, 1964).

<sup>2</sup> Healey, R. H., and Reed, J. W., "The Behaviour of Slow Electrons in Gases", *Amalgamated Wireless (Australasia)*, Sydney (1941).

<sup>3</sup> Von Engel, A., and Cozens, J. R., *Nature*, **202**, 480 (1964).

<sup>4</sup> Wright, J. K., and Swift-Hook, D. T., *Proc. Phys. Soc.*, **80**, 465 (1962).

## ELECTRON SPIN RESONANCE SPECTRA OF POLYMETHYL METHACRYLATE AFTER EXPOSURE TO NITRIC OXIDE AND NITROGEN DIOXIDE

By DR. M. EBERT and DR. J. LAW

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IT was shown by Cook *et al.*<sup>1</sup> that nitric oxide (NO) reacted with powdered samples of polymethyl methacrylate (PMMA) to give free radicals with an asymmetric electron spin resonance signal. They suggested that this might result from a reaction between the gas and the doubly bound oxygen atom in the side arm of the monomer unit. The present article extends this work and shows that the signal arises from a reaction between the gas and traces of unreacted methyl methacrylate monomer (MMA) in the PMMA samples. It is also shown that nitrogen dioxide (NO<sub>2</sub>) has a similar effect.

Preliminary work was done using samples of unplasticized PMMA as used by Boag *et al.*<sup>2</sup> for dosimetry. For the main part of the work, purified samples of PMMA

were prepared from MMA in the following way. MMA monomer was first purified by washing with a solution of 20 per cent NaCl/5 per cent NaOH to remove inhibitors. It was then washed copiously with water to remove alkali, dried over anhydrous MgSO<sub>4</sub>, distilled under vacuum and stored in the dark at about 4° C. PMMA was then made from it by three different methods: (a) some monomer was de-aerated with oxygen-free nitrogen and irradiated with cobalt-60  $\gamma$ -rays for about 16 h at about 1,300 rads/min; (b) dry benzoyl peroxide was dissolved in purified MMA (0.1 per cent) and heated to 100° C until, after about 15 min, a syrup formed; the syrup was then heated at 60°–80° C overnight and for several hours at 100°–120° C; (c) 3 per cent PMMA,



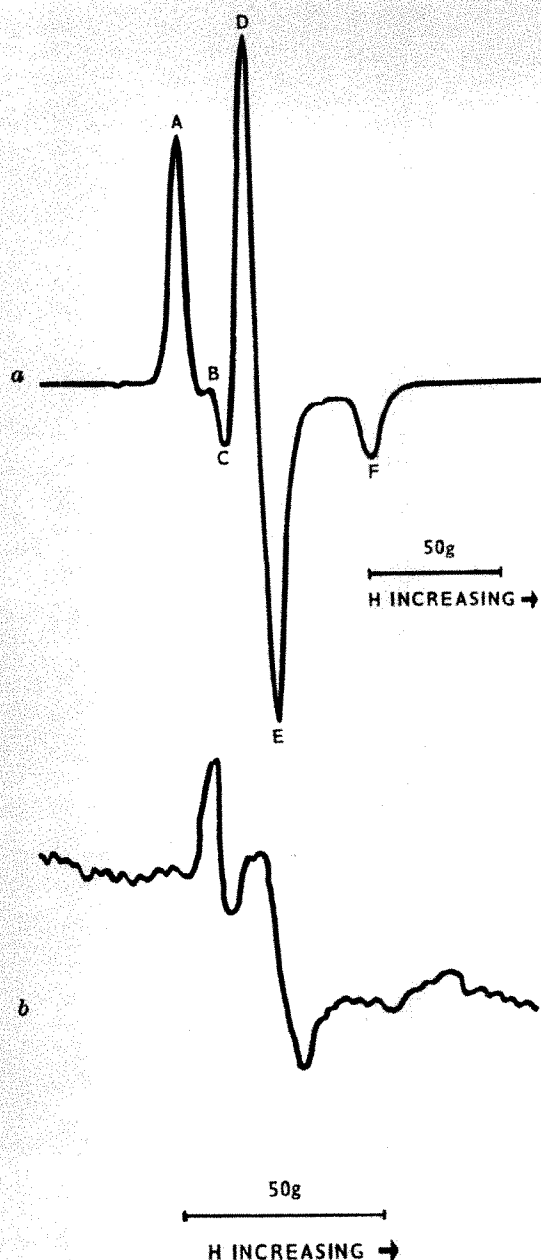


Fig. 1. *a*, Electron spin resonance signal from PMMA after exposure to  $^{14}\text{NO}$  or  $^{14}\text{NO}_2$ . *b*, Weak component of signal *a*

0.007 per cent methacrylic acid and 0.05 per cent  $\alpha\alpha'$ -azodi-iso-butyronitrile were added to purified MMA. The mixture was heated for 30 h at  $40^\circ\text{C}$  and then for several hours at  $100^\circ\text{C}$ . The solids resulting from all three methods were then purified by dissolving in benzene (7:1,  $\text{C}_6\text{H}_6$  to polymer by weight) and reprecipitating in methanol at  $-30^\circ\text{C}$  (10:1,  $\text{CH}_3\text{OH}:\text{C}_6\text{H}_6$  by volume). The polymer was then dried in vacuum at  $100^\circ\text{C}$  for 24 h, and was further purified by repeating this procedure.

Samples of purified PMMA made by these three methods and also the unplasticized PMMA were filed or ground to a powder. The resulting particles were of the order of  $100\mu$  across or less. This size allows the gases to diffuse throughout the particles in times short compared with treatment times. These powders were then evacuated to pressures of the order of  $10^{-5}$  torr for several hours and then allowed to stand in contact with NO or  $\text{NO}_2$  for 24 h.

MMA was exposed to NO and  $\text{NO}_2$  in the following way. A few millilitres of MMA in a 50-ml. flask were de-aerated by freezing and thawing under vacuum and then allowed to stand under NO or  $\text{NO}_2$  for periods varying from 10

Signal from:	Table 1 g Values of features:					
	A	B	C	D	E	F
PMMA and $^{14}\text{NO}$ , X band	2.0212 $\pm 0.0002$	2.0153 $\pm 0.0001$	2.0128 $\pm 0.0002$	2.0085 $\pm 0.0001$	2.0029 $\pm 0.0001$	1.9841 $\pm 0.0002$
PMMA and $^{14}\text{NO}$ , Q band	2.012	2.010	2.007	2.004	1.999	
PMMA and $^{15}\text{NO}$ , X band	2.0161 $\pm 0.0001$	2.0105 $\pm 0.0002$	2.0059 $\pm 0.0001$	2.0001 $\pm 0.0005$	1.9898 $\pm 0.0002$	
PMMA and $^{15}\text{NO}$ , Q band	2.010	2.008	2.007	1.999		

to 60 min. The liquid became noticeably blue but remained clear throughout. After this treatment it was frozen and the gas removed. Liquid samples were then condensed in tubes suitable for electron spin resonance measurements and sealed under vacuum, or transferred to other such tubes in air.

NO was obtained from cylinders supplied by Matheson and Co., Inc. The gas was purified by passing through KOH solution and two cold traps, one of solid carbon dioxide and a second of solid carbon dioxide and acetone mixture. The gas was then admitted to the PMMA samples, generally to atmospheric pressure.  $\text{NO}_2$  was prepared by heating  $\text{Pb}(\text{NO}_3)_2$ ; some oxygen is present in the mixture of  $\text{NO}_2$  and  $\text{N}_2\text{O}_4$ . Concentrations of  $\text{NO}_2$  quoted here refer to mixtures of  $\text{NO}_2$ ,  $\text{N}_2\text{O}_4$  and  $\text{O}_2$ .

A 50-ml. sample of  $^{15}\text{NO}$ , 99 per cent labelled, was obtained from 20th Century Electronics, Ltd. Small amounts of this were allowed to react with oxygen to form a mixture of  $\text{NO}_2$  and  $\text{O}_2$ . This mixture was in turn allowed to react with samples of PMMA and MMA as already described here.

Electron spin resonance spectra were recorded with a Hilger and Watts Microspin X-band (9,400 Mc/s) spectrometer, which uses 100-kc/s field modulation and gives first derivatives of absorption signals. In addition some Q-band (35,000 Mc/s) spectra were obtained from Hilger and Watts spectrometers in other laboratories, and are shown here as Fig. 6.

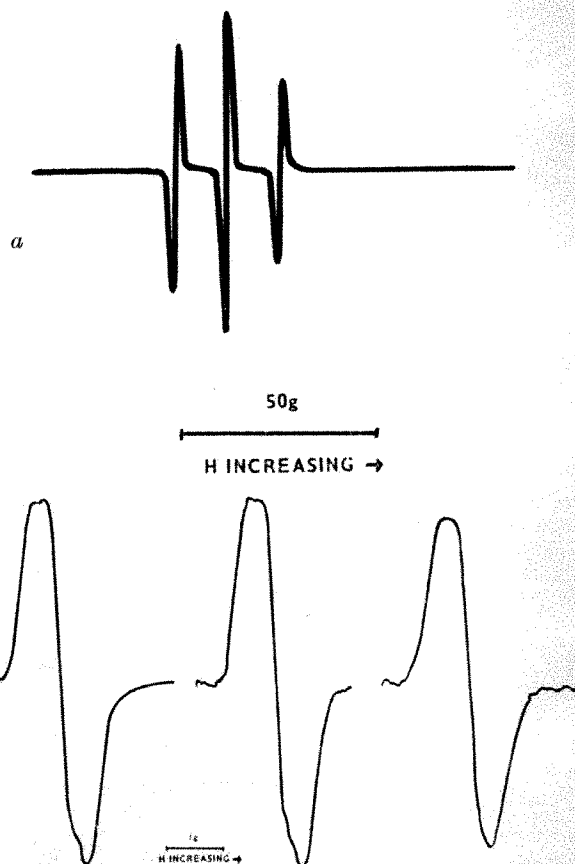


Fig. 2. *a*, Electron spin resonance signal from MMA after exposure to  $^{14}\text{NO}$  or  $^{14}\text{NO}_2$ . *b*, Separate lines of signal in *a*

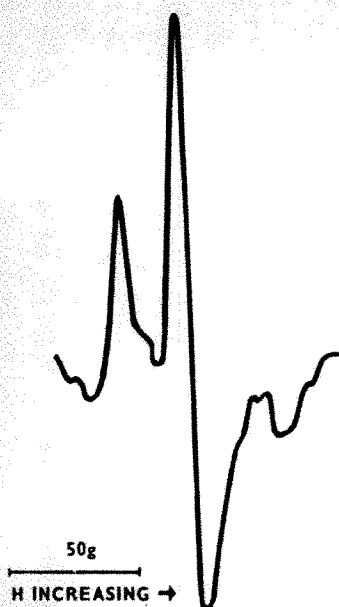


Fig. 3. Electron spin resonance signal from MMA after exposure to  $^{15}\text{NO}_2$  and subsequent freezing

All electron spin resonance measurements were made in 'Pyrex' tubes, 6 mm outer diam. and 5 mm internal diam. Any signal from the glass was negligible at the spectrometer sensitivities used.  $g$  values were estimated assuming the  $g$  value for diphenyl-picryl-hydrazyl = 2.0036.

Samples of PMMA powder showed no electron spin resonance signal before exposure to NO or  $\text{NO}_2$ , except that for a few minutes immediately after filing a very weak asymmetric signal could be detected which looked like that normally attributed to peroxy radicals  $\text{ROO}\cdot$  (ref. 3).

When powdered samples of unplasticized PMMA were exposed to NO at atmospheric pressure for 24 h, the signal shown in Fig. 1a was found, as described by Cook *et al.*<sup>1</sup>. The  $g$  values of its principal features are given in Table 1 where the uncertainties quoted are standard deviations of means. This is a mixture of two asymmetric signals, but one is very much weaker than the other. The latter is shown in Fig. 1b and is probably responsible for the small peak at B in Fig. 1a.

It was found that when PMMA was allowed to stand with excess NO, only a limited amount of gas was taken up, after which the PMMA appeared to be saturated. By weighing the PMMA before and after treatment, it was estimated that if the gas reacted with the polymer it only reacted with about 1 in 50 monomer units. Measurement of the volume of gas taken up gave an estimate of 1 in 20 monomer units.

A pressure of 1.5 cm mercury of  $\text{NO}_2$  was found to give a strong signal identical with that of Fig. 1a which was stable for a few weeks. If  $\text{NO}_2$  was used at atmospheric pressure a very weak signal of this kind was observed for about 1 h afterwards, but then disappeared.

This signal, whether produced by NO or  $\text{NO}_2$ , is stable for periods of months in either air or vacuum. It is also stable in the presence of NO for a few weeks at least, but decays in the presence of  $\text{NO}_2$ . It is unaffected by radiation at doses of at least  $10^7$  rads. Samples of unplasticized PMMA which were treated with NO or  $\text{NO}_2$  for 24 h and then re-evacuated and irradiated showed no trace of the well-known 5-plus-4 line electron spin resonance signal normally found when PMMA is irradiated *in vacuo*.

When samples of purified PMMA made by each of the three methods described here were exposed to either NO or  $\text{NO}_2$ , no electron spin resonance signal could be detected. In contrast to this, when MMA was first condensed on to these powders under vacuum, and the powders then exposed to either gas, the electron spin resonance signal of

Table 2

Signal from:	$g$ Value	Hyperfine splitting	Line widths
MMA and $^{14}\text{NO}$	2.0065 $\pm 0.0001$	$28.2 \pm 0.2$ gauss	$0.9 \pm 0.1$ gauss
MMA and $^{15}\text{NO}$	2.0065 $\pm 0.0001$	$20.0 \pm 0.2$ gauss	$1.0 \pm 0.1$ gauss

Fig. 1a was obtained from each of the three samples as with the unplasticized PMMA. A further sample of PMMA, made by irradiating MMA but without subsequent removal of unreacted MMA, also gave this signal. When monomer was condensed on to silica gel as an inert solid matrix, the silica gel showed the electron spin resonance signals of Fig. 1a after exposure to NO or  $\text{NO}_2$ , whereas silica gel alone exposed to these gases shows no electron spin resonance signal.

MMA exposed to NO or  $\text{NO}_2$  turned blue and gave an electron spin resonance signal of three lines equally spaced but appearing slightly unequal in strength in first derivative recordings, and of slightly different shape. This signal is shown in Fig. 2a and the three lines separately in Fig. 2b. It was provisionally attributed to an unpaired electron closely coupled to the nitrogen-14 atom which has spin quantum No. 1. A slight splitting can be seen in each line, presumably due to weak interaction with protons. The  $g$  value, hyperfine splitting and line width are given in Table 2.

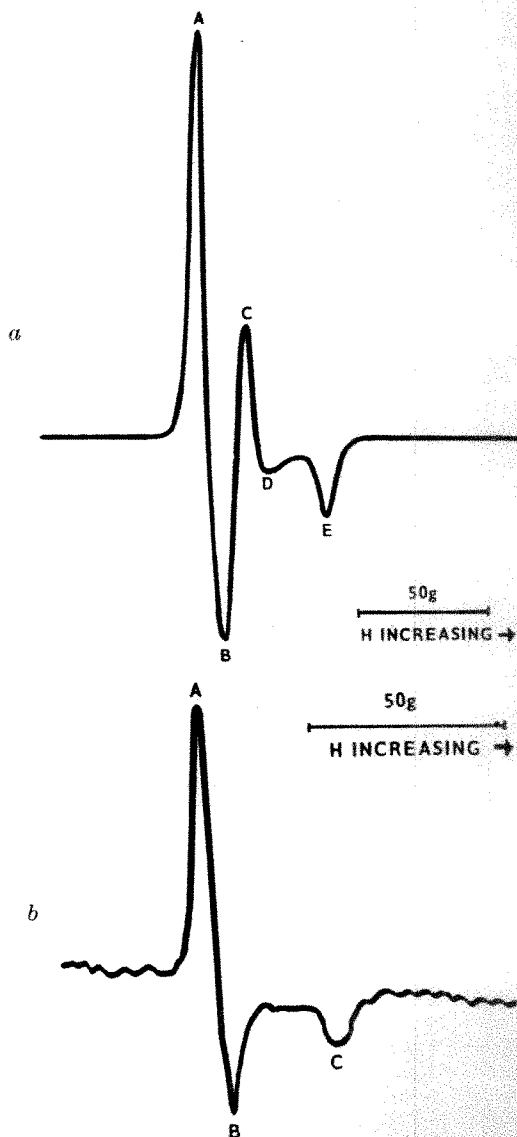


Fig. 4. a, Electron spin resonance signal from PMMA after exposure to  $^{15}\text{NO}$  or  $^{15}\text{NO}_2$ . b, Weak component of signal a.

A sample of MMA treated with  $\text{NO}_2$  and then cooled in liquid nitrogen gave an electron spin resonance signal when frozen as shown in Fig. 3. Only a relatively small sample could be accommodated under these conditions and the signal was correspondingly weak, but after allowing for the zero line drift it is the same signal as in Fig. 1a to well within the noise-level. The presence or absence of the signal of Fig. 1b could not be established. Untreated MMA gave no signal when frozen.

When powdered PMMA containing MMA was exposed to  $^{15}\text{NO}_2$  an electron spin resonance signal was obtained which is shown in Fig. 4a. Its  $g$  values are given in Table 1. This was also found to consist of two asymmetric signals, one very much weaker than the other, and the latter is shown in Fig. 4b. MMA was also exposed to  $^{15}\text{NO}_2$  and a doublet electron spin resonance signal was obtained (Fig. 5) as expected since nitrogen-15 has spin quantum number  $\frac{1}{2}$ . The hyperfine splitting and line width are given in Table 2. The hyperfine splitting is less than for the triplet signal, and the ratio of the splittings is equal to that of the nitrogen-14 and nitrogen-15 magnetic moments to within the limits of experimental error.

Electron spin resonance spectra from samples of PMMA exposed to  $^{14}\text{NO}_2$  and  $^{15}\text{NO}_2$  were also obtained at Q band frequencies. These are shown in Fig. 6. The  $g$  values of their principal features are listed in Table 1, and are unlikely to be in error by more than  $\pm 0.001$ .

Since the signals obtained from PMMA after exposure to NO or  $\text{NO}_2$  remain at the same intensity when these gases are removed, it seems likely that they result from a chemical reaction with some constituent of the polymer, rather than from gas molecules being trapped in some way in the solid. PMMA made by three different polymerization processes and purified to remove MMA does not give the electron spin resonance signal of Fig. 1a after exposure to NO or  $\text{NO}_2$ . If small amounts of MMA are present, however, samples of PMMA do show this signal whatever their mode of polymerization. This strongly suggests that the signal arises from an interaction between the gas and MMA, and does not depend on the groups which initiate the polymer chains. Furthermore, small amounts of purified MMA when condensed in silica gel as an inert solid matrix show this signal after exposure to either gas, and the same signal is obtained when purified MMA is exposed to these gases and then frozen. These results provide further support for the conclusion that the signal is given by the products of a reaction or reactions between NO or  $\text{NO}_2$  and MMA. This is qualitatively consistent with the observed uptake of NO by unplasticized PMMA. The possibility that low molecular weight polymer is involved cannot be excluded.

The most probable site at which the gases might react with MMA is the double bond between the carbon atoms, but this has not been confirmed. It seems certain, however, that the radical includes the nitrogen atom. After MMA has been exposed to either  $^{14}\text{NO}$  or  $^{14}\text{NO}_2$  it gave a triplet electron spin resonance signal which was attributed to an unpaired electron interacting with the nitrogen-14 atom. If the MMA was then frozen this triplet was trans-

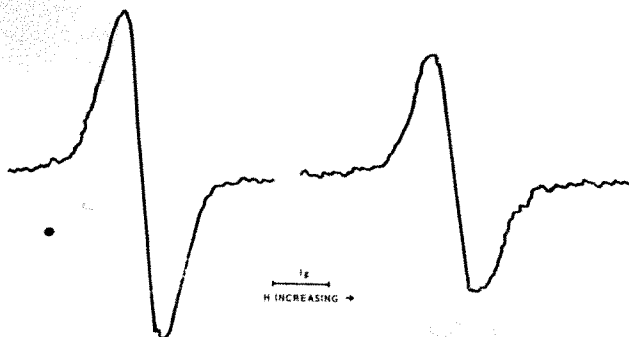


Fig. 5. Electron spin resonance signal from MMA after exposure to  $^{15}\text{NO}$  or  $^{15}\text{NO}_2$ .

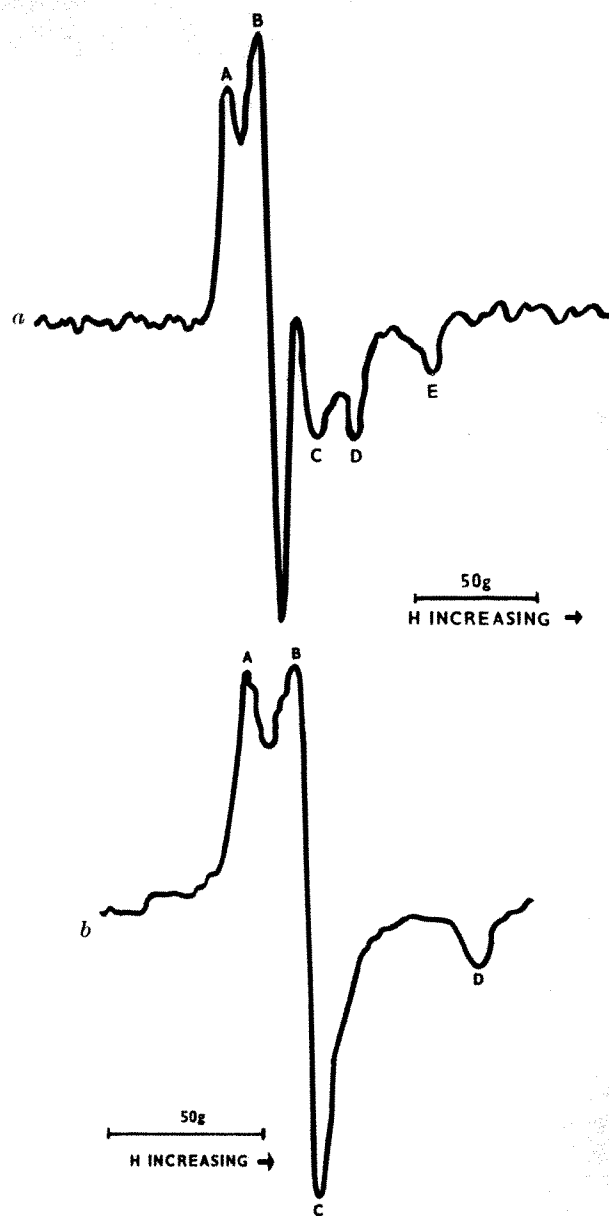


Fig. 6. Q-band electron spin resonance signal from PMMA after exposure to: a,  $^{14}\text{NO}$  or  $^{14}\text{NO}_2$ ; b,  $^{15}\text{NO}$  or  $^{15}\text{NO}_2$ .

formed to a signal like that of Fig. 1a, and returned to its original form on melting. This implies that the signal of Fig. 1a also arises from the nitrogen-14 atom. This conclusion is confirmed by the experiments with  $^{15}\text{NO}$  and  $^{15}\text{NO}_2$  in which MMA showed a doublet signal and PMMA showed a signal quite different from Fig. 1a, as seen in Fig. 4.

Several discussions took place with Dr. P. D. Cook and Mr. V. J. McBrierty of Middlesex Hospital Medical School who were working independently on a related topic [see p. 1197 of this issue of *Nature*].

We thank Dr. A. J. Swallow of this laboratory, and Dr. L. H. Sutcliffe of the University of Liverpool, for advice on the preparation and purification of PMMA samples and on other matters. We also thank Mr. H. M. Assenheim and the staff of Hilger and Watts Ltd., Dr. J. C. Evans of University College, Cardiff, and Prof. D. J. E. Ingram and Mr. S. D. Lacey of the University of Keele for help in providing the spectra shown in Figs. 6a and b and their corresponding  $g$  values.

<sup>1</sup> Cook, P. D., Ebert, M., and Mallard, J. R., *Nature*, **198**, 579 (1963).

<sup>2</sup> Boag, J. W., Dolphin, G. W., and Rotblat, J., *Radiat. Res.*, **9**, 589 (1958).

<sup>3</sup> Carrington, A., and Stein, G., *Nature*, **192**, 976 (1962).

## LETTERS TO THE EDITOR

## ASTROPHYSICS

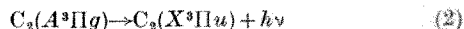
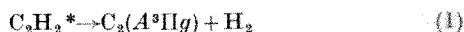
Origin of the  $C(A^3\Pi_g) \rightarrow C_2(X^3\Pi_u)$  Emission in Comets

THE observation of the  $C_2$  Swan bands ( $A^3\Pi_g \rightarrow X^3\Pi_u$ ) in emission in the coma of comets was at one time taken as evidence that the ground state of  $C_2$  was the  $X^3\Pi_u$  state since this cometary emission is known to arise by means of a fluorescence process. However, Ballik and Ramsay<sup>1</sup> have shown that the ground state of  $C_2$  is  $x^1\Sigma^+_g$  and consequently the observation of the Swan bands would be consistent with the following: (1)  $C_2$  is formed initially in the triplet system by decomposition (probably photodecomposition) of a hydrocarbon molecule. (2) The  $^3\Pi_u \rightarrow ^1\Sigma^+_g$  transition does not occur to an appreciable extent prior to fluorescence excitation by solar radiation or in the time between successive excitations. The latter implies that  $C_2$  experiences an insufficient number of collisions, or possibly no collisions, to effect the forbidden  $^3\Pi_u \rightarrow ^1\Sigma^+_g$  transition. This is not an unreasonable requirement since it is known that the rotational and vibrational distribution of  $C_2$  in cometary atmosphere corresponds to a temperature of about 2,500° K and that  $C_2$ , therefore, must experience few if any collisions between the time of its formation near the nucleus and the time of its excitation farther out in the coma.

Thus, while the second requirement seems to be met, the first requirement of the specific formation of  $C_2$  in the triplet system on photodissociation of a hydrocarbon molecule needs to be demonstrated. Any significant formation of  $C_2$  in the singlet system must be excluded since appreciable concentrations of  $C_2$  in the  $x^1\Sigma^+_g$  ground state would eventually lead to population of upper singlet states ( $b^1\Pi_u$ ,  $c^1\Pi_g$ ,  $d^1\Sigma^+_u$ ) and emission from these states (Fig. 1).

Recent work in our laboratory on the vacuum ultra-violet photolysis of  $CH_4$ ,  $C_2H_2$ ,  $C_2H_4$ , and  $C_2H_6$  suggests that photodissociation of methane and acetylene in the

vacuum ultra-violet at low pressures leads to the formation of  $C_2$  in the  $^3\Pi_g$  state. The emission from  $CH_4$  was very weak and, since a secondary process for  $C_2$  production from methane is required, the more interesting  $C_2H_2$  system was investigated in detail. A weak emission consistent with the Swan bands of  $C_2$  and the detection of molecular hydrogen formation was explained in terms of the decomposition of an electronically excited acetylene molecule<sup>2</sup>.



Emission in the regions of other  $C_2$  transitions (Fig. 1) was not observed. No emissions were observed from  $C_2H_4$  and  $C_2H_6$ .

On this basis we would suggest that the origin of the  $C_2$  Swan bands in cometary emission spectra is the photodissociation of acetylene or an acetylene-type molecule leading to an eventual accumulation of  $C_2$  in the  $X^3\Pi_u$  state.

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<sup>1</sup> Ballik, E. A., and Ramsay, D. A., *J. Chem. Phys.*, **31**, 1138 (1959).

<sup>2</sup> Stief, L. J., DeCarlo, V. J., and Mataloni, R. J., *J. Chem. Phys.* (in the press).

## PHYSICS

## A Nitrogen-14 Triplet and Nitrogen-15 Doublet as a Standard for Electron Spin Resonance Hyperfine Splitting Determinations

THE results given here follow on from an earlier communication in which it was reported that treatment of certain methacrylate polymers with nitric oxide gas induced an electron spin resonance signal<sup>1</sup>. It was later found that pure polymethyl methacrylate powder did not give a signal on treatment, and Law and Ebert<sup>2</sup> have suggested that the observed signal was due to traces of monomer in the polymer sample.

Methyl and *n*-butyl methacrylate monomers were treated with nitric oxide either by allowing the sample to stand in the presence of the gas for about an hour or, alternatively, by bubbling the gas through the monomer, the latter method being found to be the more satisfactory. The observed electron spin resonance absorption was investigated in the X-band region and the first derivative of the resulting signal (shown in Fig. 1) was found to consist of three evenly spaced peaks of equal intensities. It is suggested that the nitric oxide reacts with the carbon double bond ( $C=C$ ) and as a result of this both an unpaired electron and a nitrogen nucleus become associated with the monomer. Similar results were obtained with nitrogen dioxide.

The *g*-value of the central peak was compared with that of diphenyl picryl hydrazyl (2-0036) and was found to be

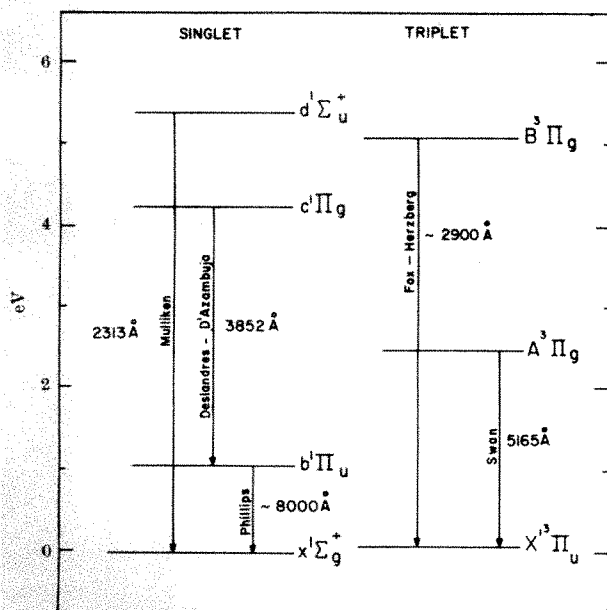


Fig. 1. Energy-level diagram of  $C_2$



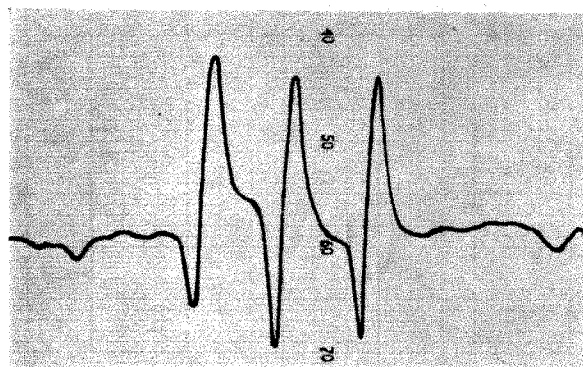


Fig. 1

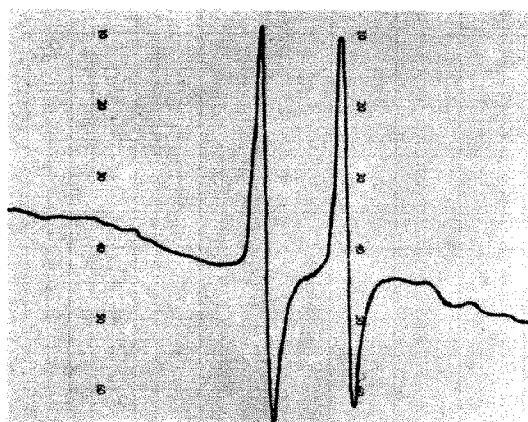


Fig. 2

$2.0058 \pm 0.0007$ . The splitting between adjacent peaks was  $14 \pm 0.5$  gauss corresponding to 39 Mc/s. The fine structure of the signal is interpreted as resulting from an interaction between the unpaired electron and the nitrogen ( $^{14}\text{N}$ ) nucleus.

The results of a further experiment support this interpretation. The interaction of an unpaired electron with a nucleus of spin  $I$  gives rise to  $(2I + 1)$  peaks. In the case of  $^{14}\text{N}$  spin  $I = 1$ . The isotope  $^{15}\text{N}$  (spin  $I = \frac{1}{2}$ ) was substituted for  $^{14}\text{N}$  in the nitric oxide gas in which case a doublet was obtained (Fig. 2). The doublet splitting was  $20.3 \pm 0.5$  gauss corresponding to 57 Mc/s. A mixture of two monomer samples treated separately with  $^{14}\text{NO}$  and  $^{15}\text{NO}$  gave a simple superposition of the triplet and doublet signals with both signals symmetrical about the central triplet peak. Assuming that the electronic wave function of the unpaired electron is the same at the site of the  $^{14}\text{N}$  and  $^{15}\text{N}$  nuclei, the results are in agreement with Fermi's hyperfine interaction theory<sup>3</sup>, that is, the ratio of the doublet and triplet splittings is equal to the ratio of the  $^{14}\text{N}$  and  $^{15}\text{N}$  nuclear magnetic moments, within the measured accuracy.

Comparison of the  $^{14}\text{N}$  triplet in nitric oxide-treated monomer and in peroxalimine disulphonate solution<sup>4</sup> has shown the former to be more stable at room temperature (there was no appreciable decay over a period of a month for a sample sealed in vacuum) and with a lower dielectric loss at X-band frequency. A sample was diluted with untreated monomer and the amplitude of the first derivative signal was found to decrease linearly with monomer dilution while the splittings remained constant to within the stated accuracy. It is therefore suggested that this sample would be more suitable for use in electron spin resonance line splitting determinations.

Measurement of the hyperfine splitting at very high frequency and zero field is being carried out, and the results will be reported in the near future.

This work was carried out independently of the work reported on related topics by Law and Ebert, although the two groups have kept in close liaison [see p. 1193 of this issue of *Nature*].

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<sup>1</sup> Cook, P. D., Ebert, M., and Mallard, J. R., *Nature*, **198**, 579 (1963).

<sup>2</sup> Law, J., and Ebert, M., private communication.

<sup>3</sup> Fermi, E., *Z. Physik*, **60**, 320 (1930).

<sup>4</sup> Pake, G. E., Townsend, J., and Weissman, S. I., *Phys. Rev.*, **85**, 682 (1952).

### Some Applications of an Improved Scanning Electron Diffraction System

An improved system for scanning electron diffraction has recently been developed in this laboratory. The main improvements on the earlier system of Grigson<sup>1,2</sup> are greater stability and sensitivity of the diffraction instrument and a more accurate display; moreover, provision is made for evaporating specimens, so that intensity profiles from a growing film are obtained; and the diffraction patterns may be scanned in two dimensions.

These improvements considerably extend the usefulness of direct-recording electron diffraction for examination of thin films: (1) The abscissae of the peaks of the intensity profiles, as measured from the recordings, are proportional to  $\sqrt{E} h^2$  to an accuracy of 0.2 per cent over the range of

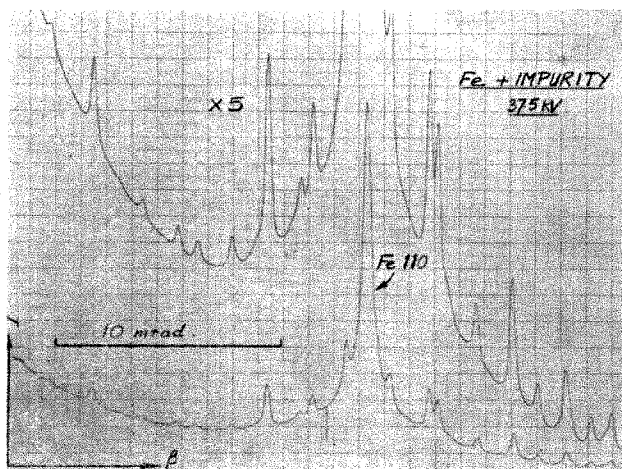


Fig. 1

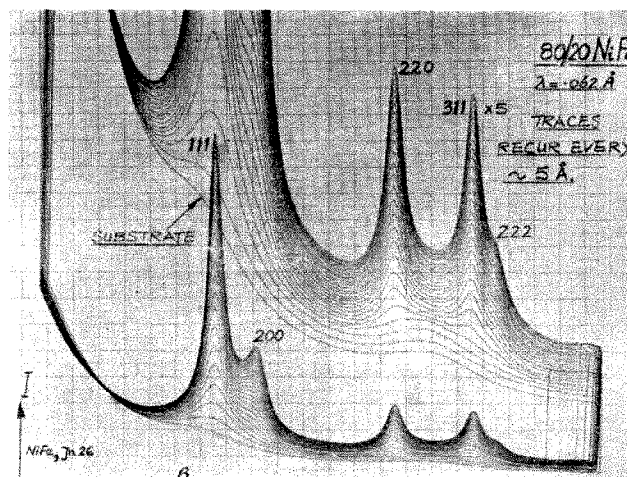


Fig. 2

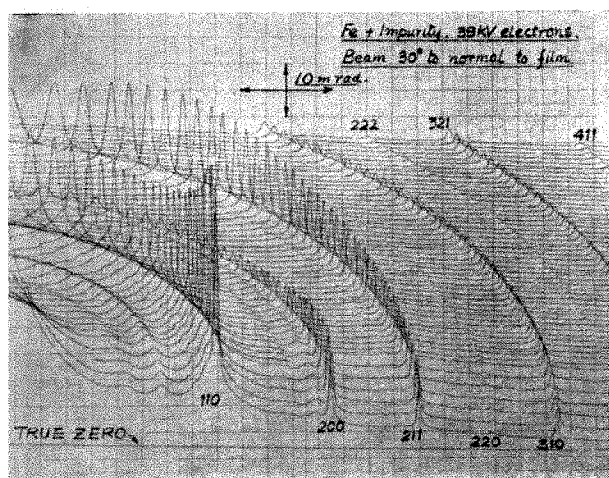


Fig. 3

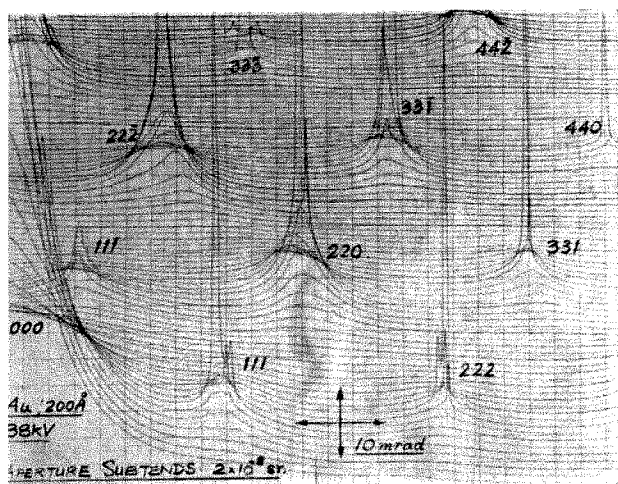


Fig. 4

scattering angles  $0 < \beta < 0.2$  radians, thus  $d$ -spacings corresponding with unknown impurities in the films must be correct to this accuracy.

(2) The high sensitivity enables weak impurity rings close to strong rings from the principal constituents of the films to be easily shown: for example, a ring of 1 per cent of the intensity of an adjacent ring, with an angular deviation of 1 milliradian away, can be clearly displayed (Fig. 1).

(3) Films may be grown<sup>3</sup> and successive diffracted intensity profiles then correspond with uniform average-thickness increments in the film. Sensitivity is sufficient to give clear signals from increments of average thickness equal to about a monolayer of atoms; see Fig. 2, which shows the growth of an 80/20 nickel-iron film.

Such increments may be observed whether the growing layers are amorphous<sup>3</sup>, or consist of crystals of very small or relatively large lateral dimensions. Thus the presence or development of small inclusions of crystalline impurity can be detected. The values of the thickness increments are obtained from interferometry and from use of a growth rate monitor<sup>4</sup>.

(4) Debye-Scherrer patterns scanned in two dimensions may be obtained. Fig. 3 is such a pattern from an iron film about 400 Å thick, inclined to the electron beam. The variation of intensity due to preferred orientation is vividly shown.

(5) Patterns from single crystal films may be displayed. Fig. 4 shows the intensities from a gold single crystal

film about 200 Å thick. Signal-levels are now about two orders greater than with powder patterns. An interesting feature is that the amount of background between the diffracted rays is high and comparable with that from polycrystalline films of similar thickness.

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### Relative Velocity in Relativity Theory

CONSIDER three co-ordinate systems,  $S$ ,  $S'$ , and  $S''$ . Let the velocities of the origins of  $S'$  and  $S''$ , as observed in  $S$ , be  $Sv_{S'}$  and  $Sv_{S''}$ , respectively, and let the velocity of  $S''$ , as observed in  $S'$ , be  $S'v_{S''}$ . Then the remaining possible velocity observations give  $S'v_S = -Sv_{S'}$ ,  $S''v_S = -Sv_{S''}$ , and  $S''v_{S'} = -S'v_{S''}$ . In principle, these six velocities can be measured directly (which is what we mean by the words 'as observed in'). If we wish to use the Lorentz transformation equations to express relations, valid in one co-ordinate system, in terms of another of these co-ordinate systems, then the appropriate one of the foregoing velocities appears in the transformation equations.

It is possible for an observer in  $S$  to measure that the velocity of  $S'$  is  $Sv_{S'}$ , and that of  $S''$  is  $Sv_{S''}$ , and so to say that the relative velocity of  $S''$  with respect to  $S'$  is  $Sv_{S''} - Sv_{S'}$ . This is not at all the same as the velocity  $S'v_{S''}$ , because the co-ordinate systems in which these quantities are measured are different, as is indicated explicitly by the left superscripts used in the present notation. Therefore, it is in principle quite invalid for the relative velocity  $Sv_{S''} - Sv_{S'}$  to be put into the Lorentz equations for transformations between  $S'$  and  $S''$ .

However, in certain specific cases it is permissible to use the relativistic velocity transformation equation:

$$S'v_{S''} = \frac{Sv_{S''} - Sv_{S'}}{1 - \frac{Sv_{S''} Sv_{S'}}{c^2}} \quad (1)$$

(or its vector generalization when the velocities are not parallel) to get the required velocity  $S'v_{S''}$  in terms of the relative velocity  $Sv_{S''} - Sv_{S'}$ . This equation is valid for transformation between inertial systems, where the velocities are uniform, and is derived for this case.

An example where equation (1) cannot be used is given by the Mössbauer experiment of Champeney and Moon<sup>1</sup>, in which source and absorber were placed at opposite points on the periphery of a uniformly rotating rotor. Let  $S$  be a laboratory system of co-ordinates,  $S'$  a system in which the Mössbauer source is at rest and  $S''$  a system in which the Mössbauer absorber is at rest. An observer in  $S$  (the laboratory) notes that  $Sv_{S''} = -Sv_{S'} = v$ , and so deduces that the relative velocity of  $S''$  and  $S'$ , observed in  $S$ , is  $Sv_{S''} - Sv_{S'} = 2v$ .

However, it is obvious that in the co-ordinate system  $S''$  (absorber), the system  $S'$  (source) is seen to be stationary, so that the velocity of the source as seen by the absorber is  $S''v_{S'} = 0$ , which is not deducible from equation (1). This conclusion is confirmed by the null result of the experiment, in which  $S''$  (that is, the absorber itself) observed no Doppler shift of the source frequency for the conditions stated (source and absorber at same pseudo-gravitational potential). We ascribe the inapplicability of equation (1) for this case to the fact that the transformation relations between  $S'$  and  $S''$  are inhomogeneous consequent on  $S'$  and  $S''$  being non-inertial systems.

Thus, Champeney and Moon's experiment does not indicate that 'the special theory is wrong', as claimed by Essen<sup>2</sup>.

It is perhaps of interest to note that interpretation of Champeney and Moon's experiment need not be dependent on special relativity. Time dilatation in a uniformly rotating co-ordinate system may be deduced if we believe that clocks in such a system can be synchronized by using the postulate of the constancy of the velocity of light. In the rotating system, if a light ray travels a go-and-return path  $2 dr$  in time  $2 d\tau$ , we have:

$$(d\tau)^2 = \frac{(dr)^2}{c^2} \quad (2)$$

while for the same events observed in a stationary system, each path length is  $[(dr)^2 + (r d\phi)^2]^{\frac{1}{2}} = [(dr)^2 + (r\omega dt)^2]^{\frac{1}{2}}$ , so that:

$$(dt)^2 = \frac{(dr)^2}{c^2 - \omega^2 r^2} \quad (3)$$

whence:

$$d\tau = \left(1 - \frac{\omega^2 r^2}{c^2}\right)^{\frac{1}{2}} dt \quad (4)$$

the same result as given by special relativity for  $v = \omega r$ .  
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<sup>1</sup> Champeney, D. C., and Moon, P. B., *Proc. Phys. Soc.*, **77**, 350 (1961).

<sup>2</sup> Essen, L., *Nature*, **202**, 787 (1964).

It is questionable whether the Lorentz transformations should be applied to any of the experiments under discussion, since accelerations are involved. If they are,  $S$ ,  $S'$  and  $S''$  in D. W. Posener's letter must be regarded as inertial systems and  $S'$  is then moving with the velocity  $2v$  relative to  $S''$ . According to the special theory the transformation can then be applied between any pair of the systems and it is impossible to differentiate between them.

Equation (4) of Posener's letter does not agree with Einstein's result,  $d\tau$  and  $dt$  being interchanged, although in both cases  $d\tau$  applies to the moving system and  $dt$  to the stationary system. The explanation arises from the inadequate definition of the symbols used in relativity theory. There are actually four measurable times, and if suffixes are used to distinguish them they are by postulate related as follows:

$$d\tau_1 = dt_2, d\tau_2 = dt_1$$

Einstein uses one pair and Posener the other, and both forms of the equation are equally correct. It was this inadequate definition of the symbols which gave rise to the 'paradox' prediction in which the relativity effect was assumed to exist between  $d\tau_1$  and  $dt_2$ .

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### Factors affecting Energy Resolution in Liquid Scintillation Counting

WHEN using  $\beta$ -conversion electron peaks to calibrate a liquid scintillation  $\beta$ -spectrometer, a number of factors which affect energy resolution must be borne in mind.

Care must be taken in selecting the photomultiplier tube, and in our case we chose an E.M.I. No. 6097 F tube. We used a silicone 30,000 centistoke grease to ensure good optical coupling between the vial containing the liquid scintillator and the phototube, and took care that air bubbles were eliminated. We found that the best results were obtained when the vial was dipped into silicone

grease, thus providing an optical coupling about 1 mm in thickness. (Systems in which the vial is separated from the phototube by an air gap do not give sufficient resolution to define the conversion electron peaks, even when this gap is very small.)

The purity of the solution is of great importance. The presence of water is of great consequence, and even if only 0.1 ml. of an aqueous solution of the isotope to be counted is added to 10 ml. of dioxane containing a liquid scintillator, the resolution is reduced to nil. We found that this difficulty could be overcome by either: (a) careful evaporation of the aqueous solution to dryness in the vial before adding the scintillator; or (b) by complexing the dry material with an organic solvent which could be mixed with the scintillator, but which does not quench the scintillation process—for this purpose di-2 ethylhexyl orthophosphoric acid was successfully used for the rare earths and tetraphenylborate in amyl acetate for caesium-137. Not all the active material, however, was complexed by this method, so the extracted portion was transferred to another vial. This technique gave better results than (a).

It is essential that the composition of the liquid scintillator should be kept as simple as possible, in order to avoid multiple-energy transfers which might reduce the resolution. The mixture xylene-PPO (2,5-diphenyloxazole) gave better resolution than xylene-PPO-POPOP (2,2-p-phenylenebis(5-phenyloxazole)) and without loss of efficiency. It is clear that there is more than one possibility of energy transfer in the case of xylene-PPO-POPOP: that is, either xylene to PPO or xylene to POPOP—both of which give a different output of light, or xylene to PPO, followed by transference of a part of its energy to POPOP, some of the energy being emitted directly in the form of light.

Finally, we found that for the best results the vial should be transparent and surrounded by a reflecting material—in our case 'Teflon' was used.

Under these conditions, the 0.625-MeV conversion electron peak of  $^{137}\text{mBa}$  for calibration of the  $\beta$ -spectrometer gave a resolution of 25 per cent.

This work was carried out under a joint contract, 3750r, between the Commissariat à l'Energie Atomique and EURATOM.

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## GEOCHEMISTRY

### Interpretation of Micro-structures in Carbonaceous Meteorites

In their recent article<sup>1</sup>, Claus and Suba-C reiterate a number of older arguments in favour of a biogenic origin of the non-birefringent, globular objects in the Orgueil meteorite. I am of the opinion that these are microchondri of glass and I have previously expounded my arguments in favour of this<sup>2</sup>.

In their new arguments for a biogenic origin for the structures, Claus and Suba-C commented that the histograms of these colourless globules show three maxima which seem to correspond to three distinct species of the hypothetical organisms. It appears that they did not see the histogram of similar objects from the same meteorite contained in my paper<sup>3</sup>, which is practically a replica of theirs. This similarity between the two histograms indicates that the size distribution of the structures in question must be remarkably uniform throughout the

meteorite, since my histogram was constructed from a different sample.

In the course of preparing my histogram, however, I noted that the size-distribution pattern of the magnetite globules, which are present in approximately double the number of the transparent globules, is exactly the same as that of the transparent globules (see Fig. 8, ref. 3). To the best of my knowledge, none of the proponents of the biogenic origin of the transparent globules attributed a similar origin to the opaque magnetite globules. However, we are forced to conclude that the agency that caused the sorting of the opaque magnetite globules must also have been responsible for the sorting of the transparent globules.

I suggest that the globules in question represent fine solidified spray particles which were ejected by gases from the interior of the parent body and gradually became arrested and sorted in the course of the gases permeating the hydrated silicate-carbonaceous phase, close-to-surface cosmic dust—through gravitational factors and the effects of random collisions with other spray particles and cosmic dust particles.

The characteristic form of the histograms with their 2–3 maxima can be explained by assuming that several gusts of gas traversed the cosmic dust at different epochs: the relatively faster gusts transported, as a rule, coarser matter and therefore produced a maximum at a higher diameter, while the slower blow-outs were responsible for the maxima at smaller diameters.

The third point which I discussed in my paper was the fact that the proportion of single crystals of olivine (olivine microchondri) increases as the diameter of the globules increases. This will be realized when we consider that with greater diameter the probability of nucleation of olivine in the glass of close-to-olivine composition progressively increases.

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<sup>1</sup> Claus, G., and Suba-C., E. A., *Nature*, **204**, 118 (1964).

<sup>2</sup> Mueller, G., *Nature*, **196**, 929 (1962).

<sup>3</sup> Mueller, G., in *Advances in Organic Geochemistry. Proc. Intern. Meeting*, Milan, 1962, 114 (Pergamon Press, London and New York, 1963).

In our recent article<sup>1</sup>, a size distribution histogram of 303 randomly selected type 1 and 2 organized elements was presented and the results were interpreted as possibly indicating a biogenic origin for these elements. Prof. Mueller in his note reiterates his older argument in favour of an abiogenical origin of these structures and suggests that the particles arose through the solidification of fine sprays and were ejected by gases from the interior of the parent body of the meteorite. He refers to his histogram, which, according to him, is practically identical to the one we presented. Several points, however, speak against the acceptability of the identity of the histograms. Since one of us (G. Claus) was a participant at the first International Meeting in Organic Geochemistry in Milan, 1962 (Prof. Mueller<sup>2</sup> mentions our presence in his paper, p. 118), naturally we are well aware of Prof. Mueller's results; however, even at that Meeting, during the discussion, it was pointed out that a basic misunderstanding exists over the identification of what Prof. Mueller calls glass particles or olivine crystals and what we call type 1 or 2 organized elements. In his histogram (*loc. cit.*, p. 114), the curve indicated as glass and homologized with the organized elements is clearly not identical with the histogram we presented. Indeed, the organized elements are never 'transparent globules', as stated by Mueller, and are definitely not composed of glass. This has been demonstrated by the electron microprobe (Nagy *et al.*<sup>3</sup>); most of, and probably all, the type 1 and 2 organized elements are structures which possibly contain limonite. It is

possible that Prof. Mueller may have missed this article and, similarly, the subsequent work on the properties of the organized elements, for example, that of Urey<sup>4</sup> in which it is stated that after acid treatment of the meteorite an acid-insoluble residue is obtained which is composed primarily of spherical bodies. The large number and size of these bodies show good correlation with the numbers and shapes of organized elements in native meteorite preparations. Mueller does not make any allusion to this finding; furthermore, he does not take into account the work of Nagy *et al.*<sup>5</sup> concerning the ultra-micro ultra-violet absorption spectra derived from single organized elements, which indicate the possible presence of proteinaceous and nucleic acid-like substances in them. Clearly these data definitely contradict his assumption about the glassy nature of the organized elements. It seems that we are dealing with a quite different group of particles from that which Prof. Mueller counted.

So far as the size distribution of the olivine crystals is concerned there is no correlation whatsoever between Prof. Mueller's histogram (*loc. cit.*) and our data.

The size distribution of the magnetite particles is interesting. However, it is not at all clear from Prof. Mueller's data whether he considered in his histogram only the really globular forms, which are rather rare, or if all kinds of magnetic 'globules' were represented. Should the latter be the case, it is easy to understand the size-distribution diagram which he presents. In an earlier paper (Nagy *et al.*<sup>5</sup>) we pointed out the differences which exist between the sizes and shapes of the magnetic particles. According to further investigations which we have carried out (unpublished results) these particles, even if one strictly separates the completely globular forms, do not show a uniform size distribution. On the other hand, if all kinds of magnetic particles are carefully measured and the elongated or more or less shapeless forms are also included, dependent on whether their longest or shortest measurement is taken, it is possible to construct histograms which have 2, 3, 4, 6 or even 8 peaks. This is just a question as to which measurements the investigator is inclined to use in constructing these curves.

The number of magnetite particles was reported by us (Nagy *et al.*<sup>6</sup>) to be approximately 7,000/mg of the meteorites belonging to Wilk's<sup>7</sup> type 1 carbonaceous chondrites. Prof. Mueller<sup>1</sup> gives a count which is approximately double the number of the organized elements. As the number of organized elements in 1 mg of meteorite is approximately 1,500, the number of the magnetic particles would be 3,000 according to Mueller. There is definitely a discrepancy between these two numbers, which again does not help to identify the granules that Mueller calls magnetite and represents in his histogram. The number of unquestionably spherical magnetite granules is only about one-seventh to one-eighth of the total number—again a much smaller number than would account for the number of magnetite granules possibly represented by Mueller.

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<sup>2</sup> Mueller, G., in *Advances in Organic Geochemistry. Proc. Intern. Meeting*, Milan, 1962, 110 (Pergamon Press, London and New York, 1963).

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## GEOLOGY

## Age of the Caledonian Orogeny and Metamorphism in Britain

RECENTLY a hypothesis on the age of the main orogenic deformation of the Dalradian rocks of Britain proposed by Fitch, Miller and Brown<sup>1</sup> was criticized on stratigraphic grounds by T. G. Miller<sup>2</sup>. I find myself in agreement with the essentials (though not the whole) of Miller's argument against a Pre-Cambrian age for the main orogenic deformation of the Dalradian rocks, and feel that some further points should be made on this aspect of the 'working hypothesis' of Fitch, Miller and Brown. A fuller account of my views on the geographical and time relationships of the 'early Caledonian' and 'late Caledonian' orogenies of Britain is being prepared to amplify the conclusions briefly indicated in the introduction of an earlier work<sup>3</sup>, but it would appear pertinent to raise briefly some of the relevant points now.

In their original article and in their later reply<sup>4</sup> to T. G. Miller the three authors reject the only published accounts<sup>5,6</sup> of detailed investigations of the Leny Limestone of Callander since the discovery by Pringle of Middle Cambrian trilobites there<sup>7</sup>. The interpretation of isotopic data by Fitch, Miller and Brown does not appear to be sufficiently unequivocal to allow the rejection of Stone's conclusion that the Leny Limestone forms an integral part of the Dalradian succession without re-mapping of the Callander area to find another structural hypothesis in agreement with the field evidence. At present the evidence of stratigraphic and structural relationships as they appear in the Callander area must be given more weight than isotopic data which are manifestly open to more than one interpretation<sup>1,8,9</sup>. Moreover, although fossils have so far been found only at this one locality, it seems to me (as it did to Anderson<sup>5</sup> and Shackleton<sup>10</sup>) highly suggestive that the Leny Grits of Arran are divided by a thin group of slates with a limestone in their middle, giving a stratigraphic succession closely similar to that advocated for the Callander area. In Arran these strata occur only a little lower in the exposed succession than the 'Arenig' rocks of the North Glen Sannox area. The advocated Arenig age of these rocks now seems uncertain in view of the evidence suggestive of Pre-Arenig folding and metamorphism in north-west Ireland and since their assignment to the Arenig was based on a lithological comparison of common rock-types and the presence of two poor specimens of a brachiopod genus which can be found in both Cambrian and Ordovician rocks. Nevertheless, whether these rocks be of Cambrian or Arenig age, it would seem relevant that they appear to have shared completely in the folding and metamorphism of the Dalradian rocks, from which they are separated by only a possible slight disconformity<sup>11</sup>; it would appear that here, as in the Callander area, the highest strata involved in the orogenic deformation contain Lower Palaeozoic fossils.

The suggestion by Fitch, Miller and Brown that the  $F_3$  phase of the Highlands may be equivalent to the  $F_1$  of Wales, Isle of Man, Lake District, etc., appears, in the present state of knowledge, attractive, but their brief quotation, without any details, of Pre-Devonian fold amplitudes of 10,000–15,000 ft. in the Midland Valley of Scotland<sup>12</sup> as supporting this link tends to obscure the true geographical relationships. It may be mentioned in passing that the concept of fold-amplitudes as defining orogeny is itself open to criticism, since the essential characteristic of orogeny is horizontal crustal shortening rather than differential vertical movement, though in the cases quoted orogenic deformation is not disputed. The main point here is that both the localities mentioned as examples of end-Silurian folding are right against the southern margin of the Midland Valley. A little farther north in the Lesmahagow district, the Hagshaw Hills and the Tinto area, and much farther north at Stonehaven,

the Silurian rocks remained horizontal and undisturbed until Middle Devonian times. It appears probable that the major part of the Midland Valley remained a stable block separating fold movements in the country to the south from possibly synchronous events in parts, in any event, of the mobile belt to the north.

The sudden nature of the boundary of this block to the south of the Hagshaw Hills and Tinto may be in part due to differences in the stratigraphic/structural level of the deformed and undeformed rocks, the marginal folds of the orogeny fading out upwards as well as laterally. Making allowance for this, however, the edge of the end-Silurian orogenic belt in southern Scotland (also apparently in eastern Wales between Welshpool and Long Mountain) was far more sudden than the available exposures would require for the southern limit of an early Palaeozoic orogeny of the Dalradian rocks of the British Isles. On this basis, and also on account of other examples of knife-edged orogenic belts in other parts of the world (as in Algeria and parts of the eastern Rockies), there appears no validity in Fitch, Miller and Brown's contention that an early Palaeozoic age for the main deformation of the Dalradian rocks is unlikely due to the absence of movements at these times in areas farther south.

In summary, while it is agreed that there is room for more work on the rocks of the southern strip of the Highlands, at present there appears reasonable evidence that strata containing Palaeozoic fossils were involved in the main phases of deformation of the Dalradian. There seems no reason to refute this evidence because the age-dates of the main orogenic movements have been largely obscured by later events, when all the ages found in the Dalradian rocks could as well result from modification of an early Palaeozoic orogeny as from partial changes of original Pre-Cambrian dates.

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## Coesite from Lake Mien, Southern Sweden

LAKE MIEN (lat. 56° 25' N, long. 14° 52' E.) is situated about 30 km north of Karlshamn, or almost on the border between the provinces of Smaland and Blekinge. The depression in which the lake is located is to a large extent filled by glacial deposits from the last glaciation. No outcrops have been found around the lake, except a few on the northern shore, which consist of granite.

During the regional mapping of Sweden, Holst<sup>1</sup> discovered that boulders of peculiar rocks, regarded by him as volcanic, occurred within an area south of the lake. Such boulders are common both in the till and in the glacial sediments. It is evident that they derive from the bottom of the lake. This geological structure is regarded in all books on the regional geology of Sweden even the most recent ones<sup>2,3</sup>, as the remnants of a young rhyolitic volcano, probably Tertiary.

A survey of the literature shows that another interpretation has also been considered. This is already evident from the original paper by Holst. He sent some rock samples from Lake Mien to the petrographer Prof. F. Zirkel for microscopical studies. In a letter to Holst

lated March 13, 1888, Zirkel says [original German letter not located, translated into English from the Swedish translation in Holst's paper (p. 28)]: "Above all I must emphasize that practically all rock samples show an extraordinarily high degree of brecciation", and speaking about the rhyolites: "The rock samples do not in any way look like normal eruptive rocks, and I would not entirely reject the idea that a completely abnormal process is responsible for their genesis". In his short petrographic description of the rocks Zirkel repeatedly points out the peculiar character of the rocks. He also notes the rapid changes in the colour of the glass present in many of the rock samples.

In 1910, at a meeting of the Geological Society of Stockholm, A. G. Högbom<sup>4</sup> suggested, with every reservation, that the Lake Mien structure has an impact origin and compared it with Meteor Crater in Arizona. In 1963, Fredriksson and Wickman<sup>5</sup> pointed out that Lake Mien is among the five known geological structures in Sweden which are possible astroblemes.

The size of the Mien structure is not exactly known but must be of the order 4–5 km, or definitely smaller than the Ries Basin in Germany.

An interesting fact is that rocks from Lake Mien and uveites from the Ries Basin are so similar in appearance that they cannot be distinguished megascopically. We have therefore tried to detect coesite in the rocks of Lake Mien. Through the courtesy of Prof. W. von Engelhardt in Tübingen we have obtained coesite-bearing samples from Ries. By selecting similar-looking samples from Lake Mien we have succeeded in showing the presence of coesite in these rocks. The idea that Lake Mien is an impact structure is thus now strongly supported. A complete description of the work will be given by one of us (N. B. S.) in *Geol. Fören. Stockholm Förh.*

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## CRYSTALLOGRAPHY

### Crystalloluminescence of Organic Compounds

THE crystallization of various inorganic compounds is known to be often accompanied by luminescence. Bright aminecence was observed visually in the crystallization of  $\text{Ba}(\text{BrO}_3)_2$  (ref. 1), in  $\text{As}_2\text{O}_3$  (ref. 2) from aqueous solutions containing HCl, in the crystallization of  $\text{K}_2\text{SO}_4$  (ref. 3) in the presence of  $\text{Na}_2\text{SO}_4$ , and in the precipitation of KCl or NaCl (ref. 4) from saturated aqueous solutions of HCl, or from ethanol. Photon counters have recorded luminescence in the visible and ultra-violet spectra of the crystallization of NaBr, KI,  $\text{BaCl}_2$ ,  $\text{NaNO}_3$  (refs. 5–8). Crystalloluminescence spectra coincide with those of photoluminescence. For example, crystallization of KI and KCl displayed luminescence in the regions of 80–480 and 240–380 m $\mu$ , respectively.

Though the detailed mechanism of luminescence excitation is not yet clear, energy considerations are no hindrance or explaining the phenomenon, as the crystal-lattice energy of ionic crystals is sufficiently high (100–170 kcal/mole), and corresponds to the luminescence region observed.

The heat of sublimation of molecular crystals is 4–15 cal/mole. The energy of one crystallization act obviously is insufficient for exciting luminescence in the visible

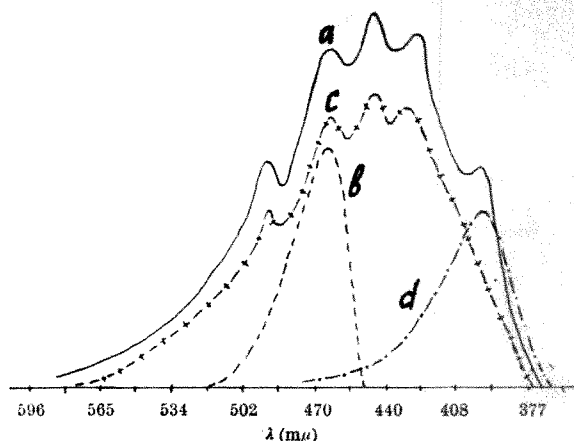


Fig. 1. Photoluminescence spectra: a, amide obtained by reaction of aniline with benzoyl chloride; b, amide purified by repeated crystallization and sublimation; c, benzoyl chloride; d, aniline

spectrum. Nevertheless, luminescence has been observed in the formation of molecular crystals<sup>9,10</sup>. It was recorded for the crystallization from organic solvents of uranine, eosine, 9,10-dibromoanthracene, of benzanilide and its derivatives, using a photometric device involving a photomultiplier as receiver of light. The spectral sensitivity of the photomultiplier was 350–610 m $\mu$ .

The crystalloluminescence of benzanilide used for kinetic studies of the reaction between aniline and benzoyl chloride<sup>9</sup> was investigated in more detail. The quantum yield of crystalloluminescence was estimated as one photon per  $10^{14}$  crystallization acts, by order of magnitude. The luminescence intensity was too low to use a spectral device. Consequently, a set of light filters was used. Spectral characteristics for the photoluminescence of solid benzanilide were obtained by means of the same light filters (Table 1). The spectra appeared to coincide. This may be seen from comparison of the transmittance ( $I/I_0$ ) of various light filters ( $I_0$  is the intensity in the absence of a light filter).

Light filter	Table 1				
	CC4	KC10	C3C-7	3C-7	C-3
Transmission band (m $\mu$ )	240–440	580–3000	380–550	380–450 500–580 550–620	380–450
Photoluminescence	0.50	0	0.88	0.37	0.30
Crystalloluminescence	0.52	0	0.87	0.37	0.30

The photoluminescence spectrum of solid benzanilide represents a broad band in the region of 380–550 m $\mu$  (Fig. 1). From Table 1, this spectrum may be considered as coinciding with that of crystalloluminescence. The same figure shows photoluminescence spectra of benzanilide, carefully purified by repeated crystallization and sublimation, benzoyl chloride and aniline. The photoluminescence spectra of benzoyl chloride and aniline were obtained at liquid nitrogen temperatures. It will be noted that the luminescence intensity of benzanilide shows a marked increase in the presence of KI, HCl, NaOH, while the spectrum remains independent of the nature of additives.

Photoluminescence was excited by the 3126 Å line of a mercury lamp. Absorption spectra of benzanilide, benzoyl chloride and aniline lie in the far ultra-violet. Consequently, it may be suggested that photoluminescence results from the crystalline nature of the systems. The additives will increase the luminescence quantum yield, as well as the absorption and emission capacities of crystals.

It may be seen from comparison of spectra (Fig. 1) that the four spectra exhibit identical luminescence bands seemingly due to the same excitation-levels.

Since the energy of a crystallization act is insufficient for excitation of luminescence, its mechanism for molecular crystals becomes even less clear. Luminescence is observed



both for crystallization and for dissolution of benzanilide, and this makes the phenomenon more complicated.

Analysis of the excitation mechanism is greatly hindered by the low value of the luminescence quantum yield ( $10^{-14}$ ), making it difficult to exclude mechanisms of low probability.

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### Resin Vapour Replication Technique for Snow Crystals and Biological Specimens

Snow flakes have always been the object of interested observation because of their beauty, simplicity and diversity. A systematic study of them was first undertaken by Bentley, who painstakingly photographed thousands of them in the field<sup>1</sup>.

In 1941 Schaefer reported that he could make lasting replicas of snow crystals by coating them with a thin film of polyvinylformal in ethylene dichloride and allowing the plastic to harden while the solvent evaporated<sup>2</sup>. After the ice in the crystals had sublimed through the 'Formvar' film, large numbers of replicas remained which could be studied at will under the light microscope.

This method has recently been still further improved by Schaefer, by dropping the specimens on to dried 'Formvar' films and then exposing them to solvent vapours just long enough to soften the film and make the desired replicas<sup>3</sup>.

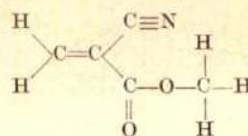
Both these processes, however, involve a liquid solution. Surface tension can cause the particles to become 'rafted'. It causes points and edges to become rounded, and uneven wall thicknesses can result. Nevertheless, these processes are admirably suited for replicating fully-grown snow flakes; but when replicating very small crystals, certain fine details internal and external are lost.

I have found that snow crystal replicas can be made with methyl 2-cyanoacrylate monomer (commercially available as Eastman's 910 Adhesive). The resin is applied through the vapour phase, eliminating the detrimental effects of a liquid medium. The monomer can polymerize on the surface of the crystal and can even penetrate into the interior via air channels (air inclusions).

Using a polarizing microscope, it was found that the replicas themselves are crystalline. Thus, it appears that the polymerization of the monomer does not take place at random sites but at precise locations governed by the molecular structure of the crystal surfaces. The replicas of the snow crystals obtained, therefore, are almost exact reproductions of the original crystals.

It can be observed that a crystal replica often has areas of intensely dark as well as very light regions. This is especially true when the crystal is examined in the electron microscope; the black areas represent areas of high electron absorption, which could result from a stereospecific polymerization. From other work (in preparation), it appears that the dark regions correspond to areas with a dominant positive charge, the light regions to areas of negative charge.

The methyl 2-cyanoacrylate molecule has two highly electronegative groups, a CN and a COOR radical<sup>4</sup>:



These two electron-rich centres of the monomer can co-ordinate directionally with the charged (ice) surfaces<sup>5</sup>. A positive surface would cause the CN and COOR groups to orient radially with the surface; the polymer would grow away from the ice in long chains. In the case of a negative surface, the monomer's electropositive carbons would tend to lie flat against the surface; polymerization could only take place horizontally with respect to the crystal surface, and a monomolecular film only one chain width thick would result. One can easily detect, therefore, the distribution of charge over the surfaces of the crystal.

If a bacterium is exposed to the resin vapour, the monomer penetrates wherever possible throughout the cell and again stereospecific polymerization is induced, with regional differentiation, as in the case of the snow crystals. As the resin can indicate the location and polarity of charged structures in the cell, it may also be of value in this application, as it has been in the case of the snow crystal.

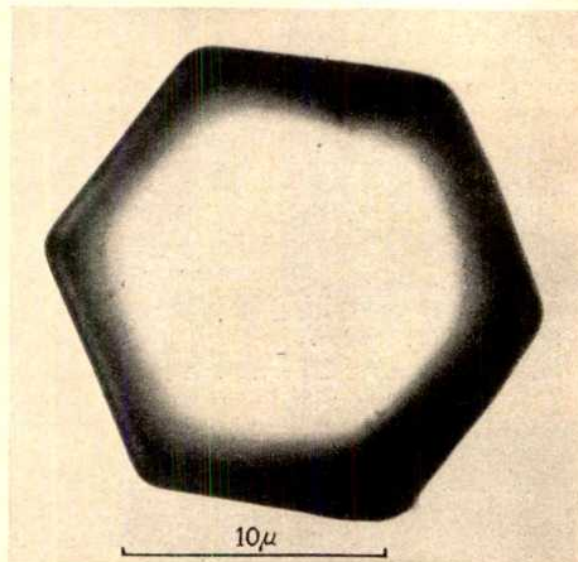


Fig. 1. Typical hexagonal platelet with positively charged periphery and negatively charged interior ( $\times 3,520$ )

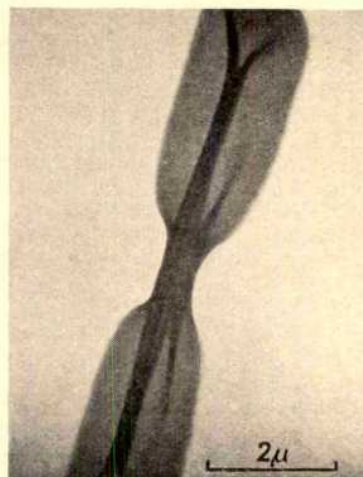


Fig. 2. Replicated ice needle, showing internal structure and charge distribution. Exterior surface is negatively charged ( $\times 8,640$ )



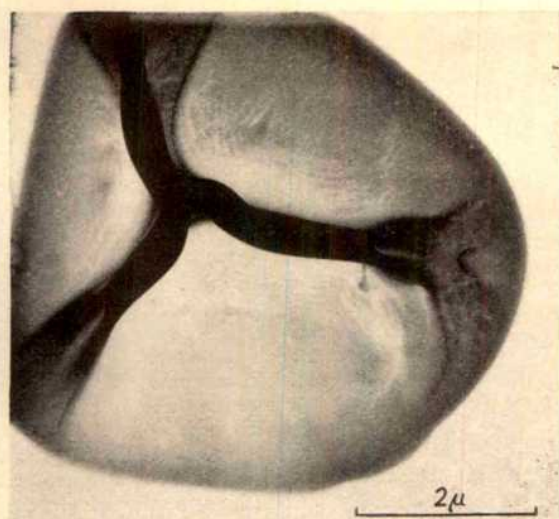


Fig. 3. Replicated frozen cloud droplet, showing considerable internal structure. External manifestations of the crystal structure are not yet evident at this early stage of development ( $\times 12,000$ )

The bacterial and crystal replicas may be best studied in the electron microscope. Here the light and dark areas can be readily identified. Replication of viruses for study with the electron microscope may also prove fruitful. Moreover, instead of replicating a cell such as a bacterium in bulk, it may be possible to prepare microsome sections of frozen cells and then replicate these thin sections with the resin; if this were done, it would be much easier to detect detail and charge distribution in the section examined.

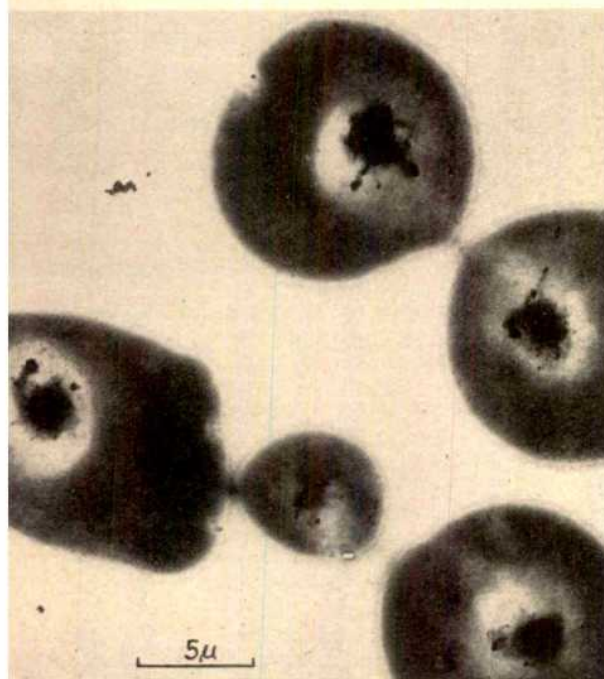


Fig. 4. Replicated bacteria, showing locations of various charged surfaces within the cells

The procedure for replication is as follows: Apply one drop of methyl 2-cyanoacrylate to the surface of a glass slide and spread it into an even layer. Immediately hold it over the sample to be replicated. For small ice crystals, an exposure from 5 to 10 sec at a distance of 1 mm from the sample is sufficient. (The replication of large crystals has been less successful due to the thinness

of the replica walls.) For cells such as bacteria (in bulk) a 30-sec exposure is enough. Following the resin deposition, the ice in the snow crystals is allowed to sublime before coming to room temperature.

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## CHEMISTRY

### Isotope Effect in the Benzene Synthesis for Radiocarbon Dating

As an alternative to the common gas sample methods for radiocarbon dating, liquid scintillation counting has been used and is being improved on. Relatively recently, a practically complete synthesis of benzene, one of the most efficient liquid scintillation materials, has been developed in Texas<sup>1,2</sup> and, independently, in the U.S.S.R.<sup>3</sup> The liquid, containing 92 per cent carbon, all of which comes from the sample being dated, can be produced in approximately 50 per cent yields in 8 h or less. The liquid scintillation counter, now in widespread use in biological and chemical tracer work, is well known for its exceptional reliability. Improvements in the photomultipliers and the introduction of fast electronics, which have substantially

Table 1. RADIOCARBON DATE PAIRS FROM THE INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTIFICAS USING THE BENZENE LIQUID SCINTILLATION METHOD

The ages are calculated as years before 1950 A.D.  $\sigma$  is the standard deviation

Sample	Sample description reference	Age	Agreement
IVIC-15; El Cerro snail shells	(8)	$170 \pm 100$ years	$0.86 \sigma$
IVIC-74; La Betania charcoal	(8)	$1100 \pm 120$ years	$1.78 \sigma$
IVIC-88; Mirinday charcoal	(8)	$1580 \pm 150$	
IVIC-106; Mirinday charcoal	(8)	$580 \pm 140$ years	$0.11 \sigma$
IVIC-108; Mechayyat wood	(8)	$150 \pm 130$	
IVIC-109; Mechayyat wood	(8)	$850 \pm 120$ years	$0.71 \sigma$
IVIC-112; La Betania charcoal	(8)	$680 \pm 120$	
IVIC-145; Tinajas charcoal	(8)	$2730 \pm 140$ years	$1.11 \sigma$
		$2420 \pm 140$	
		$2400 \pm 130$ years	$0.93 \sigma$
		$2660 \pm 150$	
		$1380 \pm 140$ years	$0.11 \sigma$
		$1350 \pm 130$	
		$< 180$ years	$1.17 \sigma$
		$330 \pm 110$	

Table 2. RADIOCARBON DATE PAIRS FROM THE UNIVERSITY OF TEXAS USING THE BENZENE LIQUID SCINTILLATION METHOD

The ground water carbonates are given as per cent of  $^{14}\text{C}$  activity in the modern standard

Sample	Sample description reference	Age or $^{14}\text{C}$ concentration	Agreement
Tx-23; Smith Shelter charcoal	(7)	$680 \pm 150$ years	$0.16 \sigma$
Tx-26; Smith Shelter charcoal	(7)	$730 \pm 170$	
Tx-91; Ground water carbonates	(7)	$685 \pm 85$ years	$0.16 \sigma$
Tx-93; Ground water carbonates	(7)	$725 \pm 170$	
Tx-94; Ground water carbonates	(7)	$76.1 \pm 1.0$ %	$1.26 \sigma$
Tx-95; Ground water carbonates	(7)	$73.7 \pm 0.9$ %	$1.00 \sigma$
Tx-127; Herttoniemi bone	(9)	$17.7 \pm 0.7$ %	$0.35 \sigma$
Tx-111; Mineral Springs charcoal	(9)	$16.5 \pm 0.5$ %	
Tx-158; Ready Bull wood	(9)	$6.72 \pm 0.68$ %	$0.62 \sigma$
Tx-140; Eagle Cave charcoal	(9)	$7.12 \pm 0.47$ %	$0.48 \sigma$
Tx-211; Ground water carbonates	(9)	$2.38 \pm 0.40$ %	$0.59 \sigma$
Tx-210; Ground water carbonates	(9)	$2.88 \pm 0.41$ %	
		$9140 \pm 290$ years	$0.59 \sigma$
		$8920 \pm 165$	
		$600 \pm 80$ years	$0.50 \sigma$
		$500 \pm 90$	
		$9500 \pm 200$ years	$0.50 \sigma$
		$9320 \pm 160$	
		$8640 \pm 170$ years	$0.59 \sigma$
		$8440 \pm 170$	
		$1.47 \pm 0.34$ %	$2.32 \sigma$
		$3.12 \pm 0.37$ %	
		$33.3 \pm 0.5$ %	$0.60 \sigma$
		$33.9 \pm 0.5$ %	



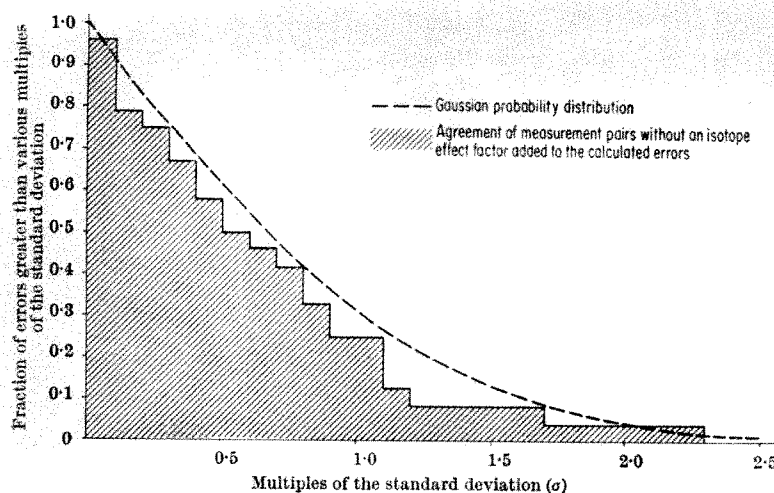


Fig. 1. Distribution of 24 radiocarbon date pairs with the calculated errors based solely on the random nature of the disintegration process

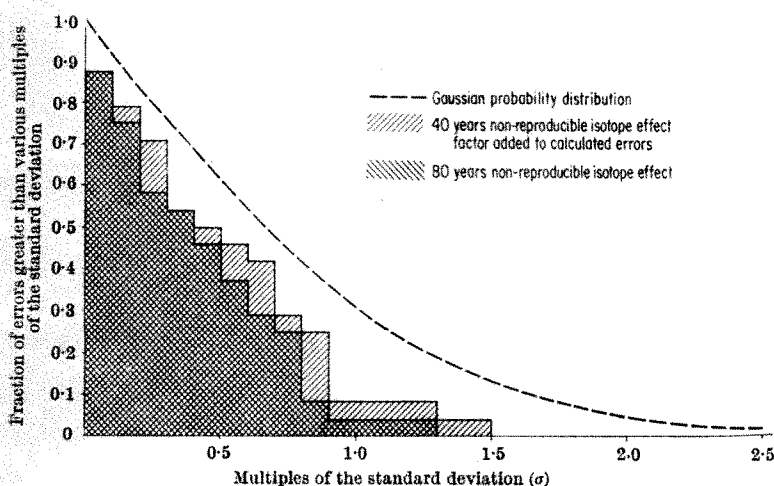


Fig. 2. Distribution of the radiocarbon date pairs with additions of 40 and 80 years to the calculated errors to account for possible isotope effect variations

reduced the resolving time of the coincidence unit, have increased the counting efficiency for radiocarbon to 75 per cent or more. This is comparable to the gas sample counters. The stability of the liquid scintillation counter seems to be as good as that of the best gas counters.

At the present time, the principal objection to the liquid scintillation counting method for radiocarbon dating lies in the incomplete yields of the chemical syntheses. It could be expected that this would give rise to widely varying isotope effects, decreasing the precision of the dating. For example, an isotope effect of only 1 per cent would result in an error of 80 years in the date obtained. This is not important for samples older than a few thousand years, but could be significant for younger materials. Isotope effects of this magnitude have been noticed in acetylene counters<sup>4,5</sup> where the chemical yields vary from 80 to 100 per cent, but should not be present in those methods using carbon dioxide or methane. The

benzene technique, with its still lower chemical yields, could have large uncertainties due to isotope effects in the chemical syntheses. It is the purpose of this work to show that the objection is not valid and that the benzene liquid scintillator method for radiocarbon dating is as reliable, in all respects, as the gas counter procedures.

Some pertinent results from two laboratories using liquid scintillation counting are presented in Tables 1, 2 and 3. This group of data consists of archaeological and geological samples on each of which two completely independent datings were effected. In order to avoid a possible error arising from an inhomogeneity in the original sample, the material was first converted to carbon dioxide gas (in the Caracas laboratory) or strontium carbonate (in the Texas laboratory) and these pure compounds divided in half for the measurement pairs. This was not done for the check sample pairs shown in Table 3 or for Tx-127. The chemical synthesis methods of the University of Texas and the Instituto Venezolano de Investigaciones Científicas (I.V.I.C.) are similar, the main difference being in the acetylene production step. Texas uses the Suess method<sup>4</sup> modified as described previously<sup>1</sup>, and I.V.I.C. the Barker-Mackay technique<sup>6</sup>. The acetylene to benzene reaction catalyst is the same in the two laboratories.

I.V.I.C. uses a Packard Benzene Synthesizer Console while the University of Texas has hand-made systems for the chemical conversions. Both laboratories carry out the radioactivity measurements on Packard Tri-carb Liquid Scintillation Spectrometers and use the NBS Oxalic Acid C-14 Modern Standard.

In the three tables, the quoted errors in the ages or radiocarbon concentrations are based solely on the random nature of the radioactive decay and the background. No error is added for the uncertainty in the half-life of carbon-14. Comparisons of each measurement pair are expressed in multiples of the standard deviation and are given in the last columns of Tables 1-3.

The agreements of the 24 measurement pairs are presented graphically in Fig. 1 and compared with the expected Gaussian distribution. The fit is good. If non-reproducible isotope effects were significant and additional factors of 40 or 80 years (to account for effects of 0.5 and 1.0 per cent, respectively, in one of the samples of each pair) had to be added to the calculated errors, the results shown in Fig. 2 would be obtained. It is seen that these curves are far from the Gaussian distribution and that the agreement of the pairs is too good to be correct. The 80 years' addition gives a distribution clearly wrong. The 40 years' addition curve has an improbable distribution, but is not completely impossible.

There is a considerable amount of other evidence, that cannot be discussed here, supporting the absence of an important non-reproducible isotope effect. This involves the agreements found in multiple runs of the modern standards and, less rigorously, archaeological evidence on a large number of samples that strongly support the ages obtained. The accumulated data can be seen in the recent date-lists of the Texas and I.V.I.C. laboratories<sup>7-9</sup>.

In conclusion, it can be stated with some assurance that in the benzene liquid scintillator method, variations in the measured radioactivities due to causes other than the random nature of the disintegration process amount to

Table 3. INTER-LABORATORY RADIOCARBON CHECK PAIRS FROM THE INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTÍFICAS AND THE UNIVERSITY OF TEXAS

	Sample references		
IVIC-5; Mordan charcoal	(8)	4400 ± 170 years	0.87 σ
Tx-54	(7)	4140 ± 130	
IVIC-18; El Cerro charcoal	(8)	790 ± 100 years	0.05 σ
Tx-188	(9)	800 ± 90	
IVIC-62; El Chao charcoal	(8)	690 ± 110 years	0.39 σ
Tx-189	(9)	610 ± 95	
IVIC-74; La Betania charcoal	(8)	1340 ± 95 years	1.17 σ
Tx-190	(9)	1100 ± 110	

certainly less than 1.0 per cent and probably less than 0.5 per cent. This is insignificant for all purposes of radiocarbon dating.

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### Steric and Electronic Effects in Electron Transfer Reactions

THERE is at present no satisfactory theory to account for the rates of electron transfer from one metal ion to another via a bridging group. Two attempts have been made, both based on the assumption that the probability of electron transfer during the lifetime of the activated complex is small. The first relationship took the form<sup>1</sup>:

$$k_{bi} \cong k_f(C_1 P_{rs} \tau)^2$$

where  $k_{bi}$  is the observed overall rate constant for the reaction,  $k_f$  is the rate constant for the formation of the activated complex,  $C_1$  is a constant, and  $P_{rs}$  the mobile bond order<sup>2</sup> between the atoms  $r$  and  $s$  associated with the metal ions.  $\tau$  is the mean lifetime of the activated complex. This conjugation theory of electron transfer was extended by including the effect of charged reactants on the rate of formation and the mean lifetime of the activated complex<sup>3</sup>, giving at zero ionic strength the relationship:

$$\ln K_0 = C_0 + \ln p_{rs}^2 - \frac{3Z_A Z_B e^2}{DkT r_{AB}}$$

where  $Z_A$  and  $Z_B$  are the charges on the ions  $A$  and  $B$ ,  $C_0$  is a constant,  $D$  is the dielectric constant of the medium,  $k$  is the Boltzmann constant,  $T$  is the temperature, and  $r_{AB}$  the distance between the metal centres. A comparison has been made<sup>4</sup> with results obtained experimentally in the chromium(II) reduction of various pentamminecobalt(III) complexes. Agreement is better with the second theory than with the first, but not good. The suggestion was made that improvement of the M.O. calculations and the electrostatic treatment might produce substantial numerical agreement. Neither theory takes into account the steric effects of substituent groups (that is, groups present as part of the ligand but not participating directly in the electron transfer path). Such groups are important: the rate constants for the reduction of  $(\text{NH}_3)_5\text{CoO}_2\text{C-R}^{2+}$  fall off from  $0.32 \text{ M}^{-1} \text{ sec}^{-1}$  to  $0.04 \text{ M}^{-1} \text{ sec}^{-1}$  as  $R$  changes from  $\text{CH}_3$  to  $\text{cycloC}_6\text{H}_{11}$ , even though the path length for the electron transfer remains unchanged<sup>5</sup>.

Fig. 1 shows a plot of some rate constants as a function of Taft's  $\sigma^*$  parameter<sup>6</sup>. In all cases, the electron transfer path is  $\text{metal}_A\text{—O—C—O—metal}_B$ . Since the van der Waals radius of a  $\text{—CH}_3$  group is almost identical<sup>6</sup> with that of a  $\text{—Br}$ , the steric effects in the two ligands should be the same, so that  $\rho^*$  in the Taft equation:

$$\log k - \log k_{\text{ref.}} = \rho^* \sigma^* + E_s$$

( $E_s$  is the steric factor) can be evaluated for the electron transfer process. The experimental value is  $-0.04$ ,

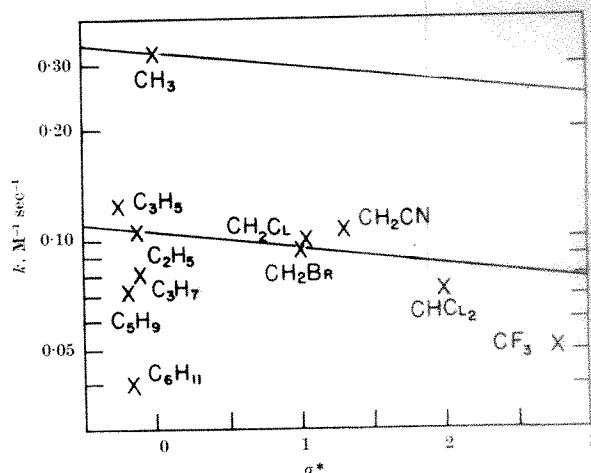


Fig. 1. Rate constants,  $k$ , for the chromium(II) reduction of carboxylatopentamminecobalt(III) complexes  $(\text{NH}_3)_5\text{CoO}_2\text{C—R}^{2+}$  plotted against  $\sigma^*$  values of  $R$ .

similar to the values obtained for the acid hydrolysis of carboxylic esters. If a line with this slope is drawn through the value for the reference compound (acetatopentamminecobalt(III),  $R = \text{—CH}_3$ ), the  $E_s$  values for the other substituents can be obtained. The values are shown in Table 1. With the exception of that for the dichloromethylene, the  $E_s$  values follow the same trend previously found in ester hydrolyses<sup>7</sup>, indicating that any electron transfer theory proposed for bridging reactions must take into account not only the mobile bond order of the bridge and the distance between the metal centres, but also the steric effects of the ligands.

Table 1. STERIC SUBSTITUENT CONSTANTS,  $E_s$

Substituent	$E_s$	Rate constant reference
$\text{CH}_3\text{—}$	0.0	
$\text{C}_2\text{H}_5\text{—}$	-0.49	
$\text{C}_3\text{H}_7\text{—}$	-0.61	8
$\text{CHCl}_2\text{—}$	-0.47	9
$\text{BrCH}_2\text{—}$	-0.49	
$\text{Cl}_2\text{CH—}$	-0.56	9
$\text{CNCH}_2\text{—}$	-0.43	9
$\text{F}_3\text{C—}$	-0.70	10
$\text{CycloC}_6\text{H}_5\text{—}$	-0.43	4
$\text{CycloC}_6\text{H}_9\text{—}$	-0.65	4
$\text{CycloC}_6\text{H}_{11}\text{—}$	-0.90	4

If the bond order is calculated without consideration of the electronic substituent effects, the empirical relationship at  $25^\circ$  and ionic strength equal to 1 is:

$$\log k_{bi} = 7.28 + \log p_{rs}^2 + \rho^* \sigma^* - \frac{3Z_A Z_B e^2}{DkT r_{AB}} + E_s$$

Similar considerations seem to apply in the electron transfer through *ortho* substituted benzoates, where the experimental data are much more limited.

The importance of the  $\sigma^*$  parameter was suggested by Dr. R. G. Linck, Wright-Patterson Air Force Base, Ohio. This research was supported by grants from the National Science Foundation and the Alfred P. Sloan Foundation.

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### Activated Chemisorption of Hydrogen on Copper Films

THE well-known dissociative adsorption of hydrogen on transition metal films has been reported<sup>1</sup> not to occur on evaporated copper films. Copper is catalytically active in hydrogenation reactions, and copper powders prepared by reduction of the oxide have been shown<sup>2</sup> to adsorb hydrogen. It is not certain that the surfaces were clean and it has been suggested<sup>3</sup> that the observed adsorption may be due to impurities. Kwan<sup>4</sup> found a slight activated chemisorption at high temperatures on very carefully reduced copper powder.

On the other hand, surface potential studies<sup>5</sup> of atomic hydrogen adsorption, together with the kinetics of the ortho-para hydrogen conversion reaction<sup>6</sup>, suggested that activated dissociative adsorption should be observable on evaporated films under suitable conditions. The failure to observe adsorption was attributed to an activation energy barrier at  $-78^{\circ}\text{C}$  or lower and to the relatively high equilibrium pressure required for moderate coverage at  $0^{\circ}\text{C}$ . The latter factor and the small surface area of copper films at  $0^{\circ}\text{C}$  render conventional gas volumetric methods insensitive, but some evidence for adsorption at  $0^{\circ}\text{C}$  has been reported<sup>7</sup>.

The presence of chemisorbed hydrogen should be revealed by its surface potential, which can be measured at high equilibrium pressures by the vibrating condenser method. A diode cell similar to that previously used<sup>7</sup> has been adapted by the addition of an extra stem to the film bulb. This stem carries a vibrating reference electrode assembly which may be lifted magnetically *in vacuo* leaving the bulb clear for film deposition and diode measurements. When the latter have been carried out the vibrating electrode can be lowered into position parallel to a flattened section of the bulb wall.

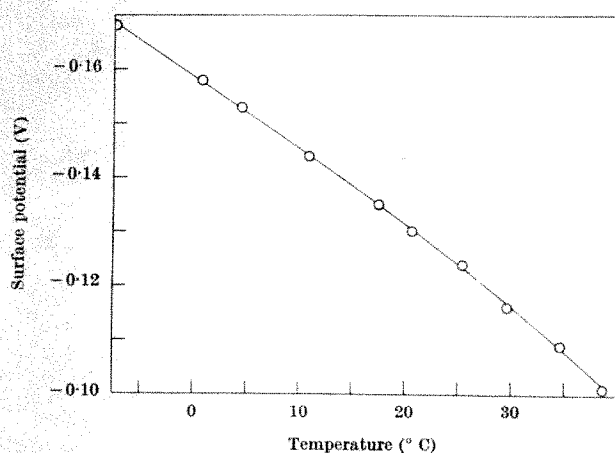


Fig. 1. Surface potential of hydrogen on copper film as function of temperature at 11 mm Hg pressure

The diagram (Fig. 1) shows how the surface potential varies with temperature at a constant pressure of 11 mm Hg on a copper film deposited at  $20^{\circ}\text{C}$ . Complete desorption occurred on pumping. Diode measurements with atomic hydrogen before and after the vibrating condenser measurements gave a rather high maximum surface potential at  $-78^{\circ}\text{C}$  of  $-0.28\text{ V}$ . It seems reasonable to suppose that the surface potential measured by the vibrating condenser was due to chemisorbed hydrogen and hence that the coverage was appreciable. A second film deposited at  $20^{\circ}\text{C}$  gave a more typical surface potential of  $-0.24\text{ V}$  at  $-78^{\circ}\text{C}$  with atomic hydrogen. At  $-4^{\circ}\text{C}$ , vibrating condenser measurements showed the adsorption of hydrogen, equilibrating within a few minutes and with a surface potential rising to about  $-0.23\text{ V}$  as the pressure was increased to 30 mm. The change of

surface potential with pressure was then very small. These results indicate that clean copper does dissociatively chemisorb hydrogen under suitable conditions. Further studies of the equilibria and kinetics of the process are planned.

I thank the Royal Society for a grant of equipment.

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### Hydration of Calcium Sulphate Hemihydrate

IN *Nature* of October 3, 1964, M. J. Ridge discusses the hydration of calcium sulphate hemihydrate on which he modifies his previous views. The mechanism proposed is somewhat similar to that put forward by me at the fourth international symposium on the "Reactivity of Solids"<sup>1</sup> and published in greater detail in subsequent communications<sup>2-4</sup>. However, Mr. Ridge's theory remains open to several objections.

(1) As was pointed out in ref. 2 the fact that the newly-formed gypsum crystals appear in the shape of plates and needles does not imply that their surface area will increase proportionately with the mass. This is not even true approximately. A plate will appear if the growth in one of three mutually perpendicular directions is considerably smaller than the other two and the needle if the growth in two is considerably smaller than the one in the remaining third. For constant, though different, speeds in all three directions the volume (and therefore the mass) will increase proportionately with  $t^3$  and the surface area with  $t^2$ .

(2) It is true that the rates of growth and dissolution may be assumed to be proportional to the surface area; however, these rates are also dependent on the under-saturation and super-saturation of the solution in the neighbourhood of the old and new phases. This depends on the concentration of  $\text{CaSO}_4$  in the suspending liquid, being at first equal to the pseudo solubility of the hemihydrate and being finally equal to the solubility of gypsum which is considerably smaller; this was shown repeatedly experimentally, for example by Birss and Thorvaldson<sup>5</sup>. Such a pronounced change of concentration cannot be expected to be without effect on the hydration. It can also be derived theoretically, as will be shown in a forthcoming paper.

(3) Mr. Ridge's modified differential equation is close to the simplified equation (15) in ref. 2. It differs only by replacing the power index  $2/3$  of  $\alpha$  by 1. This will evidently be of little consequence at the later stages of hydration when  $\alpha$  itself approaches 1 but may lead to significant differences at the beginning of the hydration period. At the beginning of the hydration Mr. Ridge's theory gives  $d\alpha/dt \sim \alpha$  as in his unmodified theory, from which it follows that  $\alpha \equiv 0$ , that is, no hydration at all unless there is some gypsum present at the very beginning ( $P \neq 0$ ). This is also the trivial solution of the modified differential equation now proposed. On the other hand, if at the beginning  $d\alpha/dt \sim \alpha^{2/3}$  as previously demonstrated<sup>2</sup>  $\alpha \sim t^3$  even if there is no trace of gypsum initially. The mass of the crystal increases proportionally to the cube of the time; this would be expected initially from a crystal growing in every direction.

(4) The one example shown fits the equation derived very well, but it should be noted that  $\alpha$  occurs in the

quation always under a cube root, and under either the  $n$  or the  $\tan^{-1}$  so that the expression is very insensitive to any error in  $\alpha$ . It is perhaps significant that the point corresponding to the start of the hydration ( $\alpha = 0.012$ ) is the worst fitting one.

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I FEEL that Schiller may be under some misapprehension regarding our previous work. The differential equation, the solution of which forms my complete equation for the hydration of calcium sulphate hemihydrate, was derived from one developed earlier to account for the self-acceleration of the reaction<sup>1-6</sup> by the inclusion of a term allowing for processes leading to the decay of the rate. Contrary to Schiller's statement (preceding communication), I have not modified my views, but have expressed them more completely in mathematical terms. The differential equation resembles one quoted by Schiller<sup>7</sup> but not derived precisely from a model. The equation mentioned by Schiller is well known for dealing with the formation and growth of nuclei of a solid product (for example, see ref. 8), and he has not tested to what extent a solution describes experimental observations in the particular system. The differences between the two equations are highly significant.

My replies to Schiller's four objections are as follows:

(1) If needles grow exclusively along the long axis and plates in two dimensions parallel to their planes the surface area is approximately proportional to mass, the approximation being better the smaller the unchanging dimensions. This is the condition for the growth of gypsum in my theoretical model, and its aptness for the accelerated and unmodified reaction is borne out by the experimental results. Schiller considers only growth in three dimensions, when a two-thirds relationship will apply as discussed earlier<sup>8</sup>. However, my model is theoretically possible, and in my opinion provides a better basis for interpreting the rate of the accelerated and unmodified reactions than does one assuming growth in three dimensions. The model leading to a two-thirds relationship between the mass and surface area of the system of growing gypsum crystals may be better for interpreting the more complex reaction occurring in the presence of inhibitors which tend to result in equi-axial crystals of gypsum, as we have suggested already<sup>9</sup>.

(2) I am aware of the questions of under-saturation and super-saturation raised by Schiller<sup>4</sup>, but they do not appear to complicate the matter seriously as shown by the accord between experimental results and a theory which ignores them. The probable reason for this is that a steady state regarding concentration gradients will be set up in the kinetically important parts of the system before much hemihydrate has been hydrated. This situation can result from the low solubility of calcium sulphate and the fact that by far the greater part of it in the system is solid.

(3) The difference in power index between my differential equation and that quoted by Schiller makes a considerable difference so far as the first half of the reaction is concerned, and particularly regarding nucleation, as Schiller admits in his third objection. The implication that "there will be no hydration unless some gypsum is present" is in accord with the heterogeneous nature of nucleation in the system. As explained elsewhere<sup>6</sup>, the population of seeding nuclei results in gypsum being present in effect at the

beginning of the reaction, although the hemihydrate need contain no gypsum as such.

(4) The mathematical argument that the expression  $F(\alpha)$  is insensitive to error is incorrect.  $F(\alpha)$  is a function of a single variable, and will respond (unless it is constant) equally to any variation in  $\alpha$  whether this is due to error in measurement or to a real change in the system.

Schiller obtains the differential equation<sup>7,10</sup> to which he refers by a considerable approximation from another equation derived from a mechanism that he develops in the series of papers quoted. In them, Schiller treats an equation (No. 11, ref. 9) as the true outcome of his method. I have tested his equation No. 11 with results obtained from the isothermal hydration of calcium sulphate hemihydrate and find that it does not represent them very well. Schiller uses the increase in temperature of the system to test his equation. It is true that we have shown theoretically and experimentally<sup>2-6</sup> that the courses of the adiabatic and isothermal reactions are not very different for the accelerated and unmodified reactions (but not for the inhibited reaction<sup>6</sup>); but it is nevertheless desirable that equations that are derived for isothermal conditions or on the assumption that the temperature coefficient is small should be tested with data from isothermal hydration, and this Schiller has not done.

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### Thermal Decomposition of Irradiated Strontium Azide

MAGGS<sup>1</sup> showed that the isothermal decomposition of strontium azide is characterized by a marked induction period during which no appreciable volume of gas is liberated. This is followed by a period of acceleration during which an exponential relationship:

$$\log p = k_1 t + c_1 \quad (1)$$

is obeyed.

Garner and Reeves<sup>2</sup> obtained a better fit of the  $p/t$  plots using the power law:

$$p^{1/n} = k_2 t \quad (2)$$

where  $n = 3$ . They also studied the effect of pre-irradiation of the azide by ultra-violet light and found: (i) that agreement with the power law (with  $n = 3$ ) improved, (ii) the induction period was shortened, and (iii) the values of  $k_2$  increased, as the times of pre-irradiation increased. Maggs<sup>1</sup> also studied the effect of the emission from radium on the rate of decomposition. The radium was kept in a glass phial adjacent to the surface of the azide during evacuation of the apparatus and also during the decomposition. At 109.5°C the induction period was halved, and  $k_1$  increased three-fold after an initial evacuation for 15-16 h.

Sviridov<sup>3</sup> found that strontium azide pre-irradiated in air at room temperature for 1 min by X-rays (25 kV and 14 m.amp) decomposed at 126°C at a greatly increased rate. In 20 min, 30 per cent decomposition occurred.



whereas with the unirradiated salt the first sign of decomposition occurred only after 3 h.

Difficulties are experienced in obtaining uniform pre-irradiation with ultra-violet light and X-rays and obviously a systematic study of the effect of the emission by radium is difficult under the conditions used by Maggs. However, pre-irradiation with  $\gamma$ -rays is capable of more precise control and has the advantage that it may be done under vacuum without any possible reaction between air and any nuclei which may be formed, and also that irradiation effects will occur throughout the solid. Marked effects on the subsequent thermal decomposition of barium azide<sup>4</sup> and permanganates<sup>5</sup> have been found after pre-irradiation with 1-MeV  $\gamma$ -rays.

Strontium azide was prepared by the method of Garner and Reeves and recrystallized three times from water. A typical  $p/t$  plot is shown in Fig. 1 with the mathematical analysis. The power law, with  $n = 3$ , fits the acceleratory period in the temperature range 124°–147° C. The decay reaction conforms to the unimolecular decay law equation:

$$\log(p_f - p) = k_3 t + c_3 \quad (3)$$

where  $p_f$  is the final pressure.

The effect of pre-irradiation by  $\sim 1$ -MeV  $\gamma$ -rays is extremely marked. Previous studies of the effects of pre-irradiation by  $\gamma$ -rays on the thermal decomposition of permanganates<sup>5</sup>, ammonium dichromate<sup>6</sup>, nickel<sup>7</sup>, mercuric<sup>8</sup> and lead oxalates<sup>9</sup>, and barium azide<sup>4</sup>, have been made and we find that the sensitivity of strontium azide to  $\gamma$ -rays far exceeds that of any of these substances.

After irradiation (20 Mrads) at room temperature, *in vacuo*, the salt is a light-grey colour. After a dose of 1 Mrad it is still white. Fig. 2 shows the effect of pre-

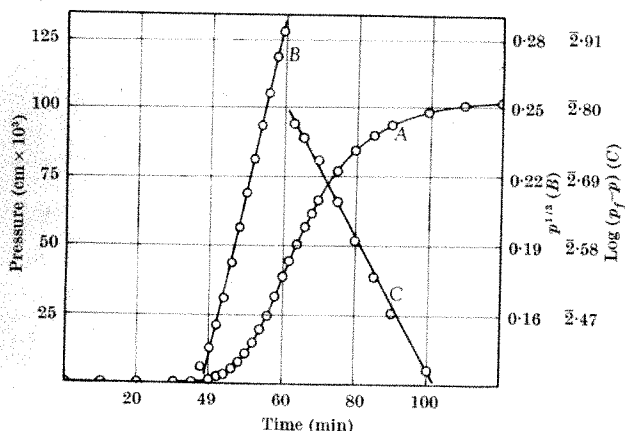


Fig. 1. Curve A, plot of pressure against time for decomposition of 5 mg strontium azide at 132° C; line B,  $p^{1/3}$  against  $t$ ; line C,  $\log(p_f - p)$  against  $t$

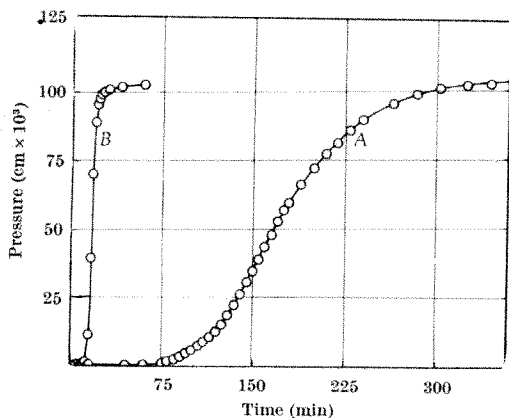


Fig. 2. Effect of pre-irradiation by  $\gamma$ -rays (dose 10 Mrads) on thermal decomposition of strontium azide at 124° C. Curve A, unirradiated; curve B, irradiated

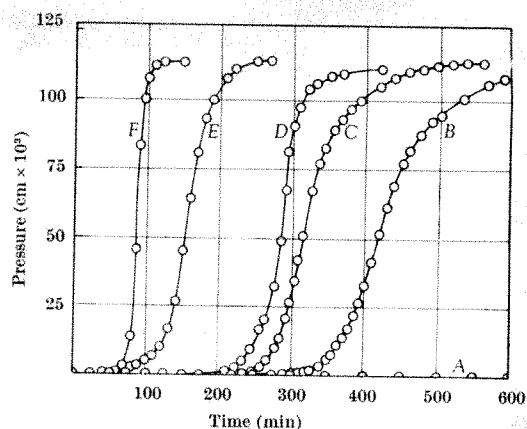


Fig. 3. Effect of varying  $\gamma$ -ray dose on the thermal decomposition of strontium azide at 96° C. Doses: A, 0 rad; B, 1,000 rads; C, 10,000 rads; D, 100,000 rads; E, 1 Mrad; F, 20 Mrads. The induction period for A ended at 700 min

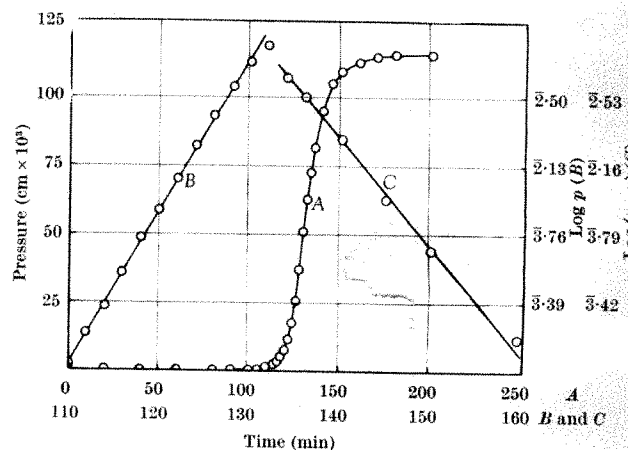


Fig. 4. Curve A, plot of pressure against time for decomposition of  $\gamma$ -irradiated (5 Mrads) at 96° C; line B,  $\log p$  against  $t$ ; line C,  $\log(p_f - p)$  against  $t$

irradiation on the subsequent thermal decomposition. The irradiation effect is produced without measurable loss of gaseous product because the final pressure, for a fixed weight of salt, is independent of the dose. Fig. 3 shows the effect of varying the  $\gamma$ -ray dose.

The power law no longer fits the acceleratory region of the  $p/t$  plots for pre-irradiated azide. Conformity to the power law with  $n = 3$  with unirradiated crystals indicates reaction at a fixed number of nuclei and the growth of these reaction centres in a three-dimensional manner. With irradiated crystals it is expected that the number of nuclei would be increased by irradiation, and as the reaction progresses this number would possibly increase as incipient nuclei become actual growth centres. Such a phenomenon would give higher values to  $n$  in equation (2). However, the power law does not fit the acceleratory period, nor does the Avrami-Erofeyev equation<sup>10</sup>. The Prout-Tompkins<sup>11</sup> equation and the modified form<sup>12</sup> of this equation do not define the plots either.

However, successful analyses are obtained using the exponential law, equation (1), for samples pre-irradiated to a dose of 1 Mrad and higher. Below this dose neither the power law nor exponential law holds throughout the acceleratory period although the extent of fit of the power law improves with decreasing dose, and with the 1,000-rads specimen the fit is from  $\alpha = 0.03$  to  $\alpha = 0.5$ . The decay reaction is described by the unimolecular decay law, equation (3). Fig. 4 shows a typical mathematical analysis.

The exponential law has been used by Garner and Hailes<sup>13</sup> in the study of the decomposition of whole crystals of mercury fulminate. It can be derived theoretically

ing the concept of nuclei as linear, branching chains, or anchoring plate-like nuclei. The formation of additional nuclei by the chain mechanism is a much more important process than the formation of fresh nuclei. The chains could be reaction ones along dislocation lines from nuclei situated there, or they could be branching plate-like nuclei at sub-grain boundaries. The latter possibility is preferable since it would account for a large percentage decomposition before the decay reaction commences; in our studies this occurs at  $\alpha = 0.50$ . The applicability of the bimolecular decay law indicates that in the remaining isolated blocks each molecule possesses an equal probability for decomposition, and thus the rate of reaction is simply proportional to the amount of substance undecomposed.

This study is being continued and electrical conductivity measurements of irradiated and unirradiated crystals are being made. The results will be presented in full elsewhere.

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## BIOCHEMISTRY

### RNA Synthesis in Normal Leucocytes, Leukaemia and Macroglobulinaemia

DIFFERENCES in the sedimentation characteristics and incorporation of radioactive precursors in the RNA of *leu*La cells, ascites tumour cells and rat liver tissue have been reported<sup>1-3</sup>. Marks *et al.*<sup>4</sup>, in the course of extensive studies on RNA synthesis in reticulocytes, reported that apidly-labelled RNA was absent from reticulocytes while it could be detected in leucocytes from normal subjects and patients with chronic lymphocytic leukaemia<sup>5</sup>. The present report describes patterns of RNA synthesis in the leucocytes from patients with leukaemia or macroglobulinaemia and from normal subjects.

Uridine-2-<sup>14</sup>C (specific activity 20–50  $\mu\text{C}/\mu\text{mole}$ ) was obtained from the New England Nuclear Corporation. Bentonite was purchased from the Fisher Scientific Co. and prepared according to Fraenkel-Conrat *et al.*<sup>6</sup>. Venous blood was treated with heparin (0.1 mg/ml.) and incubated at 37° C with 0.1  $\mu\text{C}/\text{ml.}$  of uridine-2-<sup>14</sup>C. The last hour of incubation was carried out in graduated cylinders inclined at 45° to accelerate sedimentation. At the end of 30, 90, 180 and 360 min, aliquots were removed and cooled at 4° C. Leucocytes were isolated as previously described<sup>7</sup>. RNA was extracted by the method of Eason, Cline and Mellie<sup>8</sup>, modified in the following ways: (1) All procedures were carried out at 4° C. (2) Treatment with DNase was omitted. (3) Dialysis of the RNA was usually omitted. RNA was subjected to sucrose gradient centrifugation for 13 h at 23,000 r.p.m. in a Spinco Model L ultracentrifuge using the SW 39 swinging bucket. Optical density at

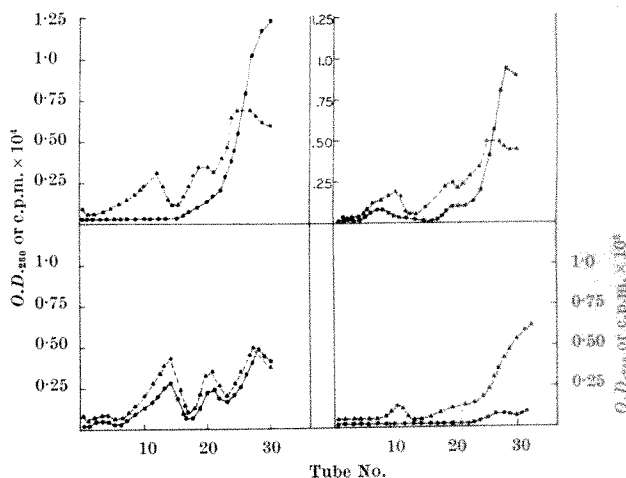


Fig. 1. Sucrose density gradient analysis of RNA from leucocytes of a patient with macroglobulinaemia. The leucocytes had been incubated with <sup>14</sup>C-uridine for 30 min (upper left), 90 min (upper right) and 180 min (lower left). The lower right curve shows the 180-min incubation pattern in RNA obtained following treatment of the patient. ▲—▲, optical density; ●—●, radioactivity.

260 m $\mu$  was determined in a Gilford spectrophotometer, and the radioactivity incorporated was quantitated with either a thin-window gas flow counter or a liquid scintillation counter. RNA was studied from the leucocytes of 7 patients with chronic lymphocytic leukaemia, 5 patients with chronic myelocytic leukaemia and 3 patients with acute leukaemia. All but 3 of the patients were studied on at least two occasions. Agreement of the specific activities of the newly formed RNA in a given untreated patient when studied on different days was within 20 per cent, reflecting the general reproducibility of the incubation, extraction and sedimentation procedure. All the patients were in haematological relapse and were either untreated or had just been started on therapy when studied. One patient with macroglobulinaemia was studied on three occasions. At the time of the first study, the leucocyte count was 60,000 cells/mm<sup>3</sup> and consisted mainly of plasma cell-like lymphocytes. Four laboratory workers served as the donors of normal leucocytes.

The sedimentation profile in sucrose gradient centrifugation of RNA extracted from leucocytes of a patient with macroglobulinaemia (J. R.) is shown in Fig. 1. In the aliquots, removed after 30, 90 and 180 min of incubation, three peaks of optical density at 260 m $\mu$  were identified corresponding to 28S, 16S and 4S material. The incorporated radioactivity, indicated with the dotted line in Fig. 1, shows that the label first appeared in the 4S material in the 30-min sample. After 90 min a small amount of radioactivity is found in the heavier RNA, and uniform labelling is seen after 3 h incubation. The counts precipitated by trichloroacetic acid became non-acid-precipitable after treatment with ribonuclease. Following therapy with chlorambucil, the patient's white blood count decreased to 13,000 cells/mm<sup>3</sup> and plasma cell-like lymphocytes disappeared from the circulation. The patient's differential count and the optical density sedimentation profile obtained at that time resembled that of normal leucocytes. There has been over a 50-fold decrease in specific activity when compared to the previous study, and there is no label incorporated in the 16S and 28S material after 3 h.

In all normal subjects studied, the optical density profile showed the same 28S, 16S and 4S peaks found in the macroglobulinaemic or leukaemic subjects. However, characteristically the specific activity of the 28S and 16S RNA was an order of magnitude lower than seen in the macroglobulinaemic cells when the patient was studied before therapy. The incorporation in J. R. after therapy is lower than that found in any normal subject.

Representative patterns after 3 h of incubation with radioactive uridine are shown in Fig. 2 for cells of chronic myelocytic leukaemia, chronic lymphocytic leukaemia, acute myeloblastic leukaemia and acute monocytic leukaemia. In contrast to the pattern found in macroglobulinaemia, incorporation of radioactivity into the RNA of the leucocytes in chronic myelocytic leukaemia and acute myeloblastic leukaemia is very low, the ratio of radioactivity to optical density being significantly less than in the macroglobulinaemic cells in the 4S material, with essentially no incorporation into the 28S and 16S RNA following an incubation period of 3 h. After 6 h incubation under the above conditions slight incorporation of label was noted in the 16S and 28S RNA, comparable in specific activity to normal leukocytes. Among the 7 patients with chronic lymphocytic leukaemia who were investigated, a consistent reproducible difference was noted in that the specific activity of the RNA in 5 of the patients ranged from 2 to 5 times that of normal leukocytes, while the other 2 patients had normal patterns. In cells of the high synthesis group label appeared in the 28S and 16S RNA between 1 and 3 h, rather than after 6 h, incubation. The profile shown in Fig. 2 (lower left) represents a patient from the group with high RNA synthesis rates, while patients in the low synthesis group would have patterns resembling that shown in the upper right of the figure. The patient with acute monocytic leukaemia shown in the lower right had active RNA synthesis.

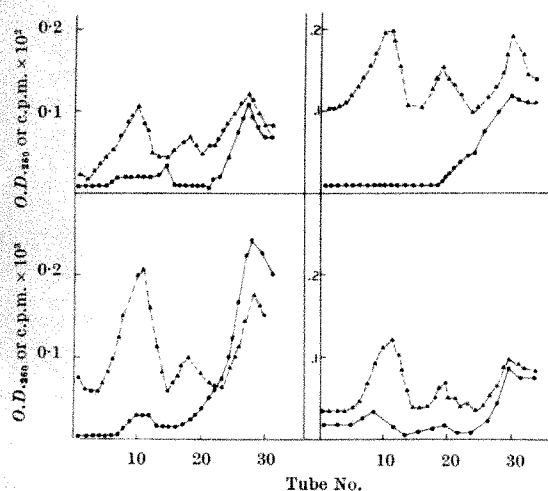


Fig. 2. Sucrose density gradient analysis of leucocyte RNA from patients with leukaemia. Leucocytes were incubated for 18/80 min with  $^{14}\text{C}$ -uridine. Upper left: chronic myelocytic leukaemia. Upper right: acute myeloblastic leukaemia. Lower left: chronic lymphocytic leukaemia. Lower right: acute monocytic leukaemia.  $\Delta$ — $\Delta$ , optical density;  $\bullet$ — $\bullet$ , radioactivity

Experiments where  $^{32}\text{P}$  or  $^3\text{H}$  uridine were used to label RNA confirmed the above results. In two experiments the extracted 4S material was concentrated and hydrolysed with 0.3 M KOH, and the 2'3-nucleotides were separated by electrophoresis<sup>9</sup>. When  $^{14}\text{C}$  uridine was used as a label, equal incorporation into U and C was noted, indicating that RNA synthesis rather than turnover of the A-C-C terminal was taking place.

The present studies reveal heterogeneity in the patterns of RNA synthesis in human leukocytes. The high rate of synthesis in macroglobulinaemic cells is not unexpected in view of the marked synthesis of protein *in vivo* and *in vitro* observed in this disorder. The evaluation of inhibitors of RNA synthesis may be relevant to therapy. Evidence of heterogeneity is found among patients with chronic lymphocytic leukaemia who have identical cell morphology. It remains to be determined whether these changes can be correlated with protein turnover, mitotic activity or infection of the cell by viral agents.

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### Determination of Purines in the Presence of Proteins: Steady-state Levels of Xanthine Plus Hypoxanthine in Rat Liver

DURING an investigation of the influence of tissue xanthine oxidase concentration on the corresponding steady-state levels of hypoxanthine plus xanthine in rat liver (cf. Haddow *et al.*<sup>1</sup>), the recovery of added xanthine, hypoxanthine and uric acid was found to be low.

This problem is not new, since the assay of serum uric acid is complicated in a similar manner<sup>2</sup>. Xanthine and purine are known to bind to certain proteins<sup>3,4</sup>. Xanthine in solution when added to the precipitated tissue proteins was only incompletely removed by repeated washing with 8 per cent perchloric acid solution (recovery was 30–50 per cent depending on the ratio of xanthine to liver). It would thus appear that the losses are due to adsorption of the purines on the precipitated proteins. Complete recovery ( $98 \pm 3$  per cent) may be achieved, however, if the protein precipitant used is lithium perchlorate. The effect is not observed if the lithium hydroxide used is replaced by an equivalent amount of sodium hydroxide.

The following procedure has been found suitable in the case of hypoxanthine plus xanthine and uric acid in liver. A modified method may prove suitable for determining the ratio of free to bound purine in tissues and may possibly be extended to similar investigations in other compounds which are non-specifically bound to proteins. The animals were killed by ether anaesthesia according to Dale<sup>5</sup>. The livers were excised and placed immediately into liquid nitrogen. Weighed amounts of frozen liver were homogenized in twice the volume of 0.45 M lithium perchlorate and the protein plug washed twice with small volumes of the lithium perchlorate solution. The supernatant and washings were combined and neutralized with potassium hydroxide<sup>7</sup>. Xanthine plus hypoxanthine was measured spectrophotometrically by conversion to uric acid utilizing xanthine oxidase<sup>8</sup>. The lowest level which could be measured under these conditions was 0.03  $\mu\text{moles/g}$  wet weight of tissue.

Using the foregoing method an attempt was made to determine the normal steady-state level of xanthine plus hypoxanthine in the liver of the female rat (C.B., Hooded and August strains), female mouse (C<sup>+</sup>) and hen (White

Table 1. DEVELOPMENT OF HYPOXANTHINE PLUS XANTHINE ( $\mu\text{g/g}$  WET WEIGHT OF TISSUE) IN RAT LIVER ON STORAGE AT VARIOUS TEMPERATURES

Time after death	0 min*	5 min	1 h	4 h
24° C	0	30	65	150
4° C	0	8	40	80
-13° C	0	0	0	8

\* Time was measured from point at which liver was removed from animal.

eghorn). A value of less than 0.03  $\mu$ moles/g wet weight was obtained. However, if the method of killing the animal was changed to cervical dislocation, then a value comparable with that obtained by Reid and Stevens<sup>5</sup> was found (0.08  $\mu$ moles/g wet weight of liver in C.B. strain rats). Dale<sup>6</sup> has commented on the effect of stress on the levels of labile metabolites and has suggested that ether anaesthesia is preferable to cervical dislocation in this respect.

Livers were also removed from animals and stored at three different temperatures ( $-13^{\circ}\text{C}$ ,  $+4^{\circ}\text{C}$  and  $+24^{\circ}\text{C}$ ) to see if any alteration in the level of the metabolites had occurred. As expected it was found that enzyme reactions must be quenched by extremely rapid cooling immediately in the animals' death (see Table 1). Even when the rat liver was stored at  $-13^{\circ}\text{C}$ , a gradual rise was observed after the first hour.

If one accepts that the low level of xanthine and hypoxanthine observed represents the true *in vivo* steady-state level, then xanthine oxidase is not a rate-limiting enzyme of purine catabolism under these conditions.

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### Structure of a Dopa Glucoside from *Vicia faba*

$\beta$ -(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA) has only been detected in a limited number of plants, and it appears to be confined mainly to leguminous species. Unlike most other phenols of low molecular weight, however, it exists in relatively high concentrations in the free state. This is particularly the case, for example, with the broad-bean, *Vicia faba*<sup>1</sup>, but Nagasawa and his associates<sup>2</sup> have also isolated an O- $\beta$ -D-glucoside of DOPA from the testa of the bean seed. The Japanese workers did not, however, determine whether it was a 3- or 4-O substituted derivative and in view of our interest in the formation and metabolism of DOPA in plants we have reinvestigated the structure and distribution of this compound.

As *V. faba* contains only small amounts of this DOPA glucoside a preliminary examination of the glucoside obtained in high yield by feeding *Pisum sativum* seeds with DOPA was undertaken and a comparison then made of this compound with the corresponding derivative obtained from the bean.

Seeds of *P. sativum* var. 'Early Onward' were soaked overnight in a saturated solution of L-DOPA. They were then washed and left on moist cotton wool for 4 days in the dark (cf. Pridham and Saltmarsh<sup>3</sup>). The resulting seedlings were extracted with aqueous ethanol (80 per

cent) and the concentrated extract was examined on paper chromatograms. This showed the presence of at least five compounds which are not normally present in the tissues. By careful fractionation of the extract on paper, using ethyl acetate/acetic acid/water (9:2:2 v/v) solvent followed by electrophoresis (0.1-M formic acid, pH 2.4, 4,000 V/1 h), a small quantity of a compound,  $P_2$ , was isolated as a syrup which was freeze-dried to a hygroscopic powder. Hydrolysis of  $P_2$  with  $\text{N H}_2\text{SO}_4$ ,  $\text{N HCl}$ , 50 per cent formic acid or  $\beta$ -D-glucoside glucosylhydrolase yielded only DOPA and D-glucose in the molar ratio of 1:1. The configuration of the glucose was determined by reaction with D-glucose oxido-reductase, and the rate of acid hydrolysis of  $P_2$  suggested that the glucosyl residue was in the pyranoid form.

$\beta$ -(3-Hydroxy-4-methoxyphenyl)-DL-alanine and  $\beta$ -(3-methoxy-4-hydroxyphenyl)-DL-alanine were synthesized as model compounds and their colour reactions with diazotized *p*-nitroaniline/NaOH and ultra-violet spectra under neutral and alkaline conditions determined. This revealed several significant differences between the 3-O- and 4-O-substituted compounds and in particular a variation in the relative absorbance at  $\sim 240$  and  $\sim 295 \text{ m}\mu$  on addition of alkali.  $P_2$  had all the characteristics of a 3-O-substituted DOPA. The absence of a free O-dihydroxyl grouping was further indicated by the fact that it gave no colour reaction with aqueous sodium molybdate and did not exhibit a characteristic hypsochromic shift in spectrum under alkaline conditions when treated with borate.

Confirmation of the position of the D-glucopyranosyl residue on the aromatic ring was obtained after methylation of  $P_2$  by the Kuhn procedure with methyl iodide and silver oxide in dimethylformamide<sup>4</sup>. Alkaline nitrobenzene oxidation<sup>5</sup> of the methylated derivative gave rise to isovanillin as the only aldehydic product. This was characterized by paper electrophoresis using phosphate buffers at pH 8.7 and 10.0, with 2,4-dinitrophenylhydrazine/NaOH as the locating reagent (isovanillin is readily distinguished from vanillin by this method as the latter possesses a more strongly dissociated phenolic hydroxyl group).

The cotyledons from 4-day-old broad-bean (var. 'Johnson's Longpod') seedlings were extracted and examined by the same procedure, and a compound identical to  $P_2$  in all respects was isolated. The conclusion is, therefore, that both  $P_2$  and the derivative from *V. faba* are  $\beta$ -[3-( $\beta$ -D-glucopyranosyloxy)-4-hydroxyphenyl]-L-alanine. The colour reactions and chromatographic and electrophoretic behaviour of this compound are summarized in Table 1.

Table 1. PROPERTIES OF DOPA GLUCOSIDE

Colour reaction with: Diazotized <i>p</i> -nitroaniline/NaOH Ninhydrin Sodium molybdate	$P_2$ and <i>V. faba</i> glucoside
	Plum red—grey green Blue-violet No reaction
<i>R<sub>DOPA</sub></i> values	
Butan-1-ol/acetic acid/water (6:1:2, v/v)	0.47
Butan-1-ol/ethanol/water (40:11:19, v/v)	0.60
Ethyl acetate/acetic acid/water (9:2:2, v/v)	0.35
Ethyl acetate/acetic acid/formic acid/water (18:3:1:4, v/v)	0.37
Ethyl acetate/pyridine/water (10:4:3, v/v)	0.56
Butan-1-ol saturated with water	0.30
<i>M<sub>DOPA</sub></i> values*	
0.1 M formic acid (pH 2.4)	0.85

\* Electrophoretic mobility relative to L-DOPA, corrected for electroendosmosis.

Nagasawa and his colleagues<sup>2</sup> reported that DOPA glucoside occurred mainly in the green testa of a late ripening variety of *V. faba* and to a lesser extent in the hilum and the inner tissue of the pod. Our own chromatographic investigations with the 'Johnson's Longpod' variety confirm their findings but also suggest that this compound is present in both the dormant and germinated cotyledons. There is no evidence, however, for its occur-



rence in either the roots or shoots of young plants or in other issues of the mature plant other than the pods.

Investigations are continuing on the DOPA-DOPA glucoside equilibrium in *V. faba*. The apparent lack of glucoside in the tissues could be due to a weakly active specific glucosylating enzyme (although other phenols are readily glucosylated<sup>2</sup>) or to a physical barrier between the enzyme and its substrate, L-DOPA.

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### Lysosomal Enzymes in Experimental Encephalomalacia

Pappenheimer and Goettsch<sup>1</sup> described the symptoms of chick encephalomalacia produced by vitamin E deficiency and indicated that capillary thrombosis may be the primary cause of the observed ischaemic necrosis. Muscular dystrophy of vitamin E-deficient rabbits may be considered the counterpart of experimental encephalomalacia in chicks since both diseases can be produced by lack of dietary vitamin E.

The activities of a number of acid hydrolases were observed to increase several-fold in skeletal muscle of dystrophic rabbits<sup>2-5</sup>. It was found in this laboratory that a neuraminidase activity (*N*-acetylneuraminyl glycohydrolase), which releases sialic acid from neuraminyl lactose, increases several-fold in skeletal muscle, spleen, and liver of vitamin E-deficient rabbits which developed muscular dystrophy<sup>5</sup>. It was also found that this enzymatic activity is concentrated in the lysosomal fraction obtained by centrifuging liver homogenates according to a technique developed by De Duve *et al.*<sup>6</sup> for tissue fractionation experiments. The presence of lysosomes in brain cells has been demonstrated histochemically by Koenig<sup>7</sup>. An important property of lysosomal enzymes is that they exhibit little activity towards their substrates so long as they remain bound to the intact particles. Rupture of lysosomes within cells can cause solubilization and activation of the bound hydrolases and may play an important part in cellular autolysis, pathological necrosis and inflammation. Evidence for the existence of a neuraminidase activity (*N*-acetylneuraminyl glycohydrolase) in rat brain and other rat organs has been reported by Carubelli *et al.*<sup>8</sup>. Morgan and Laurel<sup>9</sup> demonstrated the presence of an enzyme activity in mammalian brains which releases sialic acid from the endogenous sialic acid-containing compounds within a pH range of 3.5 and 4.0. This report describes the distribution of neuraminidase in fractions of normal chick brain, and experiments which show the effects of encephalomalacia resulting from vitamin E deficiency on the total activity and the intracellular distribution of neuraminidase and other lysosomal hydrolases.

Encephalomalacia in chicks was produced by feeding a specially formulated diet deficient in  $\alpha$ -tocopherol (vitamin E) to one-day-old White Leghorn cockerels<sup>10</sup>. The control chicks received the same diet supplemented with vitamin E. Assays for the total activity of lysosomal enzymes require pretreatment with detergent substances such as

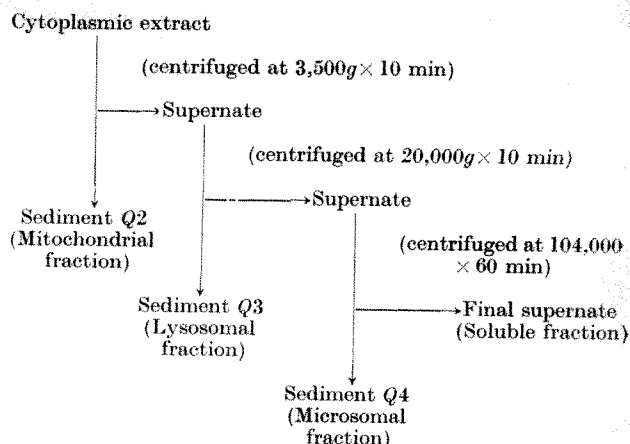


Fig. 1. Flow diagram of cytoplasmic extract fractionation

'Triton X-100' in order to activate the bound enzymes in the lysosomal particles. The total activities of several acid hydrolases were determined in brain homogenates of encephalomalacic and control chicks.  $\beta$ -Glycerophosphate phosphohydrolase (acid phosphatase) activity was determined according to a method described by De Duve *et al.*<sup>6</sup>. This enzyme is one of the original five acid hydrolases which have been identified by De Duve and co-workers as lysosomal enzymes. *N*-Acetylneuraminyl glycohydrolase (neuraminidase) activity was assayed by the method of Carubelli *et al.*<sup>8</sup> in which neuramin-lactose is used as the substrate.  $\beta$ -D-Glucuronide glucuronohydrolase ( $\beta$ -glucuronidase) activity was assayed by De Duve's modification<sup>6</sup> of a method by Fishman *et al.*<sup>11</sup> to permit measurement of total activity. Phenolphthalein glucuronide (Sigma Chemical Co., St. Louis, Mo.) was used as the substrate.

Intracellular components were sedimented by centrifuging brain homogenates according to a technique based on the procedures of De Duve *et al.*<sup>6</sup>. The homogenizing medium was 0.25 M sucrose containing 0.001 M disodium ethylenediamine tetraacetate (EDTA). Homogenization was accomplished by a single passage of the grinding tube upwards then downwards against the rapidly revolving pestle (about 1,000 rev./min) so that all the material had been forced above and then below the 'Teflon' part of the pestle once. The resulting slurry was centrifuged in the cold at 1,000g for 10 min. The sediment, which still contained a large number of unbroken cells in addition to nuclei, was re-homogenized by the foregoing procedure in the same quantity of medium used in the first homogenization and centrifuged at 800g for 10 min. The supernatants (cytoplasmic extracts) were combined and made up to volume to an equivalent of 100 mg tissue per ml. cytoplasmic extract. The sediment (Q1) constituted the nuclear and debris fraction. The cytoplasmic extract was further fractionated according to the scheme outlined here (Fig. 1) using a Spinco model L preparative ultracentrifuge to obtain three additional particulate fractions and the final supernatant. The supernatant solutions were carefully removed by aspiration. The fluffy layer on top of the lysosomal fraction was removed with the supernate to be recovered later with the microsomal fraction. The sediments were suspended in 0.1 M acetate buffer, pH 5.0, and re-homogenized to obtain the equivalent of 500 mg of original tissue per ml. of suspension. These suspensions were used to assay the total activity (free + bound) of acid phosphatase and neuraminidase in the isolated cytoplasmic fractions.

In the control chicks, the determination of acid phosphatase activity ( $\beta$ -glycerophosphate phosphohydrolase) demonstrates a distribution of activity in the cytoplasmic fraction which is typical of lysosomal enzymes (Table 1). The intracellular distribution of neuraminidase (*N*-

Table 1. DISTRIBUTION OF ACID PHOSPHATASE IN CYTOPLASMIC FRACTIONS OF ENCEPHALOMALACIC AND CONTROL CHICK BRAINS

Cytoplasmic fractions	Percentage of total activity Control	Percentage of total activity Malacic	Relative specific activity Control	Relative specific activity Malacic
nuclear and debris	14.1	9.1	0.82	0.55
mitochondrial	37.3	31.8	1.33	1.2
lysosomal	28.1	18.7	3.55	2.1
microsomal	6.9	11.2	0.45	0.78
soluble	13.6	30.2	0.45	0.90

$$\text{Relative specific activity} = \frac{(\text{percentage of total activity})}{(\text{percentage of total protein})}$$

Control chicks were fed the vitamin E-supplemented diet, encephalomalacic chicks received the vitamin E-deficient diet. The values are averages of three experiments.

Table 2. DISTRIBUTION OF NEURAMINIDASE IN CYTOPLASMIC FRACTIONS OF ENCEPHALOMALACIC AND CONTROL CHICK BRAINS

Cytoplasmic fractions	Percentage of total activity Control	Percentage of total activity Malacic	Relative specific activity Control	Relative specific activity Malacic
nuclear and debris	14.8	12.7	0.87	0.77
mitochondrial	18.1	13.0	0.71	0.49
lysosomal	31.4	13.2	3.4	1.47
microsomal	10.2	16.4	0.67	1.14
soluble	25.5	44.7	0.85	1.33

$$\text{Relative specific activity} = \frac{(\text{percentage of total activity})}{(\text{percentage of total protein})}$$

Control chicks were fed the vitamin E-supplemented diet, encephalomalacic chicks received the vitamin E-deficient diet. The values are averages of three experiments.

Table 3. ACID HYDROLASES IN CHICK BRAINS

	Specific activity Controls (5)*	Specific activity Malacic (5)
Acid phosphatase	0.95 ± 0.06†	1.72 ± 0.08
β-glucuronidase	32.5 ± 2.8	156.6 ± 11.3
Neuraminidase	0.34 ± 0.02	0.77 ± 0.06

Specific activities are expressed as follows: acid phosphatase—μmoles orthophosphate hydrolysed per mg protein per h; β-glucuronidase—μmoles phenolphthalein hydrolysed per mg protein per h; neuraminidase—μmoles sialic acid released per mg protein per h. \* Number of experiments. † Standard error.

acetylneuraminyl glycohydrolase) in control chicks is shown in Table 2, where it is apparent that the enzymatic activity is present in the soluble and not only in the lysosomal fraction. The high relative specific activity noted in the soluble fraction may indicate the presence of a second neuraminidase activity. The heterogeneity of sialic acid-containing compounds in brain (gangliosides in particular) has been demonstrated by Svennerholm<sup>12</sup>, which suggests that more than one type of neuraminidase activity may be required in the metabolism of these substrates.

The results shown in Table 3 indicate a significant increase in the total activities of acid phosphatase, β-glucuronidase and neuraminidase in brain homogenates of vitamin E-deficient encephalomalacic chicks. The increased levels of acid hydrolases (lysosomal) enzymes in the free form in brain could conceivably contribute to brain degeneration by acting on their respective substrates. However, it could not be established that lysosomes had actually ruptured *in situ* before the appearance of encephalomalacic symptoms or brain necrosis.

Calculations of the relative specific activity permit an evaluation of how the activity is distributed within the cells in relation to the observed total activity of the homogenates. The distribution patterns of brain acid phosphatase (Table 1) and brain neuraminidase (Table 2) in encephalomalacia due to vitamin E deficiency reveal a reduction in the activity of the enzymes in the particulate fractions especially in the lysosomal fraction with a concurrent increase in the soluble fraction. These results may indicate rupture of lysosomes *in vivo* resulting in an increased solubilization of the bound hydrolases. It was demonstrated by De Duve *et al.*<sup>13</sup> that five lysosomal enzymes were released rapidly in liver tissues which were rendered completely ischaemic by ligation of the blood vessels. Van Lanker *et al.*<sup>14</sup> reported similar results with respect to β-glucuronidase and acid phosphatase in autolyzing mouse liver fragments.

The experiments demonstrate that the stability of lysosomal particles is decreased in vitamin E deficiency,

but it could not be determined if the partial solubilization occurred before the malacic process began. The total activities have also increased significantly in brain homogenates of encephalomalacic chicks and may be a reflexion of the increased number of phagocytes and other reticulo-endothelial system cells in the necrotic areas of the brain. Such cells, which play an important part in pathological necrosis, have been shown to be rich sources of lysosomal hydrolases<sup>15,16</sup>.

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## Zone Electrophoresis of Human Parotid Saliva in Acrylamide Gel

THE zone electrophoresis of human parotid saliva proteins has been carried out with a variety of supporting media, including filter paper, cellulose acetate, agar gel and starch gel, with separations usually of from five to twelve fractions<sup>1-8</sup>. In a comparative investigation of these media, D'Silva and Ferguson<sup>7</sup> obtained their most satisfactory results with a combination of cellulose acetate and starch gel, thereby separating seventeen protein components from the parotid saliva of one subject. The present report concerns an examination of acrylamide gel as another medium for the zone electrophoresis of parotid saliva proteins. Preliminary experiments with acrylamide-gel strips have yielded protein patterns in which twenty or more fractions could be visually distinguished when the electrophoresis was carried out in 0.1 M tris-EDTA-boric acid buffer at pH 9.0. The tests were conducted on individual collections of parotid saliva from three subjects and on pooled collections of parotid saliva from several subjects. Fig. 1 shows the electrophoresis pattern of a sample of the pooled parotid saliva. Under the conditions of the experiment, more than half the staining components migrated anodically with generally better resolution than is evident for the cathodically-migrating components. Although disk electrophoresis on acrylamide gel has been widely used in investigations of serum proteins, gel strips were used for this investigation of parotid saliva in order to obtain the complete pattern of staining components, migrating both anodically and cathodically, for each electrophoresis run. Gel strips also permit the handling of larger quantities of protein than is feasible with disk electrophoresis, should recovery of particular fractions be desired. Salivary amylase, a troublesome factor in the starch-gel electrophoresis of saliva proteins, does not attack acrylamide gel.



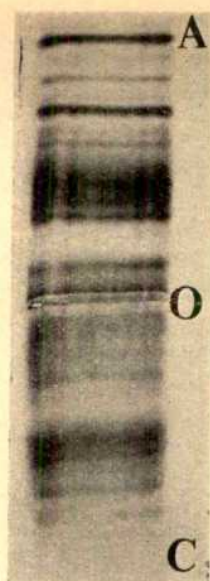


Fig. 1. Acrylamide-gel electrophoresis of pooled parotid saliva. O, origin; A, anode; C, cathode

The procedure for gel-strip preparation was adapted from the methods of Raymond *et al.*<sup>9</sup> and of Ornstein and Davis<sup>10</sup>. A 5.133 g sample of acrylamide and methylenebisacrylamide in 97.4:2.6 (w/w) proportions was dissolved in the *tris*-EDTA-boric acid buffer with 0.06 ml. of *N,N,N',N'*-tetramethylethylenediamine to a final volume of 98 ml. Two ml. of a freshly-prepared 5 per cent (w/v) solution of ammonium persulphate in the buffer was added to initiate polymerization and the resulting mixture was poured into a 'Plexiglas' mould, designed to produce a 265 × 50 × 3-mm gel strip. Polymerization was accomplished after about 30 min at room temperature.

The parotid saliva from U.S. Naval recruits 17-23 years of age was collected by paraffin mastication, using modified Lashley cups<sup>11</sup>. Individual or pooled collections of the saliva were dialysed against deionized water at 4° C to remove inorganic salts and were lyophilized. For electrophoresis, each lyophilized preparation was dissolved in the *tris*-EDTA-boric acid buffer to a final concentration of 15 per cent (w/v). After an incision of about 35-40 mm was made across the midpoint of a gel strip, a 35 × 2-mm section of filter paper (Schleicher and Schuell No. 470) was saturated with the protein preparation (c. 10-11 mg dry wt.) and positioned in the incision. The gel strip, sandwiched between 'Parafilm' sheets, was then placed in a horizontal water-cooled electrophoresis unit (Research Specialties Co., Richmond, Cal.) and electrophoresis was carried out for 2.5 h at a field strength of 8-9 V/cm strip length and a current of 4 m.amp/cm strip width. Electrical contact between the gel strip and the bridge buffer (0.05 M veronal, pH 8.6) was maintained by paired paper wicks (Schleicher and Schuell No. 470). The strip was stained for 45 min with 1 per cent amido black 10B in a 5:1:5 methanol, glacial acetic acid, water mixture. Excess stain was removed electrophoretically by the method of Ferris<sup>12</sup>, using 3.75 per cent acetic acid.

Immunochemical investigations<sup>13,14</sup> have provided evidence that parotid saliva contains serum proteins as well as proteins of apparently intrinsic salivary origin. The present interest in acrylamide-gel electrophoresis for investigating serum proteins arises largely from the influence of specific pathologies on the electrophoretic patterns obtained by this method. Acrylamide-gel electrophoresis would likewise appear to be a promising means of exploring variations in patterns of parotid

saliva protein that may accompany disturbances in an individual's metabolism.

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## PHYSIOLOGY

### 5-Hydroxytryptamine Receptors and Synaptic Transmission in Molluscan Neurones

Welsh and Moorhead<sup>1</sup> found large amounts of 5-hydroxytryptamine (5-HT) in the nervous system of different classes of molluscs and postulated its possible role as a transmitter. More recently the presence of 5-HT in land-snail nervous tissue was confirmed by Kerkut and Cottrell<sup>2</sup> and by Cardot and Ripplinger<sup>3</sup>. The interest of this finding was stressed by Ersparmer<sup>4</sup>, who pointed out that "a thorough study of the 5-HT in the nervous tissue of Molluscs may offer a key to a better understanding of the function of the amine in the brain of Vertebrates".

Various effects of 5-HT on gasteropod neurones are observed when whole ganglia are perfused<sup>5</sup>. Since some of these effects may result from interneurone interference, the present investigation is mainly concerned with the local application of the amine to individual neurones. 5-HT was injected by iontophoresis on the somatic surface membrane devoided of synapses of the monopolar central neurones of the Argentine land-snail *Cryptomphallus aspersa*. Neurones were impaled with single- or double-barrelled micro-electrodes and the bioelectrical activity recorded with a standard d.c. set. 5-HT was injected as a cation from micropipettes of 25-30 MΩ resistance filled with a 0.15 solution of 5-HT creatinine-sulphate solution, at a pH of 3.2. Sometimes a braking current was necessary to avoid desensitizing effects of drug leakage. The position of the microelectrode was tested by direct observation with a stereoscopic microscope and also by introducing the injecting microelectrode into the neurone, passing a square pulse to confirm its intracellular position, and then pulling out again the micropipette. In some cases, when a double-barrelled injecting micro-electrode containing acetylcholine (ACh) in one barrel and 5-HT in the other was used, effects obtained with ACh confirmed the extracellular location of the micro-electrode in absence of 5-HT effects.

Only a limited number of cells are responsive to 5-HT iontophoretic injection; this is in contrast to the response of a large number of cells, evoked by drug perfusion of the whole nervous system. These cells belong to a group of neurones which, besides excitatory post-



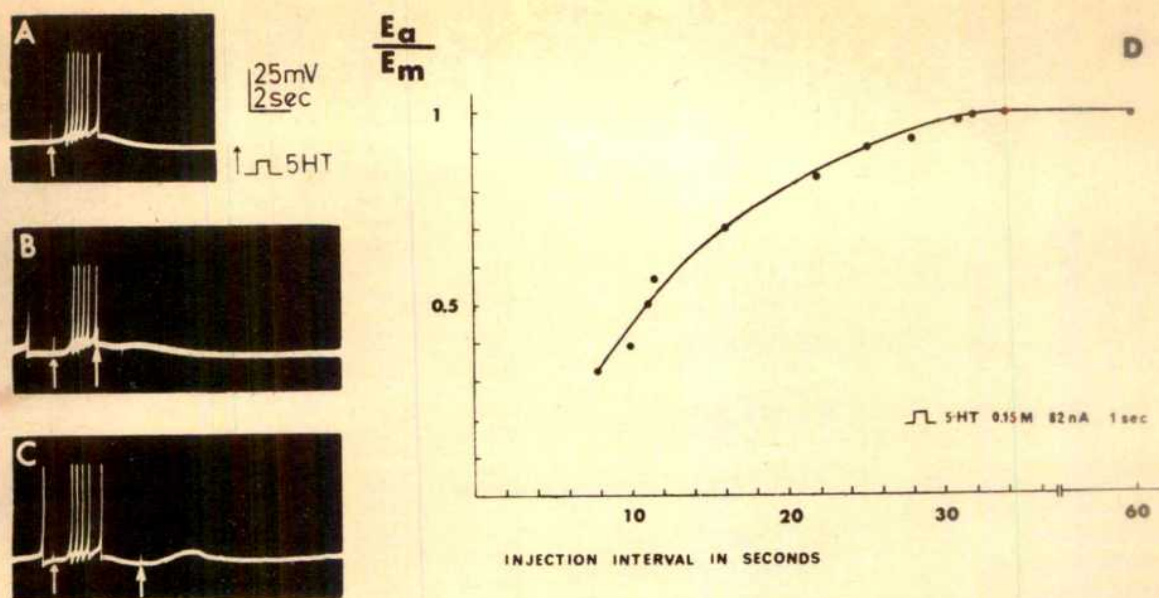


Fig. 1. Left row: intracellular recordings belonging to the same CILDA neurone. *A*, Ionophoretic injection of 5-HT on the somatic membrane (arrows) provokes depolarization and excitation of the cell. *B* and *C*, Repeating the injections at two different short intervals the effects are markedly diminished due to desensitization phenomena. *D* corresponds to a desensitization curve obtained in the same cell, hyperpolarizing it through a second intracellular microelectrode. The relation of the effect of successive injections on the membrane potential level compared with the initial one ( $\frac{E_a}{E_m} = \frac{\text{effect of injection}}{\text{initial or maximal effect}}$ ) was plotted against the injection interval in seconds. It is possible to appreciate that an interval of 34 sec is necessary to come back to the maximal effect of injections ( $\frac{E_a}{E_m} = 1$ ).

synaptic potentials (EPSP), show a peculiar inhibitory input first described by Tauc<sup>6</sup> as 'inhibition of long duration' (ILD). This inhibition is characterized by a long-lasting hyperpolarizing wave evoked by orthodromic stimulation and generally preceded by an excitatory burst. Gerschenfeld and Tauc<sup>7</sup> recognized this group of neurones with the conventional name of CILDA (cells with ILD). These neurones are characterized by the presence on their membrane of specific 5-HT receptors. 5-HT micro-injections passing currents of 10–50 nAmp during 0.5–1 sec produce transient depolarization and excitation of CILDA neurones (Figs. 1*A* and 2). Repeated micro-injections cause intense desensitization of the receptors

(Figs. 1*B* and *C*) similar to those observed, at the cellular level, in ACh receptors of both vertebrate muscle endplate<sup>8</sup> and *Aplysia* neurones<sup>9</sup> and in glutamate receptors of snail neurones<sup>10</sup>. Fig. 1*D* corresponds to a desensitization curve showing that an interval of about 40 sec is necessary to obtain maximal effects with two successive injections.

It is also possible to show that the ionophoretic injection of 5-HT only affects a restricted area of the neurone membrane, drug diffusion being negligible. In Fig. 3*C* the curve *a* represents a dose-response curve in which the dose is represented by the logarithm of the injected current in nAmp. Since the curve obtained is similar to a Langmuir isotherm and the microelectrode behaves as an 'ohmic' resistance, the plateau of the curve represents saturation of receptors, that is, the injection is recruiting a limited number of receptors from a restricted patch of the membrane.

5-HT receptors of CILDA neurones may be blocked by isomolar concentrations of lysergic acid diethylamide (LSD 25) or its derivatives and morphine (Fig. 2), that is, they share properties of both *D* and *M* receptors in other preparations<sup>11</sup>. Bromolysergic acid (BOL 148) appears to be much more active than LSD 25. All these drugs block 5-HT receptors reversibly and the same action is also shown by atropine and chlorpromazine. On the other hand, tryptamine, cyproheptadine and dibenamine block the receptor in an irreversible manner.

Relatively high concentrations (more than  $5 \times 10^{-3}$  M) of SKF trans-385 B and iproniazid, two well-known inhibitors of monoamino-oxidase (MAO), block the receptor response. SKF trans-385 B in lower concentrations enhances the effect of 5-HT micro-injections, but it is not clear whether the drug mimics and/or reinforces the 5-HT effect, or actually causes an inhibition of MAO which is located in the mitochondria<sup>12</sup>.

If 5-HT behaves as a true synaptic transmitter it is to be expected that it would alter the cell membrane conductance. Indeed, 5-HT exclusively affects the membrane conductance of CILDA neurones, increasing it to twice its value (Fig. 3*A*). This effect is highly specific because the amine does not alter the membrane constants of other types of cells (Fig. 3*B*) in the same ganglia.

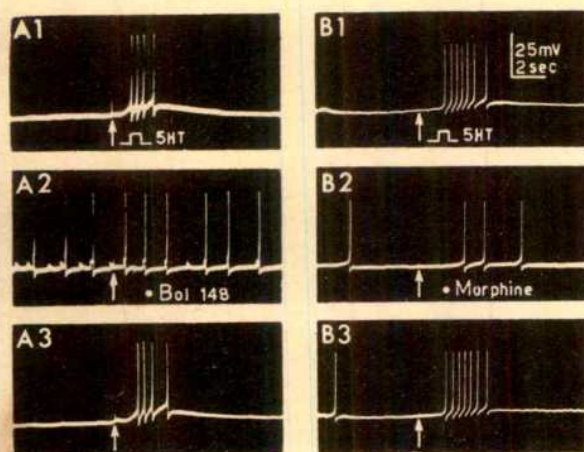


Fig. 2. Intracellular recordings of CILDA neurones ionophoretically injected on their membrane with 5-HT, using an injecting current of 75 nAmp during 1 sec. The injection microelectrode was filled with a solution of 5-HT creatine-sulphate 0.15 M at a pH of 3.2. *A1*, Control injection; *A2*, the same injection when bromolysergic acid is diluted in the bathing Ringer; the effects of the injection are blocked in spite of the slight depolarization provoked; *A3*, the effect of the injection returns when the blocking drug is removed from the bath. *B1*, Control ionophoretic injection of 5-HT; *B2*, blockade of the injection by the presence of morphine chloride in the cell environment; *B3*, the effect of morphine is reversible; effects of 5-HT injection return after washing. Concentrations used of both BOL 148 and morphine were  $10^{-4}$  M.



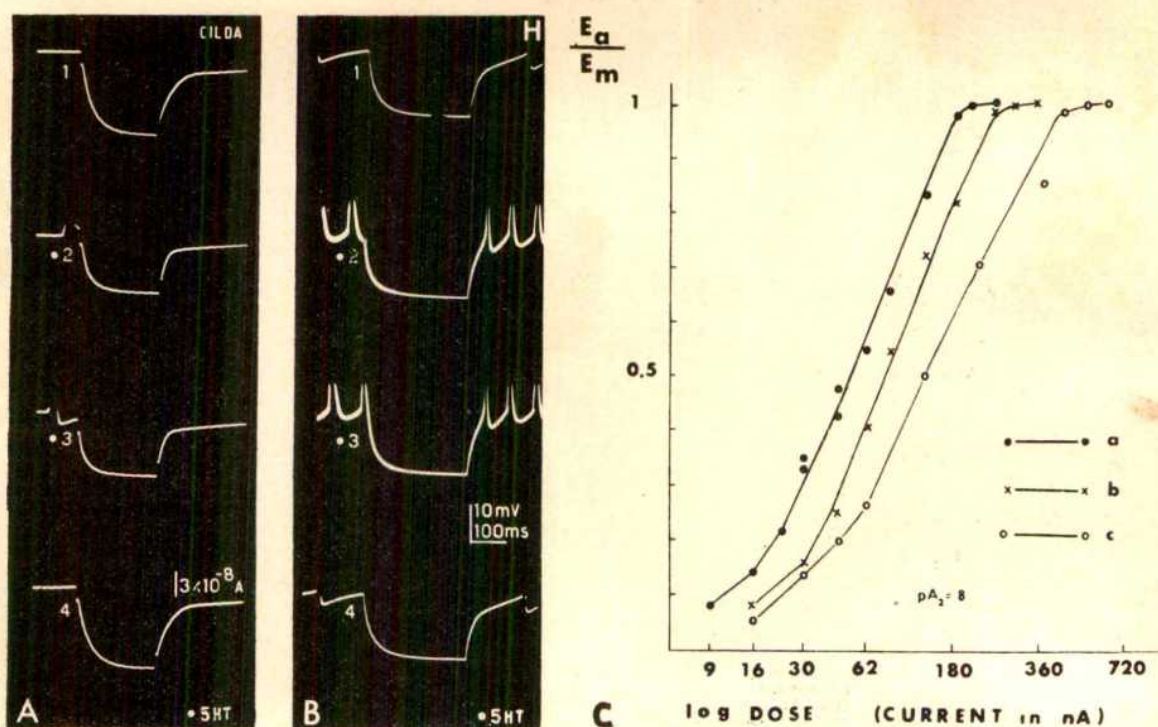


Fig. 3. In A, a CILDA neurone was impaled with a double-barrelled microelectrode. Square pulses of inward current were passed between an indifferent lead and one of the barrels in order to measure the membrane resistance. Application of 5-HT by perfusion to a cell of the type H in B did not affect the membrane resistance measured with the same technique. C, Drug-response curves of CILDA neurones to 5-HT ionophoretic injection. The cell was impaled with a double-barrelled microelectrode and artificially hyperpolarized; the membrane potential variations produced by the injections were taken as the measures of response. The abscissa correspond to the dose represented by the logarithm of the injecting current in nAmp. The ordinate is represented by the relation between the response to a determined dose ( $E_a$ ) and the maximal response obtained ( $E_m$ ). Curve a corresponds to a control dose-response curve. Curve b was obtained in the presence in the neurone environment of a  $2.5 \times 10^{-4}$  M solution of BOL 148 and curve c in the presence of a  $2.5 \times 10^{-4}$  M solution of BOL 148.

Application of the approach of the Nijmegen group<sup>13,14</sup> allows an analysis of the mechanism of blockade of CILDA neurone 5-HT-receptors by BOL 148. Comparing the control dose-response curve (Fig. 3C, curve a) obtained by injecting 5-HT on CILDA receptors with those obtained in the presence of diluted solutions of BOL 148 (Fig. 3C, curves b and c) it is possible to demonstrate that they correspond to a family of Langmuir isotherms presenting identical shape and slope and with a  $pA_2$  value of 8. These results may be interpreted as an indication that blockade by bromolysergic acid is due to an actual competitive mechanism between the antagonist and the agonist for the same receptor site.

In conclusion, the high content of 5-HT in snail ganglia, also confirmed for *Cryptomphallus aspersa*<sup>15</sup>, the presence of enzymes capable of synthesizing<sup>16,17</sup> and inactivating<sup>18</sup> 5-HT, the specific effects of the amine on the CILDA membrane conductance and the presence of specific 5-HT receptors on the membrane of the same neurone type fill some of the criteria required to consider a substance as a synaptic transmitter. Specific blockade of 5-HT receptors by LSD derivatives, first described by Gaddum<sup>19</sup> and confirmed at the cellular level in extra-synaptic receptors of CILDA neurones, provides an important clue for the investigation of 5-HT receptors at the synaptic level. The recent finding<sup>7</sup> that BOL 148 blocks excitatory postsynaptic potentials rather specifically in CILDA neurones without affecting the inhibition of long-duration input points toward the probable existence of 5-HT receptors at the postsynaptic membrane of these neurones and suggests, in addition to the other findings, that 5-HT may be the natural excitatory transmitter for CILDA neurones. However, more information regarding 5-HT synaptic receptors and 5-HT release and inactivation in molluscan nervous tissue is necessary to confirm this hypothesis.

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### Perception of Contours in the Central Fovea

Hubel and Wiesel<sup>1</sup> have shown that many neurones in the cat's striate cortex respond specifically to short black or white lines at particular orientations. A series of experiments by me, using psychophysical methods, supports the hypothesis that similar 'filter' units (FF) are to be found in the human visual system.

Various properties of these FF can be inferred from the results, concerning both permanent structures in the system and their dynamic function, and it will simplify the exposition if these hypotheses are stated first. Alternative hypothetical properties could, of course, be invoked, but the following are the most economical that are found which are consistent with all the data:

(1) Each F responds to a range of presentation orientations ( $\theta$ ); the response characteristic is bell-shaped and has extensive tails.

(2) FF vary in selectivity. Those tuned to orientations near the horizontal and vertical are the most selective.

The most selective FF are centred around two directions which differ by small angles (denoted by  $\Delta H$  and  $\Delta V$ ) from the horizontal and vertical respectively;  $\Delta H$  and  $\Delta V$  are generally not zero, nor greater than  $15^\circ$ , and vary from place to place in the field.

(3) Most FF receive inputs from both eyes.

(4) Integration of FF responses is achieved by mutual inhibition, which takes a matter of seconds to reach a steady level.

When a short-line stimulus causes FF to respond, all but a few are inhibited completely when the inhibition has had time to reach a certain level, and these respond in a proportion which determines the angle seen. If the line is sufficiently short, all but two will eventually be inhibited completely.

(5) The inhibition between FF is subject to adaptation. The sensitivity of any particular F is proportional to the average level of inhibition it has received over a period. Its own output of inhibitory signals is proportional to its present activity<sup>2</sup>. The storage period supporting this adaptation is a matter of weeks.

These properties engender some unexpected consequences, and at the same time are supported by a number of independent findings. Hypotheses (1) and (3) are in line with Hubel and Wiesel's results. Hypothesis (2) is supported by experiments which show that grating acuity<sup>3</sup>, fine-line acuity<sup>4</sup>, and vernier acuity<sup>5</sup> all vary according to orientation of the test lines; this variation has never been satisfactorily explained. My experiments show that acuity for orientation of a short line is also better in the horizontal and vertical directions than at  $\pm 45^\circ$ .

Hypotheses (1), (2) and (4) in combination lead to a surprising result. When a short line is presented for a few milliseconds only, there is no time for inhibition to be set up; consequently, any of a wide range of FF may make an appreciable response (on account of the substantial tails of the response characteristic). The resulting variation of apparent slopes is much greater than for longer exposures. This variation was observed: if a line subtending  $6'$  arc at the eye is flashed every few seconds, it is seen quite clearly at a different inclination every time; the variation is occasionally as great as  $\pm 30^\circ$ .

These errors are not normally distributed. This finding led to postulate (2) here. If  $\theta$  is, say,  $+10^\circ$  from the horizontal, an erroneous appearance is much more likely to arise from one of the less selective FF peaking between  $+0^\circ$  and  $+20^\circ$  than from one of the more selective FF peaking between  $0^\circ$  and  $+10^\circ$ . Other ranges of FF can be considered in the same way; the net result is that the mean apparent slope of a short flashed line is biased away from the horizontal (and similarly at the vertical). This phenomenon has also been observed; the results might be described as a 'stretching' of the scale of apparent orientation. In the neighbourhood of the horizontal or vertical the 'stretch factor' may be as high as 2.0. As

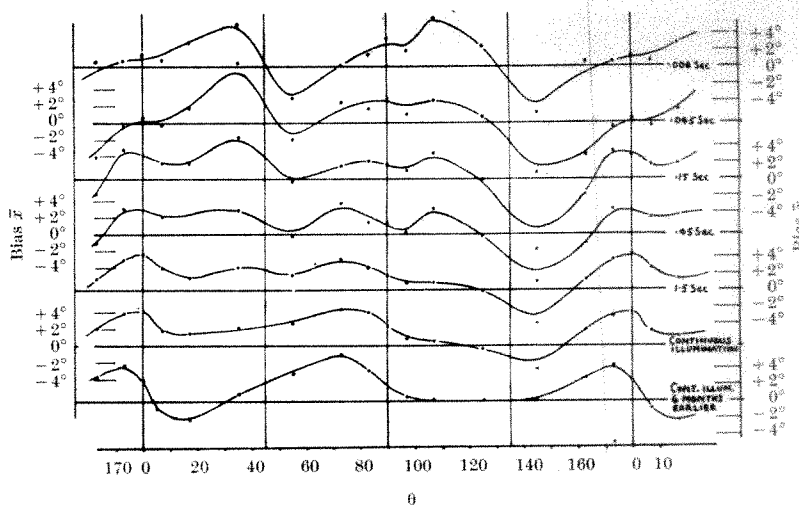


Fig. 1. Biases of perceived orientation of a short line, as a function of presentation angle and duration of exposure

interpreted in hypothesis (2), this is not an actual stretching of the metric of visual space; it is just a statistic of the error distribution. It is the distribution of mean appearance that is 'stretched', while each individual F has its peak response in proper correspondence with  $\theta$ .

When  $\Delta H \neq 0$ , there will be a mean bias at the horizontal itself. Such biases are observed, and range up to  $\pm 5^\circ$ . They vary according to the position of the line in the fovea, in an arbitrary way. Corresponding points in left and right foveas show correlated biases:  $r = 0.8$ . This provides strong support for hypothesis (3).

When the presentation period is long enough for inhibition to build up, all these effects are subject to systematic changes.

Hypothesis (5) can be elaborated: all the FF in any one small region form a dynamically stable system which in effect assigns apparent orientations to particular combinations of F-responses. It has been shown how the frequency distribution of F-responses precipitates biases of mean apparent orientation. When the F-responses are integrated, this mean bias is seen, whereas with single flashes it is a statistic of the error distribution. The adaptation suggested in (5) will continually adjust biases so that over a period the frequency distribution of apparent inclinations is uniform. This statistic is true of images chosen at random from a visual environment, so if the system integrates over a reasonably long period, the resulting scale will be satisfactory. I have suggested<sup>6</sup> that the metric of visual space is continually adjusted by a similar mechanism, which provides that contour-curvatures and contour-spacings shall be uniformly distributed throughout visual space.

Now, in normal viewing, saccades occur at intervals of order 0.5 sec (ref. 7). The adaptation equilibrium will therefore be adjusted so that there is a satisfactory scale of orientation for viewing durations of this order. For shorter viewing periods, the inhibition will not cancel the biases engendered by the selectivity distribution, and for longer periods the inhibition will over-compensate and reverse the biases found with flash stimulation. The bias does not reach a steady level for a second or two. Ratliff *et al.*<sup>8</sup> have shown that lateral inhibition takes a similar period to reach a steady level in the eye of *Limulus*. It is, in any event, desirable that inhibition should have a longer time-constant than excitatory response, if its purpose is to stabilize or integrate the latter.

This reversal of bias is observed, and was the principal reason for invoking (5). Fig. 1 illustrates how the pattern of biases changed as a function of exposure time for one subject. This transition has been mapped in detail for one subject only, but results for the two extreme durations



have been obtained for three subjects, and all showed the same net pattern of change. Details of the experiment were as follows. A short bright line subtended  $6' \times 1.0'$  at the eye, and was presented at 7-sec intervals against an illuminated background. The brightness of the short line was at that level which gave optimal acuity, which was first established for each exposure time. The subject (D. P. A.) adjusted the slope between flashes until satisfied that it matched that of a long thread near by, which was at one of 14 inclinations. This matching was repeated 17 times to give the mean bias ( $\bar{x}$ ); the *S.E.* of  $\bar{x}$  ranged between  $0.15^\circ$  and  $0.3^\circ$  (within-run), but  $\bar{x}$  is also subject to drift between runs which will affect reliability, since the experiment took several days. Each inclination of the comparison thread was used for 6 successive runs at the different exposure times. The sequences of these 6 runs and of the 14 comparison inclinations were randomized.

All measurements are in degrees, measured clockwise from the vertical for  $\theta$ , with  $\bar{x}$  measured from  $\theta$ . The graphs show the 'stretch' effects already described. Gradients around the horizontal, vertical and  $\pm 45^\circ$  directions undergo systematic changes as a function of exposure duration.

A further indication that F-outputs are subject to adaptation can be seen in the last two lines of Fig. 1, which show the  $\theta$ -bias function for continuous exposure, and the same function six months earlier. Although the general shape of the function is the same in the two cases, the actual values of bias differ considerably. This drift is co-ordinated: the biases at neighbouring orientations have drifted by similar amounts. Similar drifting has been found to affect the biases depending on the position of the stimulus line in the field; in this case, the drift was co-ordinated spatially over a foveal region  $40'$  square.

This drifting is evidently held within limits of some kind, or the pattern of the results would change as well as the actual values. The adaptation hypothesis (5) is an economical explanation of these phenomena. Such a mechanism has the advantage that the nervous system does not have to encode exact values of orientation, which frees it from a difficult engineering problem. So long as the sequence of orientation values is preserved reasonably well throughout the transmission lines, the system can supply a satisfactory scale of perceived orientation at the receiving end.

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### True Venous Pulse Wave

THE classical jugular phlebogram (JP) is a composite record of arterial and venous pulsations. Mackenzie<sup>1</sup> noted that if the carotid arterial pulse wave could be subtracted from the JP we would have what he called 'the true venous pulse wave'. He was, however, unable to obtain such a record experimentally.

An approach to this problem was made as follows. Pulse recording elements were placed, first, in the position where classically the JP is recorded (in the angle formed by the origin of the right sternomastoid muscle and the upper and inner margin of the clavicle) and, secondly,

over the right carotid artery as high as was feasible in the neck. A technique was devised to ensure the temporal alignment of the carotid pulsations from both recording sites. The magnitude of the pulsations was also balanced. The subject was supine on a tilting table  $10^\circ$  head-up. These two sets of pulsations were recorded through a differential gauge made by Statham, Puerto Rico (P 23 H, No. 113). Fig. 1A shows a typical record made with a Grass Polygraph. Reading from the top the records are ECG, jugular phlebogram, true venous pulse (TVP) and right carotid. The differential record of the TVP is obtained by subtracting the carotid record (line 4) from the JP (line 2).

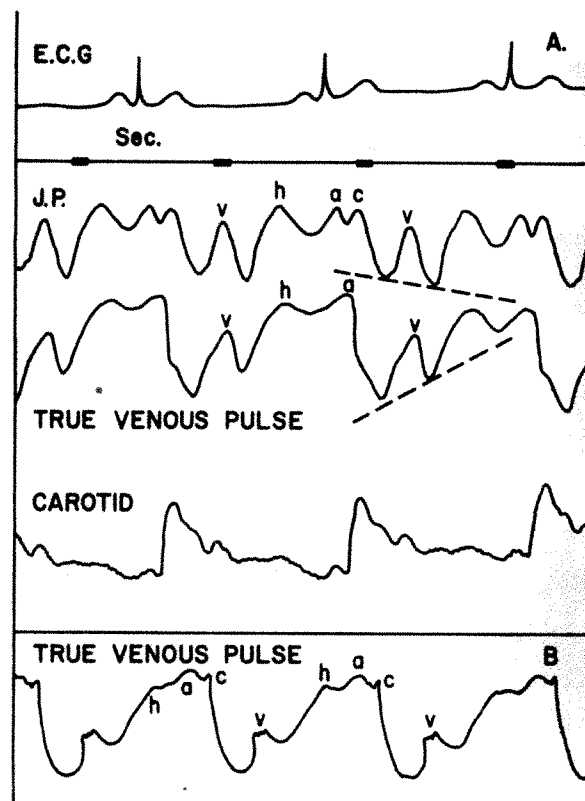


Fig. 1

The most obvious difference between the JP and the TVP records is the change in the 'c' wave. This may be absent. If it is present it is small and coincident with the early part of the anacrotic limb of the carotid pulsation (see Fig. 1B). The 'a', 'v' and 'h' waves are superimposed on a rising base line which starts at the bottom of the X<sup>2</sup> wave and rises to the summit of the 'a' wave. This base line in the JP is usually horizontal. Another feature of the negative wave X<sup>2</sup> is that it is more prominent, with a rapid descending limb from the summit of 'a'.

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<sup>1</sup> Mackenzie, J., *The Study of the Pulse, Arterial, Venous, and Hepatic and of the Movements of the Heart*, Fig. 178, 183 (Young J. Pentland; Edinburgh, 1902).

## Uptake of Formalin by Brain Tissue from Dogs killed at various Stages of Development

EARLIER work<sup>1</sup> with fresh canine brains has shown that there is a linear increase in the dry matter content from birth to 6 weeks of age (10 per cent to 19 per cent) and then a more gradual maturation to the adult level of 20 per cent. A rapid increase in brain volume up to 6 weeks of age was also found.

Histological examination of the occipital and frontal cortex revealed changes in cell density with age<sup>2-4</sup>, there being a great reduction in cell density from birth to 6 weeks of age (250-60 cells per unit area) followed by a gradual reduction to the adult density of 40-45 cells per unit area.

Fourteen inbred Beagle dogs ranging from birth to adulthood were used in the present investigation; under deep urethane anaesthesia seven dogs were perfused with paraffinized normal saline followed by formalin; the brain matter was removed from the brain, which was then immersed in formalin for 4 weeks. After this time the brain was dissected and the parts were weighed before and after maceration and desiccation for 5 days in a drying oven. The parts consisted of medulla and pons (up to the superior colliculi) and cerebral cortex (excluding olfactory lobes). After desiccation the remaining weight of each part was determined and expressed as a percentage of the original formalin-fixed weight before desiccation (Fig. 1). Even controls of corresponding ages were similarly treated, with the omission of formalin perfusion and fixation.

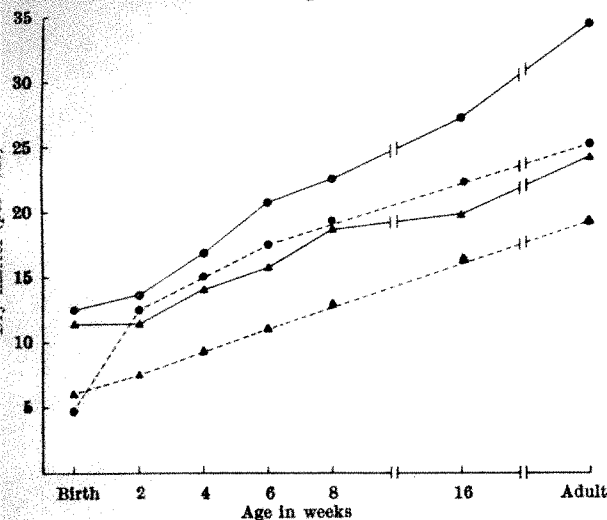


Fig. 1. Percentage of dry matter in different parts of the brain of the dog at different ages and after formalin fixation. ●, Medulla; ▲, cerebrum. Broken lines, formalin fixed

It was found that the percentage of dry matter was least at birth and increased gradually with age. Some evidence of caudocephalad encephalization was found in various parts of the brain, the most caudal region having the greatest dry matter content.

It would appear that the amount of formalin taken up by central nervous tissue is a function of cell size and changes in cell density with age. In young specimens where the cell density is greatest there is consequently a reduction in the intercellular space, and because the average size of the neurone does not reach adult dimensions until 4 weeks of age (neuroblastiform at birth in the cerebrum), the interperikaryal space is consequently less in younger specimens<sup>4</sup>. The reduction in dry matter content following fixation is at present under investigation; it has been suggested that phospholipids are leached during fixation.

Calculation of the dry matter content after desiccation of fresh and formalin-fixed specimens therefore gives an indirect estimate of the intra- and extra-cellular content of the brain which can be correlated with other aspects of

post-natal development. Higher dry matter percentages are found in the more caudal parts of the brain in accordance with greater aggregation and packing of neurones and nerve tracts as compared with the cytoarchitectonics of the cerebral cortex<sup>5,6</sup>.

These findings were correlated with previous data on the developing canine brain. The percentage dry matter of formalin-fixed tissue was directly correlated ( $P < 0.01$ ) with cell density and a 10 per cent loss in the percentage dry matter content of fresh brain predictable after fixation.

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## Calcium Exchangeability in Frog Sartorius Muscle during Potassium Depolarization

THE release of calcium from the membrane or from endoplasmic reticulum is regarded as the main process in the mechanism provoking muscle contraction. The system of membrane control of contractile activity in the fast muscle fibres has several properties for which similar terminology was proposed as for the membrane control of Na conductance<sup>1</sup>. In this work some properties of the Ca-releasing system were examined by using <sup>45</sup>Ca and the results are discussed in terms of membrane control of Na conductance.

Sartorii of the frog *Rana temporaria* were used. The experiments were carried out in Ringer's solution containing: NaCl, 115 mM; KCl, 2.5 mM; CaCl<sub>2</sub>, 1.8 mM; Na<sub>2</sub>HPO<sub>4</sub>, 2.15 mM; NaH<sub>2</sub>PO<sub>4</sub>, 0.85 mM. High-CaCl<sub>2</sub> Ringer's was made without phosphates. When increasing the K concentration the corresponding amount of KCl was substituted for NaCl. One of the pair of sartorii of each animal was used as an experimental muscle; the other as a control. Two series of experiments were carried out. In the first series the control muscle was exposed for 15 min to <sup>45</sup>Ca Ringer's solution (RS). The experimental muscle was exposed for 5 min to <sup>45</sup>Ca RS and for 10 min to <sup>45</sup>Ca RS with high K. The muscles were washed four times for 5, 10, 25 and 50 min in non-radioactive RS to remove <sup>45</sup>Ca from the extracellular space and from the superficial binding sites of the fibres<sup>2</sup>. They were then ashed and the radioactivity was counted. In the second series the control muscle was immersed for 40 (or 10) min in non-radioactive RS, then the <sup>45</sup>Ca RS was applied for 15 min. The experimental muscle was exposed for 40 (or 10) min to non-radioactive high-K RS, then the high-K <sup>45</sup>Ca RS was applied for 15 min. To prevent twitching, 10<sup>-4</sup> M cocaine was added in all solutions.

In preliminary kinetic experiments it was found that the estimation of Ca influx in the sartorii of *Rana temporaria* on the basis of the slow component does not represent the real exchange of cellular Ca. For this reason the changes in Ca movements were evaluated only as the changes in Ca exchangeability. The values in Fig. 1 concerning the values between 20 and 50 mM K do not, therefore, represent quantitative relation between Ca exchange and K concentration.

The changes in Ca exchangeability at different values of depolarization are shown in Fig. 1. A big increase in Ca exchangeability was found both at the beginning of depolarization and after 40 min depolarizing with 15-20 mM K. At greater depolarization a big increase in ex-



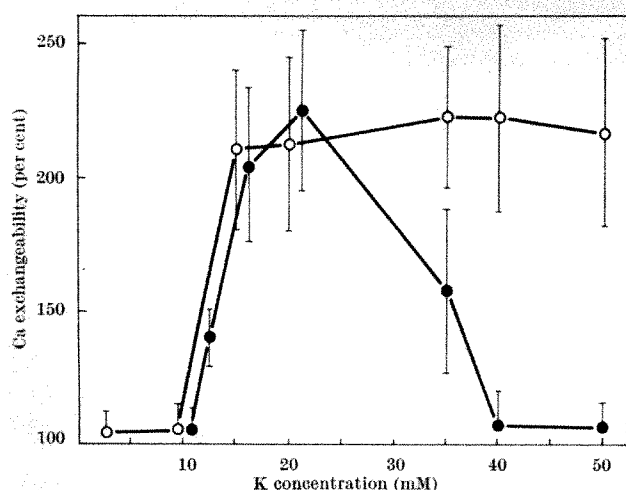


Fig. 1. Ca exchangeability at different values of K depolarization. Radioactivity in control muscles is taken as 100 per cent. Open circles, Ca exchangeability at the beginning of depolarization; filled circles, after 40 min.

changeability was found only at the beginning of depolarization. Maintained increase in Ca influx in 20 mM K RS was reported also by Bianchi and Shanes<sup>3</sup>.

In further experiments the influence of procaine and  $\text{CaCl}_2$  on Ca exchangeability was investigated (Fig. 2). It was found that both procaine and  $\text{CaCl}_2$  suppressed Ca exchangeability at low depolarization (20 mM K, that is, about 50 mV of membrane potential). At a higher level of depolarization (40 mM K, that is, about 30 mV) their effect was found to be different. Procaine still favours the suppression of exchangeability.  $\text{CaCl}_2$ , on the other hand, does not act in the sense of suppression, but causes a long-lasting increase of exchangeability. The same effect as for procaine was found for phenobarbital and physostigmine<sup>3</sup>. It must be mentioned that the concentrations of the drugs and  $\text{CaCl}_2$  used in our experiments do not substantially influence the sensitivity of the muscle fibre membrane to K depolarization<sup>4</sup>. An inhibitory effect of procaine on Ca influx induced without depolarization by caffeine was reported by Feinstein<sup>5</sup>.

Summarizing our findings, we think that the terminology used for the control of Na conductance in excitable tissues can be accepted. We suppose that the system releasing Ca from its bound state is activated for a long period when the membrane is depolarized to about 50 mV. At higher depolarization values the process of activation lasts for a shorter period and is followed by inactivation of the system. The time-course of these processes seems, however, to be much slower than those governing Na conductance.

The experiments with procaine and  $\text{CaCl}_2$  strongly support the use of terms accepted for Na conductance.

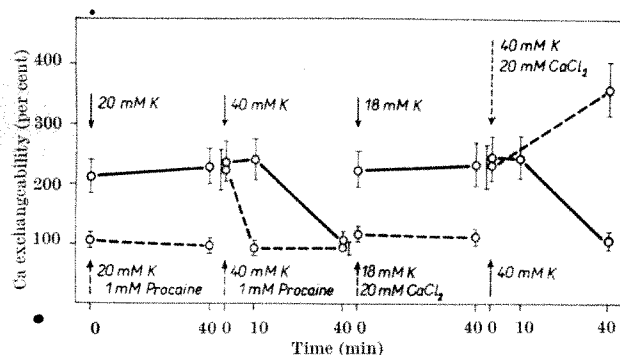


Fig. 2. The effect of procaine and  $\text{CaCl}_2$  on Ca exchangeability at different values of K depolarization. Radioactivity in control muscles is taken as 100 per cent. The exposure to  $^{45}\text{Ca}$  is described in the text. In experiments with high  $\text{CaCl}_2$  both control and experimental muscles were exposed to 20 mM  $\text{CaCl}_2$ .

On the basis of electrophysiological examination, it is supposed that at low depolarization values both procaine and  $\text{CaCl}_2$  decrease Na conductance<sup>6-9</sup>. At higher values of depolarization the effect of these two compounds is different. Procaine favours inactivation of Na conductance, while  $\text{CaCl}_2$  favours activation. It seems that a similar mechanism of the action of procaine and  $\text{CaCl}_2$  on the Ca releasing mechanism can be inferred from our experiments.

The effect of  $\text{CaCl}_2$  on the release of Ca agrees very well with the effect of  $\text{CaCl}_2$  on the contractile properties of the fast frog muscle fibres observed by Pausching and Brecht<sup>10</sup>. These authors state that extracellular Ca increases the duration of K contracture caused by the prolonged activation of the Ca-releasing mechanism.

The results presented in this communication and those reported earlier<sup>4</sup> also suggest that the extra oxygen consumption caused by K depolarization might be directly connected with the increase of free Ca in the muscle fibres.

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### A Possible Cytological Basis for the 'R' Membrane in the Vertebrate Eye

THE 'R' membrane was the name coined by Brindley for a hypothetical retinal barrier whose penetration by a microelectrode advancing from the vitreous chamber results in a marked drop in electrical resistance and decrease in capacitance. Later, Tomita, Murakami and Hashimoto<sup>2</sup> found that this barrier could be demonstrated in eyes from which the retina was removed, thus placing the barrier behind the retina either at the level of the pigment epithelium or at Bruch's membrane. This was afterwards confirmed by Brindley<sup>3</sup>. Since the membrane of Bruch appears to be a substantial structure in light microscopy, it was a likely candidate for the 'R' membrane. Electrode marking methods employed by Brown and Tasaki<sup>4</sup> gave results compatible with this hypothesis and ruling out the retina although in fact no clearly marking Bruch's membrane as distinct from the pigment epithelium. Cohen<sup>5</sup>, using electron microscopy cast doubt as to the role of Bruch's membrane in this phenomenon by pointing out that Bruch's membrane consisted of the basement membranes of the pigment epithelium and choroid capillaries together with a layer of loosely interwoven connective tissue. Since there is basement membrane at the retinal-vitreous junction where no electrical change of a comparable type is noted it seemed unlikely that Bruch's membrane had the requisite structure for significant electrical resistance.

Two other potential barriers are the external limiting 'membrane' and Verhoeff's 'membrane'. These names respectively designate the lines of terminal bars between the receptors and glial cells of Müller in the case of the external limiting 'membrane' and between the cells of the pigment epithelium in the case of Verhoeff's 'membrane'. These terminal bars are structures of presumed adhesive function and in nervous tissue occur at the surface junctions of all cells facing on the vertebrate cerebrum.



intricles both embryonically<sup>8,9</sup> and in the adult<sup>10</sup>. Since the cavity of the optic vesicle is cerebral ventricle and since the space between the external limiting 'membrane' and Verhoeff's 'membrane' is a persistence of this cavity, it is not surprising that terminal bar systems occur here. Previous considerations of the possibility that these structures formed the basis of the 'R' membrane phenomenon were based on the assumption that the external limiting 'membrane' and Verhoeff's 'membrane' were structurally identical. As the external limiting 'membrane' is a retinal structure and as the 'R' membrane lies behind the retina, it followed that an identical structure in both the pigment epithelium and retina could be ruled out. However, recent examination of the terminal bar systems in these two 'membranes' shows that they are not identical and that they differ significantly in an important structural detail. Moreover, they differ in a direction suggesting that the movement of ions between cells of the pigment epithelium alone might be restricted.

The vitreal chambers of eyes of anaesthetized (hypothermia) macaque monkeys were perfused with 5 ml. of a 5 per cent glutaraldehyde solution in Earle's balanced line (pH 7.4) and then enucleated. The anterior segment was removed and the rest of the eye submerged in fixative. While submerged the sclera was loosened with a spatula and cut free. After an hour's fixation the tissue was used briefly in the buffered Earle's saline and re-fixed in 2 per cent osmium tetroxide in the same buffer for 1 h, then dehydrated, embedded in epoxy resin and sectioned. The sections were stained with lead hydroxide and photographed with an RCA-3F electron microscope.

Fig. 1 shows the junction of two cells of the pigment epithelium at their inner (vitreal) aspect. After the terminology of Farquhar and Palade<sup>11</sup>, the junctional specializations observed consist of a tight junction or zonula occludens (zo) and sclerad to this a region of wider gap designated as a zonula adherens (za). Desmosomes or maculae adherens occur elsewhere between these membranes and are not illustrated here. Inasmuch as mic fixation generally shows a single dark line representing the inner or cytoplasmic line of unit plasma membrane structure<sup>12</sup>, and since the distance between the dark lines of the tight junction is 145 Å, the intercellular space can be regarded as virtually non-existent at the tight junction. Since this represents a section of what is continuous 'seal' between the edges of the inner faces

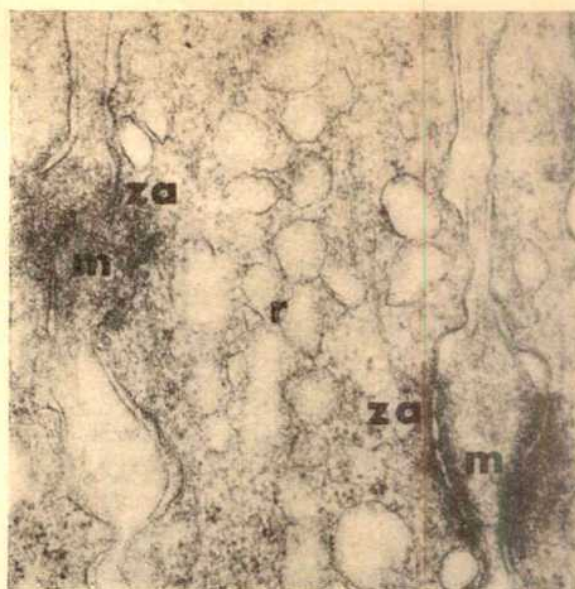


Fig. 2. The junctional area of the inner segment of a rod (r) and two glial cells of Müller (m) showing only a zonula adherens (za) ( $\times 46,900$ ).

of the cells of the pigment epithelium, the significant movement of ions and molecules across the inner face of the pigment epithelium may perhaps be confined to movements through the cell rather than around them. On the other hand, as shown in Fig. 2, the homologous junctions between the cells of Müller and the receptors, or between cells of Müller, fail to have tight junctions and show zonulae adherens structure only, the narrowest intercellular gap being of the order of 200 Å. Kuffler and Potter<sup>13</sup> have recently emphasized the significant part that such narrow intercellular clefts may play in movements of substances in the central nervous system. Thus for ion movements, only the tight junction of the pigment epithelium seems to constitute an adequate barrier to explain the 'R' membrane resistance. The 'R' membrane capacitance would follow from the extensiveness of cell surfaces in the pigment epithelium due to the long processes of the pigment cells which interdigitate with the receptor outer segments. The tight junctions of the pigment epithelium probably contribute significantly to the blood-ocular barrier which, on the basis of the extent of dye penetration, Rodriguez-Peralta<sup>14</sup> placed at the inner face of the pigment epithelium. The variations of terminal bar structure exhibited here suggest that such variations may occur elsewhere in the central nervous system and possess physiological significance.

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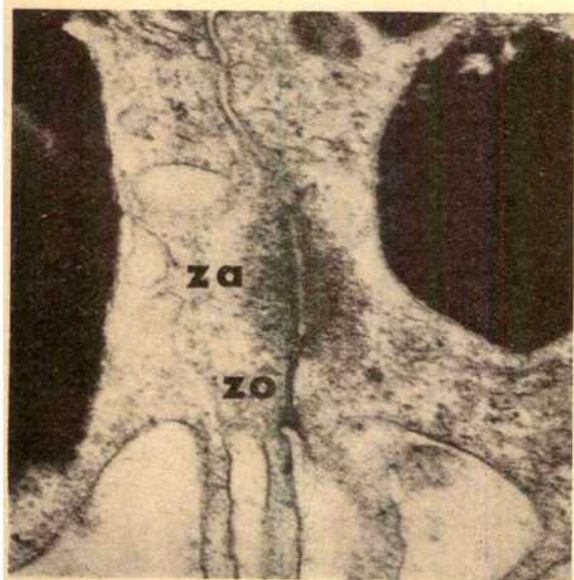


Fig. 1. The junctional area at the inner face of two cells of the pigment epithelium showing the zonula adherens (za) and zonula occludens (zo) ( $\times 46,900$ ).



### Evidence for the Fenn Effect in Skeletal Muscle exposed to Low $pO_2$

FENN<sup>1</sup>, and later Hill<sup>2</sup>, showed by heat measurements the isolated frog sartorius muscle liberated extra energy in proportion to the mechanical work done by the muscle during contraction ('Fenn effect'). More recently, analysis of the breakdown of high-energy phosphates also revealed that more energy is utilized by a contracting sartorius muscle, which is allowed to shorten against a load (isotonic contraction), than by a muscle which is not allowed to shorten (isometric contraction)<sup>3,4</sup>.

Efforts to establish the presence of the Fenn effect by measuring the oxygen consumption of muscles contracting isotonically vs. isometrically have not been in agreement. Fischer<sup>5</sup> found that when the work was near maximal extra oxygen was consumed during isotonic contractions, but that muscles which shortened a great deal against a light load actually consumed less oxygen than muscles contracting isometrically. Whalen and Collins<sup>6</sup> confirmed the latter finding, but found no evidence of the Fenn effect, even with the muscle performing near maximal work. In an attempt to explain the difference between my results and those from heat measurement and chemical analysis, I considered the possibility that in my work the resting respiration provided a pool of 'extra' energy which was ordinarily converted directly to heat. If isotonic contractions used more of this surplus energy than isometric ones, evidence might not have been seen of it. When we found that the resting respiration could be suppressed without apparent detriment to the muscle<sup>7,8</sup> the possibility seemed less remote. It suggested that the Fenn effect might become obtrusive when larger muscles, or a lower  $pO_2$  or temperature, were used. Indeed, previous work had been performed with larger muscles than I had used, at lower temperatures (0°–17° C) and, often, lower  $pO_2$  (ref. 6). It is suggestive that Fischer "could find no evidence of the Fenn effect when the temperature was more than 17° C". The present communication is a report of the attempt to suppress the resting respiration by low  $pO_2$  and a somewhat lower temperature than I used previously, with the expectation that the extra energy for work would become apparent.

Except as mentioned, the experimental conditions and the muscles used were similar to those in my previous report with Collins<sup>6</sup>. Oxygen consumption was measured volumetrically in chambers designed to accommodate the frog sartorius muscle full-length rather than looped as previously<sup>6</sup>. Each of three chambers contained 9 ml. Ringer's bicarbonate solution with glucose as the substrate. The medium was agitated by a magnetic rotor in the chamber. The content of carbon dioxide in the gas phase (about 5 c.c.) was maintained near 2 per cent by using as the carbon dioxide 'absorber' a mixture of 70 parts of 3 M potassium bicarbonate and 30 parts of 3 M potassium carbonate plus carbonic anhydrase<sup>9,10</sup>. At the bath temperature of 22.4° C the pH was  $7.2 \pm 0.1$ . A mercury seal in the chamber top enabled the measurement of muscle tension or shortening to be made<sup>6,11</sup>.

The muscles (obtained from small *R. pipiens*) had a mean dry weight of 8.8 mg (cf. ref. 6). One of a pair of muscles was mounted at *in situ* length in one chamber, and its counterpart in another. The third chamber served as a control. On alternate days the chambers were gassed with either 98 per cent oxygen–2 per cent carbon dioxide, or 25 per cent oxygen–73 per cent nitrogen–2 per cent carbon dioxide. After gassing for 15 min, 45 min were allowed for a state of equilibrium to be reached, then readings were taken at least every 30 min, for a total of 6 h. After the oxygen consumption stabilized, both muscles were stimulated at a rate of 150/min for 3 min. One muscle was allowed to shorten and lift a near optimum load (5 g), the other merely exerted tension on a strain gauge. At the end of the experiment the muscles were removed, the pH of the medium determined;

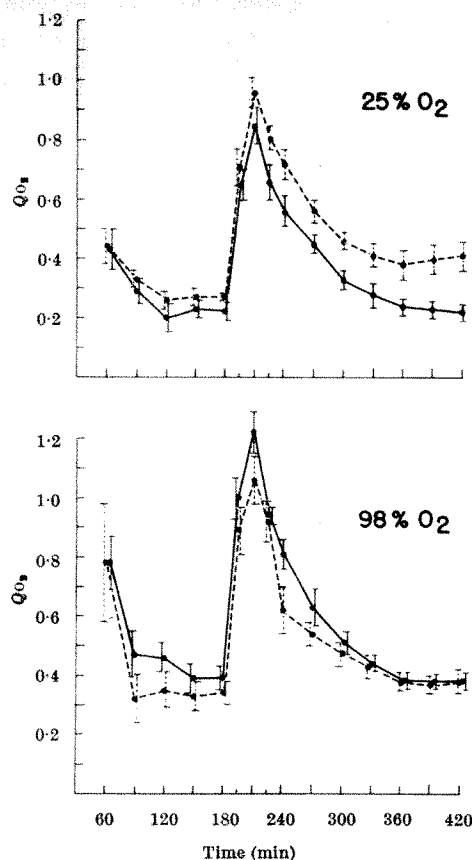


Fig. 1. Mean  $Q_{O_2}$  and S.E. (vertical bars) of 8 pairs of muscles exposed to 25 per cent oxygen and 8 pairs to 98 per cent oxygen. (The S.E. includes interindividual variation.) Stimulation at 150/min for 3 min began 180 min after placing the muscles in the chamber. —, Tension; ---, work

and, usually, aliquots taken for lactic acid analysis. The muscles were dried overnight at 100° C and weighed. Oxygen consumption is expressed as ' $Q_{O_2}$ ', that is,  $\mu V$  (s.t.p./mg (dry wt.)/h).

The resting  $Q_{O_2}$  was at first high, especially in 98 per cent oxygen, and dropped in about an hour to a steady level (Fig. 1). As expected from our previous investigations, the  $Q_{O_2}$  in 25 per cent was significantly lower than the value in 98 per cent oxygen<sup>6</sup>. We also confirmed our previous results<sup>6</sup> that, in 98 per cent oxygen, muscle which performed substantial amounts of mechanical work consumed no more oxygen than muscles which did essentially none (Fig. 1). On the other hand, muscles which contracted isotonically in 25 per cent oxygen consumed significantly more oxygen than their isometrically contracting mates. The work performed or tension developed by the muscles was independent of the  $pO_2$ , as was lactic acid liberation, in confirmation of previous results. There was a tendency for the lactic acid liberation to be higher in muscles which did work. However, no experiments would have to be performed to establish any significance.

Since the  $Q_{O_2}$  of the muscle which contracted isotonically in 25 per cent oxygen did not return to the control level, it is impossible to quantitate the work done with the extra oxygen consumed. Indeed, if the hypothesis is correct, that resting respiration represents a relative large pool of available energy, no correlation would be meaningful unless one knew the extent to which isotonic contractions dipped into the pool. That others were able to find evidence of the Fenn effect could be due to the fact that the investigators used larger muscles, low  $pO_2$ , or a low experimental temperature<sup>1-3</sup>, all of which would be expected to reduce the pool of 'resting' energy.

It seems probable that the same explanation underlies the failure to find evidence for the Fenn effect in isolated rat muscle<sup>11</sup>. In this connexion, Lentini<sup>12</sup> found that isolated rat trabeculae the oxygen consumption was the same whether the strips were contracting or quiescent. Likewise, in nerve, apparently normal conduction can go on for hours without apparent additional energy cost<sup>13</sup>. These functions may simply use 'pool' energy.

The concept of a pool of 'excess' energy would tend to found interpretation of investigations which attempt to relate consumption of oxygen to other cell functions, such as the active transport of sodium<sup>14,15</sup>. Obviously, the efficiency of the transport system, calculated on the basis of consumption of oxygen, would vary considerably, depending on the experimental conditions. It seems pertinent that the effect of metabolic inhibitors on ion transport depends very much on the temperature<sup>16</sup>. In investigations of energetics it might be necessary to investigate the process, in nitrogen<sup>17</sup> or *in vivo*, where the energy may be supplied according to the demand and less energy-utilization of oxygen is likely. However, even *in vivo*, Fales, Heisey and Zierler<sup>18</sup> were unable to find evidence for the Fenn effect in dog skeletal muscle. I thank Mrs. W. Bosch for her help in this work, which is supported (in part) by U.S. Public Health Service Grant H5390.

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## PHARMACOLOGY

### Influence of Amino-oxidase Inhibitors on the Psychomotor Depression produced by an Intraperitoneal Injection of Adrenaline in Mice

In earlier papers<sup>1,2</sup> the psychomotor depression produced by an intraperitoneal injection of catecholamines in mice was described. We found that the depression produced by adrenaline did not depend on peripheral autonomic phenomena and was not influenced by the inorganic blocking agent phentolamine ('Regitin'); it is, however, inhibited by methylamphetamine<sup>3</sup>.

It seemed to us worth while to examine the effect of amino-oxidase inhibiting substances on the psychomotor depression produced by adrenaline. We used two inhibitors having different mechanisms of action, that is, iproniazid, a hydrazine derivative which acts by chemical inactivation of the amino-oxidase, and tranyleypromine, an amphetamine derivative, antagonizing it competitively. The method used in these experiments has been described in our preceding papers<sup>1,2</sup>. Iproniazid was injected in doses of 100 and 200 mg/kg. The dose of

100 mg/kg was injected 2 and 18 h before injecting adrenaline. The dose of 200 mg/kg was injected 18 h before the administration of adrenaline. All injections were applied intraperitoneally. Tranyleypromine was injected in doses of 1, 2, 5 and 10 mg/kg, always 2 h before the administration of adrenaline. The psychomotor activity of the animals was registered 15 min after the injection of adrenaline for 10 min in an actophotometer.

The results are shown in Table 1. Each group consisted of 7-21 animals.

Table 1

Distilled water	Adrenaline 1 mg	Adrenaline 2 mg
51 ± 8	17 ± 4	5 ± 1
Iproniazid 100 mg 2 h before	46 ± 15	10 ± 4
Iproniazid 100 mg 18 h before	40 ± 11	13 ± 3
Iproniazid 200 mg 18 h before	59 ± 8	11 ± 3
Tranyleypromine 1 mg	17 ± 4	7 ± 2
Tranyleypromine 2 mg	67 ± 16	12 ± 5
Tranyleypromine 5 mg	78 ± 15	29 ± 19
Tranyleypromine 10 mg	100 ± 15	81 ± 17

The values in Table 1 correspond to the actophotometer readings  $\pm$  S.D.M. These values show that iproniazid in doses of 100 and 200 mg does not effectively modify the psychomotor activity.

The same substance given 2 or 18 h before an injection of adrenaline has no significant effect on the psychomotor depression caused by the last substance.

The situation with tranyleypromine is different. This substance in doses of 2 and 10 mg/kg enhances somewhat the psychomotor activity and in doses between 1 and 5 mg/kg eliminates completely the psychomotor-depressing action of 1 mg/kg adrenaline, but not that of 2 mg/kg of this substance. 10 mg/kg of tranyleypromine not only inhibited the depressing action of adrenaline (even that of the dose of 2 mg/kg) but also produced an enhanced psychomotor activity.

We suppose that tranyleypromine acts in these experiments not by inhibition of the amino-oxidase, but as an amphetamine derivative showing an effect similar to that of this drug. Our opinion is based on the fact that iproniazid manifested no clear antagonism to the psychomotor depressing action of adrenaline.

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### Formation of Two Spots caused by 'Secondary Adsorption' in Paper Chromatography of Primycin

We have shown<sup>1</sup> that primycin, an agent of strong antituberculous effect belonging to the group of guanidino antibiotics, is not identical with any of the known antibiotics. On the basis of its colour reactions and of its infra-red and ultra-violet spectra<sup>2</sup>, the molecule of primycin ( $C_{19}H_{37}O_7N_5$ ) contains an N—H group and several O—H groups; it is inclined to molecular associations and it discloses adsorptive properties. On subjecting primycin to paper chromatography, the formation of a second spot was observed. This was due to the so-called 'secondary adsorption' of an accompanying substance present in minute amounts.



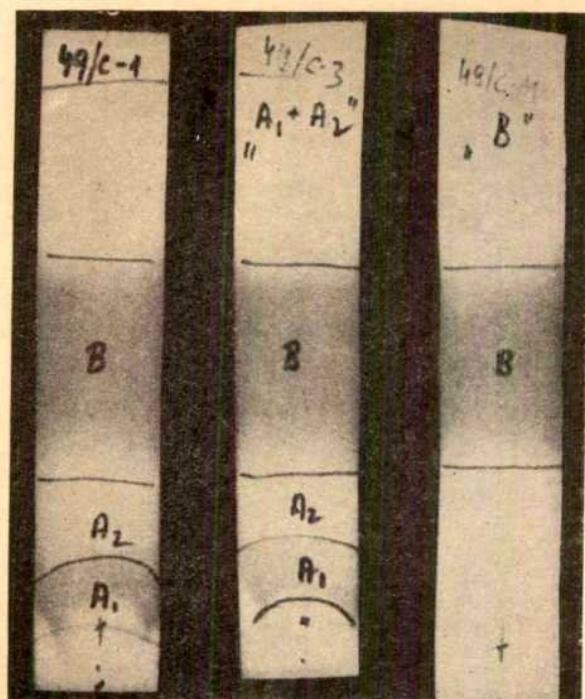


Fig. 1

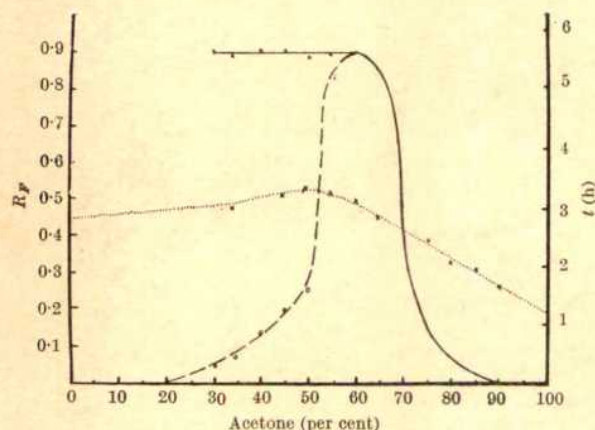


Fig. 2. Changes in  $R_F$  values and migration periods ( $t$ ) in hours, plotted against acetone concentration. —, Spot 'A'; ---, Spot 'B'.

The anomalous phenomena of 'tail formation' and of formation of two or more spots in the paper chromatography of a single substance have already been dealt with in the case of sugars<sup>3</sup> and antibiotics<sup>4</sup>. Though the phenomenon has been observed and described by several authors, its interpretation has been based solely on assumptions. However, it was proved by Soholsky *et al.*<sup>5</sup> that in the case of novobiocin, depending on the nature of the dropping solvent, the solvate or hydrate of novobiocin was actually adsorbed by cellulose as a separate spot. Primycin, dissolved in any of its solvents and allowed to dry on paper, retained its property of forming two spots. Thus, in the mechanism of paper chromatography, in addition to partition effects, other competitive influences such as 'secondary adsorption' may also emerge.

**Experimental technique.** The paper strip technique generally applied in the case of antibiotics was followed, while the circular method was used for the partition of larger quantities. Chromatography was carried out by ascending development at room temperature, using  $10 \times 180$  or  $10 \times 290$  mm strips of filter paper (Schleicher and Schüll '2043/b'). The amount of primycin dissolved in butanol/ethanol/water (1 : 1 : 2 v/v) (BEW)<sup>2</sup> and dried on

paper, indicated by 'autobiography' against *B. subtilis* ranged from 0.5 to 10  $\mu\text{g}$ , while on detection by the N-bromo-succinimide modification of the Sakaguchi colour test<sup>2</sup> it varied from 50 to 200  $\mu\text{g}$ . The  $R_F$  values were established from the mean values of spots which were allowed to run parallel.

The adsorption mechanism of the formation of two spots in the case of primycin was statistically supported by the data of chromatograms<sup>6</sup> investigated by 'autobiography' in 40 different solvent systems, with three exceptions (7.5 per cent), only a single spot was observed. A 45 per cent mixture of acetone and water proved to be the most suitable for the investigation of the causes which lead to the formation of two spots (Fig. 3, strips 1-2). The two spots also appeared on paper previously subjected to exhaustive extraction by benzene and methanol (Soxhlet) proving that the phenomenon is not essentially affected by the contaminations of filter paper. As to the effect of the quantities of substance transferred on the paper, it was found that a single zone appeared with 1  $\mu\text{g}$  of primycin whereas two spots formed with 2, 4, 6  $\mu\text{g}$  or more. In contrast, on running amounts of 50-200  $\mu\text{g}$  of primycin for example, in the system *n*-BuOH-Ac.ac.-H<sub>2</sub>O (40 : 10 : 50), only one spot was observed ( $R_F$ : 0.56-0.61). To determine how these two spots of primycin could have been formed, the antibiotic was allowed to run to various frontal distances in the critical acetone-water solvent mixture. The antibiotic content of the upper 'B' spot (of higher  $R_F$  value) proved to increase gradually with the progress of the solvent while that of the lower 'A' spot proportionately decreased. Consequently, changes took place in the antibiotic content of the spots during migration. Thus, the presence of another active substance or of an eventual isomer of primycin cannot be assumed. When the position of the spots was simultaneously established on control strips, the chromatogram was correspondingly divided and run again in the same solvent, all the 'A' and 'B' spots appeared at an identical  $R_F$  value (0.85), indicating that they originate from one single type of primycin. However, the problem of the 'two active agents' of primycin still remained doubtful in the case of two-dimensional chromatography.

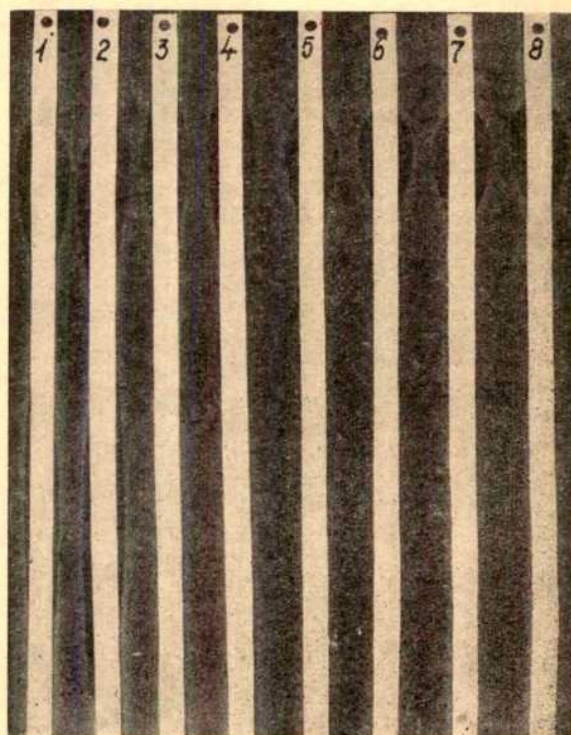


Fig. 3



In applying paper strips and *n*-butanol saturated with ter, chromatograms with only one spot were always served. When larger amounts (200  $\mu$ g) of primycin re subjected to partition by the circular method, and Sakaguchi reaction<sup>2</sup> was used for indication, spots and 'B' formed with this solvent (Fig. 1). On cutting these spots and eluting them with methanol, the plates were allowed to run separately with *n*-butanol on the paper strips. Spot 'B' remained unchanged while 'A' formed two. Consequently, the accompanying contamination formed a separate system on the paper per + contamination + primycin), resulting in the titting of primycin. Later, the effect of the solvent tern acetone-water on the fractionation of primycin s investigated by 'autobiography'. Two spots formed h acetone concentrations from 35 to 55 per cent; ler identical conditions only one spot appeared h acetone concentrations of 60-90 per cent (Fig. 2). Fig. 2 the changes in  $R_F$  values and the migration iods of the solvent mixtures were plotted against concentrations of acetone (in steps of 5 per cent). It be seen that in solvents which migrate slowly (up 50 per cent acetone) two spots of primycin formed, ile in solvents which migrate more quickly (50-90 cent acetone) only one spot appeared, presumably to the absence of the accompanying substance. If conclusion drawn from the experimental data is rect, no two spots may reappear when the single spot primycin obtained by running in 70 per cent acetone g. 3, strips 3-4) is again chromatographed in 45 per acetone (strips 5-8). In fact, on carrying out this ond chromatography in paper strips of double length, phenomenon of 'secondary adsorption' due to the ged contaminant was no longer observed. n further experiments, the purity of primycin was ified not only by paper chromatography but also by titution column chromatography, and was supported exact data obtained by counter-current extraction aig) partition and fractionated crystallization. In all cases only one active substance was detected which closed constant analytical values.

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## HAEMATOLOGY

### Specific Technique for the Isolation of Human $\alpha_2$ -Macroglobulin

ORMAL human serum contains two kinds of macrobulin, the  $\gamma_1$ M (or  $\beta_2$ M)-group and  $\alpha_2$ -macroglobulin; latter was first isolated and described by Schultze l co-workers<sup>1</sup>. The macroglobulin isolated by Brown d. from Cohn's Fraction III<sup>2</sup> seems to be the same tein<sup>3</sup>. Although  $\alpha_2$ -macroglobulin corresponds to ut 3 per cent of total serum protein, its biological ifficance is still unknown. This may be a sufficient lanation for the rather scarce literature concerning it. wever, it seems to us that the main reason might be the iculty of obtaining this protein in a pure state and in ficient quantity. This involves a complete fractionation

scheme<sup>1,2</sup>, or the use of small-scale techniques involving ultracentrifugation or preparative electrophoresis<sup>3-5</sup>. A specific and simple isolation technique is still lacking.

The technique we propose is based on the precipitation of a protein fraction containing the  $\alpha_2$ -macroglobulin by 'Rivanol' (diamino-ethoxyacridin-lactate) adsorption of the contaminating plasminogen on bentonite and adsorption of impurities on DEAE-cellulose. Table 1 gives the details of this fractionation scheme.

Table 1. ISOLATION TECHNIQUE FOR  $\alpha_2$ -MACROGLOBULIN

Plasma	Proteins 5 per cent; pH 8.6
Supernatant	1 vol. + 1.8 vol. 'Rivanol' 0.5 per cent pH 6.4
Precipitate	1 vol. + 0.44 vol. 'Rivanol' 0.5 per cent Supernatant discarded Dissolution (1/5 initial volume) 3 per cent NaCl Insoluble material is discarded Dialysis against water (2 h) Re-precipitation with 'Rivanol' (0.38 vol.)
Precipitate	Supernatant discarded Washing with distilled water Dissolution (1/10 initial volume) 3 per cent NaCl; 2-h dialysis against water Dilution to 1.5 per cent protein concentration; $\Delta t = -0.14^\circ$ ; pH 8.6 Absorption of plasminogen by bentonite (3 per cent) for 30 min
Supernatant	Centrifugation pH 5 (with dilute acetic acid) Adsorption of contaminating proteins on DEAE equilibrated at pH 5 against acetate buffer 0.015 M (60 g pressed, humid cellulose for 1 g of protein)
Pure $\alpha_2$ -macroglobulin	remains in solution

The first precipitate, obtained by addition of a 0.5 per cent 'Rivanol' solution at pH 8.4, is discarded, and the second precipitate, obtained at pH 6.4, contains the  $\alpha_2$ -macroglobulin. This precipitate is dissociated by 3 per cent NaCl and the protein solution is separated by centrifugation from the insoluble material (mainly 'Rivanol'), and after a short dialysis against distilled water the second precipitation step is repeated. The resulting precipitate is washed with cold water, dissociated with 3 per cent NaCl as before, and dialysed.

At that stage starch-gel electrophoresis showed that  $\alpha_2$ -macroglobulin was mainly contaminated by some haptoglobin and other  $\alpha_2$ -globulins; immunoelectrophoresis revealed, in addition to these proteins, the presence of  $\beta_2$ A-globulin.

Some years ago we isolated haptoglobin by chromatography on DEAE-cellulose at pH 5 in the presence of 0.03 M acetate buffer<sup>6</sup>. A small modification of the original technique allows adsorption of haptoglobin as well as the  $\alpha_1$ -globulins and  $\beta_2$ A-globulin, whereas  $\alpha_2$ -macroglobulin is not adsorbed. Unfortunately, routine testing for proteolytic activity showed that the  $\alpha_2$ -macroglobulin prepared by this method contains considerable amounts of plasminogen. There seems to be a real molecular affinity between the pro-enzyme and  $\alpha_2$ -macroglobulin, and plasminogen proved to be a very tenacious contaminant. The best result has been obtained by adsorption of plasminogen on bentonite, the adsorption step being carried out between precipitation by 'Rivanol' and the adsorption of impurities on DEAE-cellulose.

Several preparations submitted to analytical ultracentrifugation (the analytical ultracentrifugation data were provided by the Station Centrale d'Ultracentrifuga-

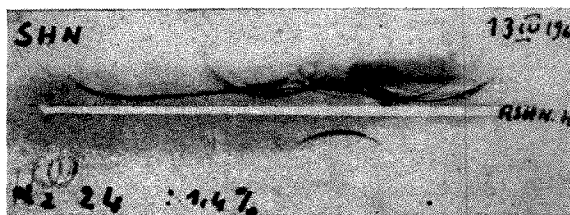


Fig. 1. Immuno-electrophoresis: 1.4 per cent solution of  $\alpha_2$ -macroglobulin. An anti-human serum from the Netherlands Red Cross Blood Transfusion Centre



tion du C.N.R.S.) revealed 91–94 per cent of homogenous 19 S component. By a second chromatography on DEAE under the same conditions we obtained a preparation containing 97 per cent heavy component. The residual 2 per cent of the preparation corresponds to a 12 S component which might eventually be found to correspond to a degradation product of  $\alpha_2$ -macroglobulin. Indeed, immunoelectrophoresis of a 1.4 per cent solution of  $\alpha_2$ -macroglobulin revealed a single precipitation line when tested with whole anti-human serum (Fig. 1). A similar observation has been made by Filitti-Wurmser and Hartmann for  $\alpha_2$ -macroglobulin obtained by ultracentrifugation and dialysis.

Thus, the proposed technique provides a means of obtaining pure  $\alpha_2$ -macroglobulin, the procedure needing neither preparative ultracentrifugation nor preparative electrophoresis. The total yield is about 50 per cent calculated on the basis of a theoretical concentration of 2.1 g/l. of plasma.

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## HISTOLOGY

### Haarscheiben in the Skin of Sheep

ON the skin surface of many mammals, including man, there are mounds of epidermis, called 'haarscheiben', which are characterized by several layers of epithelial cells overlying an area of connective tissue that is highly innervated and vascularized<sup>1,2</sup>. The haarscheibe is an integrated part of the highly specialized tylotrich follicle<sup>3,4</sup>. Although the formation and morphology of sheep hair follicles are well known<sup>5</sup>, haarscheiben have not been found. The present communication describes haarscheiben in sheep skin and shows that they are associated with a particular type of hair follicle.

Skin samples were removed from the dorsum and venter of three 8-month-old  $F_1$  hybrid sheep (Cheviot  $\times$  Suffolk). Each skin specimen was clipped, depilated with barium sulphide, and examined under a low-power microscope for the presence of haarscheiben. Circular haarscheiben were clearly visible on the skin surface (Fig. 1). Associated with each of these structures was a hair, which generally emerged slightly caudal to the centre of the haarscheibe (Fig. 1). The mean diameter of ventral haarscheiben was larger than that of those found on the dorsum (Table 1). Similar results were observed in the mouse<sup>6</sup>. Many of the haarscheiben on the dorsum of the sheep contained clumps of pigment granules, but this was not seen on other parts of the skin surface.

Table 1. DIAMETER OF SHEEP HAARSCHIEBEN

Skin area	Haarscheiben		S.E.	t*	P
	No. observed	Mean diam. (mm)			
Caudal dorsum	20	0.263	$\pm 0.007$	8.48	< 0.001
Venter	30	0.380	$\pm 0.010$		

\* Based on Fisher's small-sample *t* test.

About forty haarscheiben with some surrounding tissue were excised from the skin specimens. Some tissues were impregnated with gold chloride<sup>7</sup>. Others were placed in



Fig. 1. Haarscheibe on the ventral skin surface of the sheep. The orifice of the hair canal is marked with an arrow. Another hair shaft which lacks a haarscheibe is shown on the right ( $\times 475$ ).

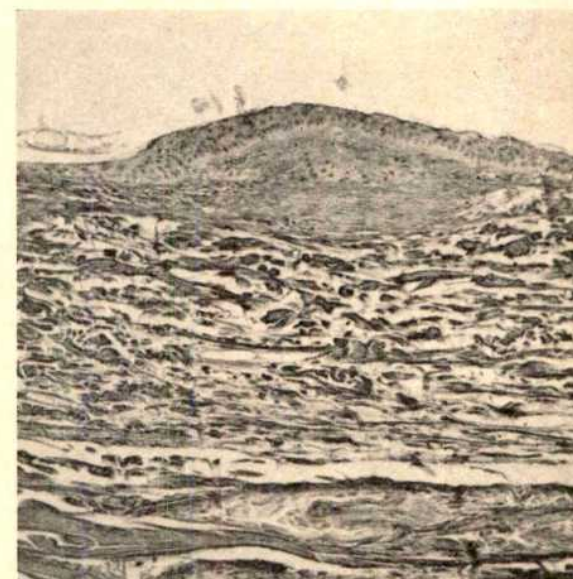


Fig. 2. Longitudinal section through a haarscheibe, showing the firmer connective tissue under the thickened epidermis. Section stained with alum haematoxylin and eosin ( $\times 134$ ).

either Bouin's or Lavdowsky's fixatives. All tissues were embedded in paraffin, sectioned serially at  $7\mu$ , and stained with alum haematoxylin and with either eosin or van Gieson's picric-acid fuchsin stain.

The haarscheibe of the sheep is composed of several layers of epithelial cells, the number of cell layers decreasing abruptly on both sides of the haarscheibe (Fig. 2). The lower cell layers of epidermis are morphologically distinct. The basal layer consists of highly specialized cells containing large amounts of cytoplasm (Fig. 3). The next layer contains elongated epithelial cells with spindle-like nuclei, many of which stain deeply with haematoxylin. Underlying the epidermis is a specialized region of dermis composed of fine connective tissue fibres. Throughout this region are many capillaries and fine nerves (Fig. 2).

In addition to the haarscheiben, tylotrich follicles characterized by a specialized area of tissue just below the level of the sebaceous glands. This sensory area,



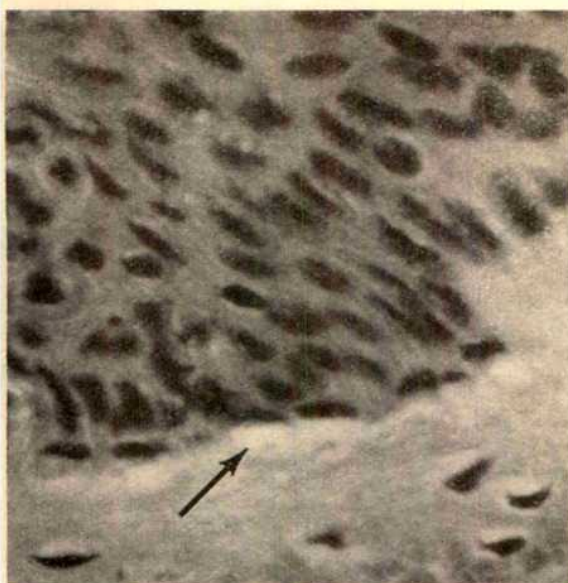


Fig. 3. A tangential section through a haarscheibe showing the specialized cells at the base of the epidermis, one of which is marked with an arrow. Section stained with alum haematoxylin and eosin ( $\times 1,105$ )

annular complex, consists of a prominent network of nerve fibres surrounded by an annular arrangement of highly vascularized connective tissue<sup>3,4</sup>. The annular complex is observed most readily in the resting phase of the hair growth cycle; during the growth phase it is depressed and obscured<sup>4</sup>. All the sheep follicles examined were growing, and the annular complexes were not observed. Nevertheless, sheep haarscheiben are associated with distinct hair follicles.

Sheep hair follicles are classified into primary follicles, which possess sweat glands and arrector pili muscles, and secondary follicles, which lack these accessory structures<sup>5,6</sup>. Each follicle associated with a sheep haarscheibe possesses a sweat gland and arrector pili muscle, indicating that tylotrich follicles are in the primary follicle group. Primary follicles are divided further into central primary follicles, the first follicles to appear in the foetus, and lateral primary follicles, which begin to form a few days later<sup>5</sup>. Which of the two sheep primary follicle types possesses haarscheiben is not known, but by comparison with mouse tylotrich follicles, which are the first to appear in the foetus<sup>6</sup>, it is suggested that the central primary follicles have the haarscheiben.

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### Survival of Chromaffin Cells in Organ Culture of the Adrenal Medulla

AUTOGRAFTS of the adrenal medulla are known to survive for weeks in the anterior chamber of the eye, retaining their cytological properties and catecholamine content<sup>1,2</sup>. On the other hand, few attempts have been

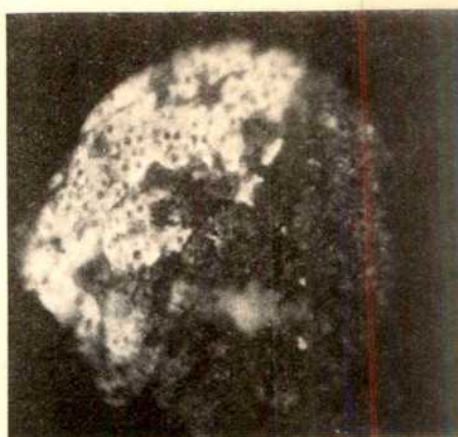


Fig. 1. Formaldehyde-induced fluorescence in a piece of the adrenal of a new-born rat after cultivation for 20 days. Medullary cells exhibit an intense cytoplasmic fluorescence, while cortical cells are essentially non-fluorescent ( $\times 50$ )

made to cultivate adrenal medullary tissue *in vitro*<sup>3,4</sup>. Experiments carried out in our laboratory led to the development of a simple technique by which it is possible to keep adrenal medullary cells alive in culture for several weeks.

Small pieces of adrenal medulla of adult Sprague-Dawley albino rats, and pieces of whole adrenals of new-born rats, were dissected under sterile conditions. The fragments were cultivated in a plastic Petri dish on a chicken plasma and embryo extract coagulum. The culture medium consisted of a mixture of 2 volumes of Hanks's balanced salt solution with 5 mg/ml. of lactalbumin hydrolysate, one volume of amino-acid-Parker and one volume of Seitz-filtered calf serum inactivated for 30 min at 56° C. The Petri dishes were incubated in a

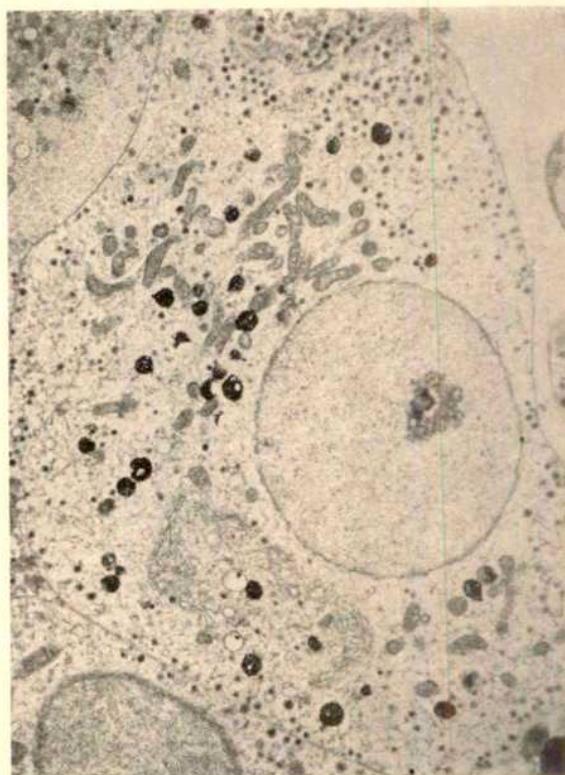


Fig. 2. Electron micrograph showing a medullary cell from a 39-day culture of the adrenal of a new-born rat. Note the small electron-dense vesicles typical of the adrenal medulla, the mitochondria above, and the Golgi apparatus below the nucleus. Palade's fixative, embedding in Epon ( $\times 4,000$ )



humidified air atmosphere continuously supplied with a flow of  $\text{CO}_2$  sufficient to keep the pH at 7.4 in the culture medium.

After varying periods of cultivation, pieces were removed from the culture and they were examined histochemically, using the chromaffin reaction or formaldehyde vapour-induced fluorescence for the demonstration of catecholamines<sup>5</sup>, or by electron microscopy after fixation in Palade's solution and embedding in 'Epon'. Pieces of fresh adrenal glands were similarly treated and served as controls.

The intensity of the chromaffin reaction weakened in the medullary cells during the first 1-3 days of culture. Thereafter the reaction intensity was again similar in the surviving cells and in fresh controls. After 2-3 weeks' culture, the chromaffin reaction of the medullary cells of new-born rats was sometimes even more intense than normal. Formaldehyde-induced fluorescence (Fig. 1) also indicated at least normal catecholamine content in pieces cultured for up to 60 days. Although many cells thus clearly retained their ability to store and possibly to synthesize catecholamines, other cells obviously died during cultivation, especially in cultures of pieces taken from adult animals; pieces from new-born rats survived better.

The ultrastructure of the cultured cells (Fig. 2) corresponded to that of the controls. Droplets containing catecholamine, and surrounded by a membrane, were of typical structure, as were other cytoplasmic organelles, such as mitochondria.

Cultures of this kind may facilitate the investigation of, for example, direct effects of drugs on adrenal medullary cells.

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## HISTOCHEMISTRY

### Staining of Bacteriophage Nucleic Acids with Acridine Orange

THE use of the fluorescent stain acridine orange for the quick identification of viral nucleic acids has been described by Mayor and Hill<sup>1</sup>. In this valuable technique colour differences permit differentiation between double-stranded deoxyribonucleic acid (DNA), on one hand (fluoresces green), and ribonucleic acid (RNA) and single-stranded DNA on the other (fluoresces red). The latter nucleic acids can be separated by their respective sensitivities to RNase and DNase. While the procedure of Mayor and Hill is simple compared with the techniques of biochemical analysis, it involves numerous treatments and the use of fluorescence microscopy. Furthermore, acridine orange staining is particularly sensitive to pH levels, and, because of this, some difficulty may be experienced in obtaining the correct colours. In addition, the procedure does not differentiate between single-stranded DNA and RNA, using the stain alone. This communication describes some improvements and modifications with these points in mind. Direct viewing under ultra-violet light is substituted for the fluorescence microscope, the pre-staining treatment is simplified, and possible unreliability due to pH sensitivity is eliminated.

The method is as follows. The reagents required are Carnoy's fixative, 0.1 M citric acid, 0.15 M  $\text{Na}_2\text{HPO}_4$ , 1

per cent acridine orange in water and buffered saline ( $\text{Na}_2\text{HPO}_4$ , 1.27 g/l.;  $\text{KH}_2\text{PO}_4$ , 0.41 g/l.; NaCl, 7.36 g/l. pH 7.2).

Droplets of a virus suspension ( $10^{10}$ - $10^{12}$  particles/ml in buffered saline) are dried down on to microscope slide. The slides are transferred to Carnoy's fixative for 5 min then rinsed in absolute alcohol and dried in a stream of warm air. The material is stained with acridine orange for 5 min in the following modified McIlvaine's buffer: 6 ml. 0.1 M citric acid; 4 ml. 0.15 M  $\text{Na}_2\text{HPO}_4$ ; 0.1 ml. 1 per cent acridine orange; pH 3.8. The slides are rinsed in 6 ml. 0.1 M citric acid + 4 ml. 0.15 M  $\text{Na}_2\text{HPO}_4$ , and transferred to 0.15 M  $\text{Na}_2\text{HPO}_4$  for 15 min. Excess liquid is shaken off and the slides are examined under an ultraviolet lamp (wave-length 2570 Å), the colour of the smears being noted. If the smears are red, the slides are placed in 0.1 M citric acid and examined in ultra-violet light at intervals of 1, 2 and 3 min, the colour change being noted.

The results obtained with various bacteriophages, etc are summarized in Table 1.

Table 1

Specimen	Nucleic acid	$\text{Na}_2\text{HPO}_4$	Citric acid
Phage T4	2-stranded DNA	Yellow-green	—
Phage $\phi$ L <sup>2</sup>	Unknown	Yellow-green	—
Mouse liver DNA	2-stranded DNA	Yellow-green	—
Mouse liver DNA (denatured)	1-stranded DNA	Flame-red	Stays red but fades noticeably
Phage $\phi$ R <sup>3</sup>	1-stranded DNA	Flame-red	Goes pale green then fades
Phage ZJ/2 <sup>4</sup>	1-stranded DNA	Flame-red	Stays red but fades noticeably
Phage $\phi$ 12 <sup>*</sup>	Unknown	Flame-red	Fades completely
Phage ZIK/1 <sup>4</sup>	RNA	Flame-red	Stays red, does not fade
Phage ZJ/1 <sup>4</sup>	RNA	Flame-red	Stays red, does not fade

\*A filamentous form similar to ZJ/2.

It can be seen that viruses with double-stranded DNA are quickly distinguished from others by their colour after treatment with phosphate. The green is bright and quite unmistakable, though there is sometimes a trace of red in the centre of the smear if the phage concentration is high. If green is obtained there is obviously no need for treatment with citric acid. In fact, this causes the colour to change to red and fade. Both single-stranded DNA and RNA phages produce flame-red colour after phosphate treatment. The colour is almost crimson and there is no trace of green. The citric acid treatment helps to distinguish these two types of nucleic acid. The criterion is that RNA phages do not fade over the 3-min period whereas the single-stranded DNA phages fade noticeably and may be accompanied by a change to green. While this test is to a certain extent subjective, it can be used to confirm other indications as to the type of nucleic acid. For example, a phage which is subject to plaque inhibition by RNase<sup>4</sup>, the red fluorescence of which does not fade in citric acid, undoubtedly contains RNA. If additional confirmation is required, however, Carnoy fixed slides may be treated with RNase or DNase<sup>1</sup>. Subsequent staining will produce no fluorescence with the appropriate nucleic acid.

The procedure must be strictly followed for satisfactory results and virus preparations should be free from contaminating nucleic acids.

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## IMMUNOLOGY

Prevention of Immunological Tolerance to *Shigella* Antigens in New-born Mice by Immune Spleen Cell Extracts

IMMUNOLOGICAL capability may be restored in tolerant animals by means of spleen or lymph node cell transplants from normal or immune donors<sup>1,2</sup>. In somewhat similar situations, establishment of tolerance to *Shigella dysenteriae* antigens<sup>3</sup> and to bovine serum albumin<sup>4</sup> has been prevented in neonatal mice by simultaneous administration of normal spleen or thymus cell suspensions obtained from either neonatal or adult donor mice. In the *Shigella* system, it was found that viable lymphoid cells were apparently necessary for interference with establishment of tolerance. Heat-killed normal lymphoid cells, and non-lymphoid tissue suspensions from liver, lung, kidney and brain, were not effective<sup>3</sup>. During the course of these investigations, it was found that transfer of spleen cell suspensions from adult donor mice previously immunized to *Shigella* antigens was more effective in blocking tolerance than transfer of cells from either neonatal or normal adults<sup>3</sup>.

It was not clear whether such interference was due exclusively to the transferred immune cells, or could be attributed to passive transfer of anti-*Shigella* antibody. In order to clarify this point, experiments were performed to test the ability of cell-free extracts from *Shigella* immune mice to prevent establishment of tolerance. For this purpose, spleen cell suspensions were obtained from groups of NIH adult mice which had been immunized 4–7 days previously with a single intramuscular injection of 0.1 ml. *Shigella* soluble antigen (20 µg N), derived by trypsin digestion from alcohol-killed *Shigella* organisms<sup>5</sup>. For preparation of 'immune' extracts, spleens were obtained only from mice exhibiting serum agglutinin titres of 1:1,024 or higher. The spleens were washed in cold, sterile Hanks's solution, trimmed free of excess fat, and 'teased' into Hanks's solution, pH 7.2, containing 20 per cent sterile calf's serum. A 10-ml. suspension containing 5 to 10 × 10<sup>7</sup> viable nucleated cells per ml. was subjected to alternate freezing and thawing for 10 min. The cell debris was removed by centrifugation at 6,000g for 10 min. The clarified supernatant was filtered through 'Millipore' membranes (0.45 µ), dialysed against saline for 4 h in the cold, and lyophilized. The extracts were stored at -20° C until used. Control extracts were prepared from (a) immune spleen cell suspensions which had been heated for 30 min at 56° C, and (b) spleen cell suspensions prepared from normal, non-immune adult NIH mice.

All spleen cell extracts were rich in mouse γ-globulin as revealed by gel diffusion and immunoelectrophoretic analysis using rabbit anti-mouse whole serum of high titre. The immune extracts had anti-*Shigella* agglutinin titres of 1:10,240 or higher as a solution of 1 mg extract or ml. saline. The heated extracts had titres of 1:640–1:2,560, while non-immune extracts had titres of 1:128 or less. These extracts were tested for their ability to interfere with the establishment of tolerance in new-born mice injected with *Shigella* antigen. Mice were injected intraperitoneally (i.p.) within 12 h of birth with 0.1 ml. *Shigella* soluble antigens (20 µg N). Control mice from the same litters were injected with saline. Groups of new-born mice receiving either *Shigella* or saline were then inoculated i.p. with the concentrated extracts obtained from (a) *Shigella*-immune spleen cells, (b) heated *Shigella*-immune spleen cells, (c) normal spleen cells or (d) saline only. Each mouse received 0.1 ml. extract containing 0.8–0.5 mg N. Five to six weeks thereafter, all surviving mice were challenged by means of an intramuscular injection of *Shigella* antigen. Blood samples were obtained by retro-orbital puncture before challenge, and at close intervals hereafter for 2–3 weeks. The samples were tested for

anti-*Shigella* agglutinins by standard test-tube agglutination procedure or in micro plastic cups using calibrated wire loops and 0.025 ml. volumes of serum dilutions and killed *Shigella* organisms.

Table 1 presents results obtained in a typical series of experiments. New-born mice injected with *Shigella* antigen only at birth were markedly deficient in ability to respond to challenge immunization with *Shigella* at five to eight weeks of age (group A). Similarly treated mice which received spleen cell extracts from *Shigella*-immune donor mice were competent to form anti-*Shigella* agglutinins following challenge injection (group C). Such extract-treated mice receiving *Shigella* antigen at birth responded nearly as well as control mice (group B) which received no inoculations at birth. Administration of extract from normal mice had no effect on establishment of tolerance (group F). However, extracts from heat-treated spleens still retained some ability to prevent establishment of tolerance (group E). Transfer of extracts to mice not receiving an injection of *Shigella* at birth (to induce tolerance) had no detectable effect on agglutinin formation after challenge injection (group D).

Table 1. EFFECT OF TRANSFER OF VARIOUS CELL-FREE EXTRACTS FROM DONOR IMMUNE SPLEEN CELLS ON ESTABLISHMENT OF TOLERANCE TO *Shigella* IN NEW-BORN RECIPIENT MICE

Group	No. of mice	<i>Shigella</i> injection at birth*	Extract transferred at birth†	Agglutinin response after challenge injection No. positive‡ No. injected	Mean peak titres
A	17	+	Saline	2/13	1:28
B	14	—	Saline	11/12	1:417
C	18	+	Immune spleen	11/14	1:372
D	11	—	Immune spleen	8/9	1:402
E	18	+	Heated immune spleen	6/15	1:220
F	15	+	Normal spleen	1/11	1:29

\* 0.1 ml. *Shigella* soluble antigen (20 µg N) injected intraperitoneally within 12 h of birth.

† 0.1 ml. of saline or spleen cell extract (0.8 to 1.5 mg N) injected intraperitoneally in recipient mice within 12 h of birth.

‡ No. of mice with agglutinin titre of 1:128 or higher after challenge injection with *Shigella* antigen at 5–6 weeks of age.

Graded doses of immune spleen cell extracts were administered i.p. to various groups of new-born mice. Injection of 5, 2, 1, 0.5 and 0.1 mg N extract was found to be effective in preventing establishment of tolerance. Administration of lower dosages was ineffective. Absorption of concentrated extract with an equal volume of packed, alcohol-killed, *Shigella* micro-organisms for 30 min at 37° C, followed by high-speed centrifugation to remove the organisms, removed all activity capable of blocking tolerance. Such absorption also removed detectable anti-*Shigella* agglutinins from the extract. Treatment of extract with sheep red blood cells or packed *Salmonella typhosa* antigen had no effect.

The results presented here indicate that immunological tolerance induced in new-born mice by inoculation of a large dose of *Shigella* antigen may be prevented by a simultaneous inoculation of cell-free spleen extracts prepared from *Shigella*-immune adult donor mice. Such extracts contain relatively high levels of *Shigella* agglutinins. Absorption of agglutinins by incubation with *Shigella* vaccine eliminates the biological effectiveness of the extract. Non-immune extracts were not effective in preventing unresponsiveness. Other experiments in progress on the prevention of tolerance have suggested that mouse or rabbit anti-*Shigella* immune serum of high titre may also block tolerance. These findings suggest that tolerance to a non-living antigen such as *Shigella* soluble antigens may be prevented in new-born mice not only by lymphoid cell transplants, but also by specific antibody. The mechanism of such inhibition is not clear. Immune spleen cell extracts may contain an active antibody-forming mechanism (nucleoprotein-RNA-coding complexes) which actively induces agglutinin formation in new-born mice<sup>6</sup>. On the other hand, passively trans-

ferred extracts may contain antibody which inhibits postulated antigen-antibody complexes capable of blocking the induction of immunity in otherwise competent cells<sup>7</sup>.

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### Immune Globulins in Human Viral Infections

EXTENSIVE investigations in animals, including man<sup>1-3</sup>, have shown that the first immune globulins which appear after a primary antigenic stimulus are of high molecular weight and can be readily inactivated by sulphydryl-reducing compounds such as 2-mercaptoethanol (2-ME). In most systems these antibodies, which will be referred to as 19S, are replaced within a few weeks by 7S  $\gamma_2$ -globulins which resist reduction. The 19S antibodies can be demonstrated in early post-immunization sera by sedimentation characteristics or more easily by titration of antibody activity before and after treatment with 2-ME. In late sera or following a secondary stimulus, 19S globulins make up no more than a small portion of total antibody so that 2-ME treatment does not result in over-all titre reduction.

It seemed that the relative amounts of 19S and 7S antibody might provide a valuable index for recognizing recent viral infections in humans and differentiating between primary and secondary immunological experiences. This has been tested and confirmed following measles, mumps and Coxsackie virus infections. Those vaccinated against measles were also examined to provide sera collected at accurately known intervals after infection.

Sera collected at various times after measles were fractionated by ultracentrifugation on sucrose gradients. Fractions were titrated for antibody content by the haemagglutination-inhibition (HI) method of Rosen<sup>4</sup>.

Table 1. SULPHYDRYL SENSITIVITY OF MEASLES HI ANTIBODIES IN POST-VACCINAL SERA

Days post vaccination	No. tested	No. sensitive
12	1	21 (100%)
13	3	
14	1	
16	9	
19	2	
21	5	
42	5	2 (40%)
60	37	

Table 2. SULPHYDRYL SENSITIVITY OF ANTIBODIES FOLLOWING VIRAL INFECTIONS

Patient	Infecting virus	Days post-hospital admission	Antibody untreated	Titre 2-ME treated	Fold reduction
1	Mumps	1	60	30	2
		3	240	120	2
2	Mumps	1	120	40	3
		15	320	160	2
3	Mumps	1	80	40	2
		36	640	640	0
4	Coxsackie B5	1	8	<8	—
		3	24	<8	>3
5	Coxsackie B5	1	64	<4	>16
		7	512	8	64
6	Coxsackie B5	1	<8	<8	—
		9	>640	<8	>80
		16	>640	8	>80
7	Coxsackie B5	1	256	16	16
		19	2,048	256	8
8	Coxsackie B4	1	<10	<10	—
		22	512	128	4
		100	1,024	1,024	0

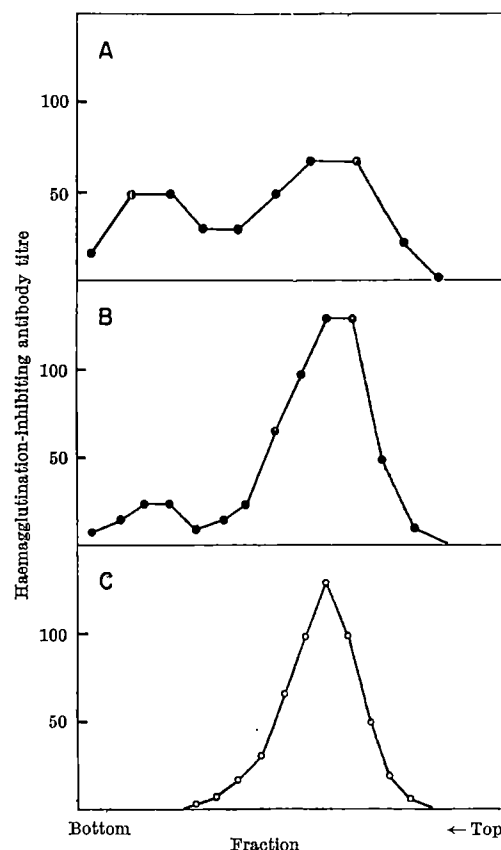


Fig. 1. Sedimentation analyses of measles antibodies in sera collected at various times after measles. A, 4 days after rash; B, same patient as in A, 19 days after rash; C, 1 year after measles. Each serum was layered on a five-step gradient ranging from 12.5 to 37 per cent w/v sucrose and centrifuged at 35,000 r.p.m. for 16.5 h in a Spinco SW-39 rotor. Fractions were collected through a hole pierced in the bottom of the tube.

Two peaks of antibody activity were found in sera following recent infections. The lower peak, shown on the left in Fig. 1, corresponded in position with heterophile antibody centrifuged under the same conditions as previously characterized as 19S globulin<sup>5</sup>. Acute phase sera (A, Fig. 1) contained relatively large amounts of 19S antibodies. 'Convalescent' sera (B, Fig. 1) contained progressively less macroglobulin until only 7S antibodies were detectable in specimens collected more than six days after measles (C, Fig. 1).

Even though measles 19S antibodies as determined by centrifugation rarely made up half the total HI activity, two-fold or greater reductions in antibody titre could be detected consistently after 2-ME treatment of sera collected during the first three weeks after immunization with live measles vaccine (Table 1). The sedimentation characteristics of that portion of the 2-ME-sensitive antibody that is not in the 19S fraction has not yet been determined. Sulphydryl sensitivity was demonstrated after treating sera with 1/10 volume 1 M 2-ME for 30 min at 37° C. Tests on treated and untreated samples were done in duplicate and in parallel and scored from 0 to 4-plus for haemagglutination. Using 2-plus agglutination as end-point, reproducibility in replicate titrations was within 0.5 log<sub>2</sub> and two-fold reductions in titre could regularly be confirmed.

Results obtained by determining sensitivity of mumps HI antibodies and Coxsackie neutralizing antibodies in sera collected after natural infection were similar to results with measles (Table 2). Titres of these antibodies in sera from healthy adults showed no 2-ME sensitivity, whereas acute and early convalescent sera from patients were uniformly sensitive to 2-ME. As in measles, titre reductions after mumps were consistent but never more

han four-fold. In the Coxsackie system reductions up to 4-fold were found.

Secondary immune responses were elicited in measles-immune persons by injection of inactivated measles vaccine. Following immunization 7S antibodies increased, but no 19S antibodies were detectable. Thus, the primary character of an antibody rise may be determined.

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### Simplified Procedure for Passive Haemagglutination Tests with Protein Antigens

FOR passive haemagglutination tests with proteins, it has been necessary to couple these antigens to erythrocytes by agents such as *bis*-diazotized benzidine<sup>1-3</sup>, polyene-2,4-diisocyanate<sup>4</sup> and tannic acid<sup>5</sup>. During the course of investigations with formalin-treated erythrocytes, we observed that bovine serum albumin (BSA) could adsorb on formalin-treated chicken erythrocytes (FCE), as well as on formalin-treated sheep erythrocytes (FSE) without recourse to such coupling agents. This finding suggested a means of simplifying the procedure for coating erythrocytes with proteins for passive haemagglutination tests.

When protein antigens are adsorbed on tannic acid-treated erythrocytes, Coombs's serum cannot be used since globulins of the antiserum are non-specifically adsorbed. This is overcome by preliminary incubation of the coated, tanned erythrocytes with normal serum of the different species from that providing the direct anti-protein serum<sup>6</sup>. The present method is advantageous in that Coombs's serum reacts specifically with the coated erythrocytes that have previously reacted with specific anti-protein serum and greatly augments the sensitivity of the test. Chicken erythrocytes were obtained from White Leghorn hens 20 weeks old. FCE were prepared according to the method of Weinbach<sup>7</sup> and resuspended in 0.1 M acetate buffer, pH 5.0. FCE were coated with BSA by mixing 5 ml. of 2 per cent FCE suspension with 5 ml. of acetate buffer containing 1 mg of BSA and the mixture was kept at 24° C for 4 h with constant rotation. The cells were then washed 3 times and resuspended in 0.1 M phosphate buffer, pH 7.2, as a 1 per cent suspension.

Haemagglutination tests were performed by incubating, for 1 h, mixtures of 0.1 ml. of 1 per cent BSA-coated FCE and 0.1 ml. of appropriate dilutions of chicken anti-BSA

Table 2. SPECIFIC INHIBITION OF HAEMAGGLUTINATION REACTION OF BSA-COATED FCE BY BSA

Anti-BSA serum was incubated with BSA or HGG followed by addition of BSA-coated FCE. After recording the titre, Coombs's serum was added.

BSA added	Antiserum dilution titre	
	Before Coombs's	After Coombs's
None	1/400	1/40,000
0.03 µg	1/120	1/12,000
0.1 µg	1/120	1/12,000
1.0	1/20	1/40
3.0	> 1/20	> 1/20
HGG added		
500 µg	1/400	1/40,000

serum diluted in phosphate buffer containing 2 per cent normal rabbit serum. After the agglutination reactions were recorded, 0.1 ml. of Coombs's serum (rabbit anti-chicken γ-globulin serum) was added, the tubes were shaken and then incubated for an additional hour. As shown in Table 1, before and after the addition of Coombs's serum, the chicken antiserum used gave titres of 1/600 and 1/60,000, respectively. FCE coupled with BSA via *bis*-diazotized benzidine gave the same antiserum titres.

Inhibition of the reaction by BSA, but not by human γ-globulin (HGG), attests to the specificity of the reaction. BSA in amounts ranging from 0.01 to 3 µg were incubated with 0.1 ml. of the antiserum for 0.5 h; 0.1 ml. of the reaction mixture was then reacted with 0.1 ml. of coated FCE. The data in Table 2 show that the reaction with antiserum was completely inhibited by 3 µg of BSA whereas even 500 µg of HGG was without effect.

Preliminary experiments have shown that two other antigens would coat formalin-treated erythrocytes. Using the foregoing procedure, rabbit γ-globulin was found to coat FCE as well as FSE. Ovalbumin coated FCE at pH 4 but not at pH 5.

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### Quantitative Estimation of the Reactivity of Normal and Hodgkin's Disease Lymphocytes with Thymidine-2-<sup>14</sup>C

SINCE lymphoid blood cells play an important part in the mediation of delayed hypersensitivity and homograft rejection<sup>1-4</sup>, it is reasonable to expect that further understanding of these forms of immunological responsiveness will come from appropriate *in vitro* investigations of circulating lymphocytes. Lymphocyte reactivity *in vitro* may also provide insight into the pathogenesis of diseases involving immune or autoimmune mechanisms and provide a measure of the efficacy of immunological suppression. It has been the particular goal of several laboratories<sup>5-7</sup> to apply such lymphocyte investigations to the

Table 1. ANTISERUM DILUTION TITRE OF CHICKEN ANTI-BSA SERUM AGAINST BSA-COATED FCE WITH AND WITHOUT COOMBS'S SERUM

	Controls			Final dilutions of anti-BSA serum								
	Diluent	NCS* 1/10	Coombs's	1/20	1/60	1/200	1/600	1/2,000	1/6,000	1/20,000	1/60,000	1/200,000
Before Coombs's	—	—	—	+	+	+	+	—	—	—	—	—
After Coombs's	—	—	—	+	+	+	+	+	+	+	+	—

\* Normal chicken serum.



problem of donor selection for human organ transplantation, and for this purpose several techniques for the culture of lymphocytes and the measurement of their reactivity have been developed. Inasmuch as the methods being used to determine human lymphocyte reactivity, that is, conversion of lymphocytes to large immature cells<sup>5,6</sup>, counting of mitoses<sup>5,6</sup> and autoradiography with tritiated thymidine<sup>6</sup> are laborious and subject to several sources of error, a simple quantitative technique for following lymphocyte reactions *in vitro* would be of considerable value. The present communication describes such a method which is based on the incorporation of <sup>14</sup>C-labelled thymidine into the deoxyribonucleic acid (DNA) of reacting cultured lymphocytes. This method was developed to investigate the lymphocytes of patients with Hodgkin's disease, a condition in which delayed type (cell-mediated) hypersensitivity and homograft rejection are regularly depressed<sup>8</sup>.

The technique of Bain, Vas and Lowenstein for human lymphocyte culture has been closely followed<sup>5</sup>. Heparinized venous blood was allowed to sediment for 60 min at 37° and then centrifuged at 25g for 5 min at room temperature. Into each tube 1 ml. buffy coat (or for mixed cultures 0.5 ml. of buffy coat from each of two individuals) was placed and diluted with 3 ml. of TC-199 medium containing 100 units of penicillin and 100 µg of streptomycin per ml. (obtained from Microbiological Associates, Bethesda, Maryland). Total and differential counts were done on the buffy coat by standard haematological technique, and the lymphocytes were found to constitute 30–80 per cent of the leucocytes. Because heavy aggregates form when Hodgkin's sera are cultured, the buffy coat from these individuals was centrifuged at room temperature for 5 min at 800g and the cells resuspended in an equal volume of normal human serum before diluting. After 5 days' incubation at 37° in 20 × 125 mm screw-cap glass culture tubes, 2 µc. of thymidine-2-<sup>14</sup>C in 15.9 µg (specific activity 30.5 µc./µmole, obtained from the New England Nuclear Corporation, Boston, Mass.) was added to each tube. Following an additional 90-min incubation at 37° C, the tubes were chilled and centrifuged in the cold (2° C) for 5 min at 1,300g. The lymphocyte pellet was then washed three times by resuspension in 4 ml. of cold 0.9 per cent sodium chloride and recentrifugation under the same conditions. 4 ml. cold isotonic saline was next added and the pellet homogenized briefly with an all-glass Potter-Elvehjem homogenizer. A 0.5 ml. portion of the homogenized lymphocyte preparation was then pipetted into a 'Millipore' microanalysis filter holder fitted with a 24-mm 'HA Millipore' filter disk and the cells trapped by vacuum filtration. The cells were then washed in place on the filter disk with 5 aliquots (5 ml. each) of cold trichloroacetic acid. Once the filter disks were dry they were mounted on "Tracerlab E-7B" rings and disks which were in turn placed on planchet holders and assayed for radioactivity in a Nuclear-Chicago thin end-window low background counter.

Preliminary results established that the uptake of <sup>14</sup>C-thymidine under the conditions of the incubation of five-day cultures was linear for 2.5 h and that duplicate analyses from the same culture checked to within ± 5 per cent. All cultures were performed in duplicate and separately analysed. While under certain conditions, found in incubations which contained phytohaemagglutinin (PHA), variations of ± 25 per cent were seen in duplicate cultures, in most circumstances under which the cells were investigated (unstimulated, mixed culture and after antigen addition) results from the two cultures checked within ± 10 per cent. The results of the duplicate cultures were averaged for inclusion in Table 1. An occasional grossly contaminated tube, indicated by change in colour of the indicator dye, was discarded.

The results with normal and Hodgkin's disease lymphocytes are presented in Table 1. The Hodgkin's patients

Table 1. REACTIVITY\* OF NORMAL AND HODGKIN'S DISEASE LYMPHOCYTES

Donor	Control	Phytohaemagglutinin	Mixed culture	Tuberculin (PPD)
<b>Normals</b>				
AA-1	0.03		5.3 (BW-1)	0.25 (+)
AA-2	0.53	130.0		
AA-3	0.06	21.3		
AA-4			1.8 (BW-3)	
AA-5			0.2 (BW-4)	
BW-1	0.06	17.9	5.3 (AA-1)	
BW-2	0.20	89.0	3.8† (PM-1)	
BW-3			1.8 (AA-4)	
BW-4	0.09		12.1 (NB)	
RW	0.20	67.5	0.2 (AA-5)	0.37 (-)
DG	0.56	28.2	5.2 (DG)	0.60 (-)
			7.2 (RW)	
NB	0.22	55.7	5.1† (HJ)	
MS	0.10	153.0	12.1 (BW-3)	
GF	0.14			3.9 (+)
KB	0.43			4.5 (+)
LM	0.04			0.10 (-)
WS	0.23			0.31 (-)
<b>Hodgkin's disease</b>				
PM-1	0.59	71.0	0.03 (AW)	3.7 (+)
			3.8† (BW-2)	
PM-2	0.90		5.5 (SP)	
EM	0.08	315.0	10.3 (VD)	0.12 (-)
WD	0.12	331.0	10.3 (EM)	0.12 (-)
HJ	0.06	5.1	7.1† (DG)	0.87 (-)
AW	0.00	2.0	0.03 (PM-1)	0.33 (-)
RS	0.05	0.12	0.05 (RP)	0.05 (-)
RP	0.01	5.2	0.05 (RS)	0.02 (-)
SP	0.56	174.0	5.5 (PM-2)	0.59 (-)
RS	0.25	7.0	0.25 (LS)	0.20 (-)
LS	0.45	11.0	0.25 (RS)	0.55 (-)

\* Reactivity is expressed in c.p.m. per  $1.25 \times 10^6$  lymphocytes. The si in parenthesis in the tuberculin column represents the status of the conventional tuberculin test (0.1 ml. of 1 to 1,000 old tuberculin intracutaneously).  
† Mixed Hodgkin's/normal lymphocyte cultures.

were the same individuals whose cutaneous anergy (depressed delayed hypersensitivity) had been established in an earlier work<sup>9</sup> by failure to accept sensitization with dinitrochlorobenzene. The results are expressed in c.p.m. per  $1.25 \times 10^6$  lymphocytes. Prior autoradiography confirmed the results of others<sup>5,6</sup> that few granulocytes survive in 5-day cultures, and that thymidine incorporation is solely within lymphocytes.

A small but measurable rate of incorporation of thymidine is seen in the unstimulated (control in Table 1) normal cultures which is increased fifty- to three hundred-fold on addition of phytohaemagglutinin (0.1 ml. Bacto-phytohaemagglutinin 'M', Difco Laboratories, Detroit, Mich. Resting incorporation by cells of a particular individual on repeat determinations displays some variability. Lymphocyte reactivity (thymidine incorporation) is markedly stimulated in mixed cultures of normal human lymphocytes over that of the individual normal control and PPD ( $2 \times 10^{-4}$  mg of purified protein derivative of tuberculin, Lilly) stimulates thymidine incorporation in individual normal cultures. While PPD stimulation correlates with tuberculin status, it is not certain that the stimulation is antigen-specific since the group of individual investigated is small (preliminary results suggest that for individuals with no history of prior antigen exposure typhoid vaccine stimulated the lymphocytes).

The response pattern of lymphocytes from anergic Hodgkin's disease patients is variable. Some show a normal response (resting, PHA, mixed culture and antigen stimulation), but most Hodgkin's individuals display depressed lymphocyte reactivity under one or more of the conditions under investigation. Thus the lymphocyte of 6 of the 10 Hodgkin's patients show depressed reactivity to PHA, and 3 of the 5 mixed Hodgkin's lymphocyte cultures show little stimulation. Likewise, the degree of PPD stimulation was minimal in the Hodgkin's lymphocytes with one exception. It is of interest that Hodgkin's lymphocytes tend to be unreactive in a number of circumstances. Since in this condition there is a defect in delayed hypersensitivity<sup>8</sup> and since there is a considerable body of evidence that lymphoid cells are concerned with the mediation of this form of immunological responsiveness<sup>1-4</sup>, this finding is not unexpected. The results are also in keeping with observations, to be reported separately.

tely, that local skin reaction following transfer of Hodgkin's lymphocytes is depressed<sup>10</sup>. Though details of the mechanism remain obscure, the immunological defect of Hodgkin's disease does appear to reside in the lymphocyte.

The technique described here offers a simple quantitative method for the evaluation of human lymphocyte reactivity. Without a substantial body of data correlating *in vitro* lymphocyte reactivity with specific sensitivity of the donor individual, the immunological significance of the former must remain in doubt. The published data at present available are inconclusive in this respect<sup>6,11</sup>. It is evident that mitosis, deoxyribonucleic acid synthesis, or large cell transformation of immunologically competent cells, cannot be equated to immunological reactivity; that is, immunologically competent cells can react to non-immunological stimuli. This conclusion in no way negates the importance of information to be gained from *in vitro* investigations of human lymphocytes, or the great significance of cell proliferation in immunological reactivity<sup>12</sup>; it merely indicates that the new information so gained must be carefully integrated into the existing body of immunological data. The technique described will perhaps make this process less difficult.

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## PATHOLOGY

### Lack of Diurnal Variation in Tritiated Thymidine Labelling Index of Human Leukaemic Blast Cells

VARIOUS renewal systems of mammalian cells exhibit diurnal variations in proliferative activity as evidenced by rhythmical fluctuations in mitotic index and labelling index (percentage of cells labelled) after 'flash-labelling' with tritiated thymidine. In other tissues, it is not possible to demonstrate such variations<sup>1-4</sup>. In general, it appears that diurnal variations in proliferative activity are found in cell systems with a low rate of cell renewal but not if the cell renewal rate is high. In tumours, diurnal variations in mitotic index have been reported<sup>5</sup>, but in other investigations such fluctuations have not been demonstrated<sup>1,6</sup>. In some animal tumours, the specific activity of deoxyribonucleic acid (DNA) after tritiated thymidine labelling is higher at night than during the day<sup>7</sup>, thus indicating diurnal changes in rate of DNA-synthesis per cell, changes in fraction of total cells in DNA-synthesis, or both.

The purpose of the work recorded here was to determine whether there is any diurnal variation in the proliferative activity of human leukaemic cells. The existence of such

variations would imply partial reinterpretation of data on the kinetics of leukaemic blast cells after labelling with tritiated thymidine *in vivo*, since in these investigations<sup>8</sup> the tracer was injected in the morning hours. Moreover, anti-leukaemic chemotherapy might be rendered more effective if medication was scheduled to yield maximal drug concentration during possible peaks of DNA-synthesis.

Four patients with untreated acute leukaemia were studied. From each patient, five blood samples were drawn 6 h apart during a 24-h period. 3-5 ml. of blood was immediately transferred to a test-tube containing 50 i.u. of heparin (containing no preservative), 1.0 ml. of 6 per cent dextran in saline and 2  $\mu$ c. of tritiated thymidine (1.9 c./mmole, Schwarz BioResearch, Inc.). The tube was incubated at 37° C for 50 min. Then the supernatant plasma was removed and briefly centrifuged to obtain a white cell concentrate. After exposure for 1 h to tritiated thymidine, smears of the cell concentrate were prepared; these were fixed in absolute methanol and processed for autoradiography with Kodak 'NTB-2' emulsion. After exposure for 1 week, the slides were developed and stained with Giemsa at pH 5.75. All slides from one experiment were processed simultaneously. Cells with five grains or more were considered labelled. Since the autoradiographs were deliberately over-exposed, most labelled cells had 50 grains or more, and overlap between 'labelling' due to background and true labelling did not occur. At least two slides from each sample were evaluated, and the percentage of labelled cells was determined from a total count of 1,000-4,000 blast cells. All counts were made by one observer on slides which were coded until the counting had been completed.

Table 1

J.J. (F), 52y, ALL N = 2,000		A.N.J. (M), 68y, AML N = 1,000		G.B. (M), 54y, AML N = 4,000		E.M. (M), 56y, AML N = 2,000	
Time	Labelled (%)	Time	Labelled (%)	Time	Labelled (%)	Time	Labelled (%)
8:40	2.0	9:45	6.8	18:00	1.7	20:30	6.5
14:30	1.7	15:45	6.2	23:50	1.8	2:30	6.0
20:25	1.6	21:40	6.0	5:40	1.8	8:40	6.0
2:15	2.2	3:30	6.1	11:55	2.1	14:30	6.3
8:20	1.6	9:15	6.6	17:55	2.1	20:25	6.5

Percentage of circulating leukaemic blast cells labelled with tritiated thymidine *in vitro* at various times of the day. Patients' initials (italics), sex (in parenthesis), age and type of leukaemia are included. ALL=acute lymphoblastic leukaemia, AML=acute myeloblastic leukaemia. N=number of blast cells counted at each time interval.

It can be seen from Table 1 that the labelling index of leukaemic blast cells in the blood shows no diurnal variation. Similar investigations on bone marrow blast cells have not as yet been possible because of patients' reluctance to submit to repeated bone marrow aspirations. An interesting difference between blast cells in the marrow and in the blood is that, with few exceptions, the former have a higher labelling index than the latter<sup>8,10</sup>. It is improbable, however, that this is a fundamental difference between blast cells in different sites. The most likely explanation of the observed difference is that, in part, leukaemic blast cells have lost the capacity to divide further and that the non-proliferating cells are preferentially released from the marrow, similar to the conditions pertaining to normal haemopoiesis. In this way the ratio of non-proliferating:proliferating cells becomes higher in the blood than in the marrow, which explains the observed differences in labelling indices without invoking differences between proliferating cells in various sites<sup>9</sup>. Experimental evidence exists to show that human leukaemic blast cells can enter the blood before going into mitosis after they have been labelled with <sup>3</sup>H-thymidine extravascularly, that is, they enter during the latter part of their DNA-synthesis period or during the G<sub>2</sub>-period<sup>8</sup>. Together with the fact that DNA-synthesizing blast cells are present in the blood, this indicates that cells can enter the blood while they are traversing the generative cycle.

Therefore one would expect that significant variations in the labelling index of bone marrow blast cells would be reflected by the labelling index in the blood. It is likely, then, that the lack of diurnal variation in the labelling index applies to all leukaemic blast cells irrespective of where they are located.

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### Pathological Excretion of 4-Hydroxy-3-methoxyphenyllactic Acid

ONE of the 'spots' frequently appearing on paper chromatograms of the phenolic acids in human urine has been examined in these laboratories for some six years, since it was recognized as being of complex nature. Normally it appears to consist of a mixture of 4-hydroxy-3-methoxy- and 3-hydroxy-4-methoxyphenylhydracrylic acids, both probably largely derived from dietary chlorogenic acid, the latter isomer sometimes being observed in relatively large amounts following the consumption of citrus fruits containing the flavonoid hesperidin<sup>1</sup>. However, at least two other strongly acidic substances having rather similar  $R_F$  values have occasionally been detected, particularly in extracts prepared from urines which have been refluxed with acid in order to destroy the hydracrylic acids. The chromatographic properties of these compounds are recorded in Table 1.

Table 1. CHROMATOGRAPHIC PROPERTIES OF 3 PHENOLIC ACIDS

	3-Hydroxy-4-methoxyphenyl-hydracrylic acid	Compound A	Compound B (4-hydroxy-3-methoxyphenyl-lactic acid)
$R_F$ in anisole-acetic acid-water (70:29:1)	0.34	0.33	0.34
$R_F$ in benzene-propionic acid-water (125:72:3)	0.15	0.12	0.14
$R_F$ in dioxan-methyl isobutyl ketone-pyridine-water (40:40:12:20)	0.44	0.30	0.32
$R_F$ in isopropanol-ammonia-water (8:1:1)	0.31	0.35	0.35
Colour with diazotized <i>p</i> -nitroaniline	Violet-blue	Blue	Grey
Colour with diazotized sulphanilic acid	Red-orange	Violet-red	Violet-red
Colour with 2:6-dichloro-quinone chloroimide-borax	Blue	Blue	Negligible

Compound A was investigated in a case of neuroblastoma in which it was excreted in relatively large quantity. A similar spot has been detected in only one other subject, a case of post-operative stress maintained on a noradrenaline drip. The possibility that the substance is a drug metabolite cannot be excluded.

Compound B was particularly prominent in the case of malignant phaeochromocytoma previously reported<sup>2</sup>. Its chromatographic properties are identical with those of 4-hydroxy-3-methoxyphenyllactic acid, itself conveniently isolated as its sodium salt after reduction of

4-hydroxy-3-methoxyphenylpyruvic acid in aqueous ethanol with sodium borohydride. The acid was detected in small amount, in only one other of 30 phaeochromocytoma urines examined. However, this metabolite 3:4-dihydroxyphenylalanine was also detected readily in one case of neuroblastoma and small amounts appear to be excreted in 5 of 10 further cases of this condition (cf. ref. 3).

A variety of pathological urines have been examined for the presence of 4-hydroxy-3-methoxyphenyllactic acid using diazotized *p*-nitroaniline for its detection after paper chromatography<sup>4</sup> of extracts of acid hydrolysed urine equivalent to 1 min excretion or to 1 mg of creatinine excreted. The acid was thus detected in 2 cases of cancer one of gross kidney deficiency and one of rheumatoid arthritis in a total of 72 subjects. The foregoing urines were selected, usually because abnormalities in phenol acid excretion were revealed, from a much larger series which were examined without recourse to acid treatment many such urines did not contain interfering hydracrylic acids, but in none of these was the lactic acid detected. It has not yet been encountered in any normal urine or in urines from subjects under stress. Nevertheless its presence in traces in all urines may be inferred since the closely related 4-hydroxy-3-methoxyphenylpyruvic acid appears to be invariably present in small quantities detectable as the derived hydantoin formed by condensation with urea under acid conditions. However, excretion of the lactic acid in readily detectable amount seems to be a very uncommon phenomenon which is particularly if not invariably, associated with the abnormal production of 3:4-dihydroxyphenylalanine; it also appears to be usually associated with high excretion of *p*-hydroxyphenyllactic acid.

Allowance for possible confusion with compound B should be made if 4-hydroxy-3-methoxyphenyllactic acid is to be detected on paper chromatograms with diazonium reagents only. In particular, the grey colour obtained with diazotized *p*-nitroaniline (Table 1) may be distinctly blue if the coupling is not carried out under alkaline conditions. Compounds identified as the lactic acid have been reported to yield 'blue-violet'<sup>5</sup> or 'light blue' colours by other workers.

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### Enhancing Effect of Thymectomy on Hepatotumorigenesis in Swiss Mice following Neonatal Injection of 20-Methylcholanthrene

UNTIL recently, liver has proved refractory to tumour induction by carcinogenic hydrocarbons. Klein<sup>1</sup> reported in 1959 that the increased incidence of liver tumours was attained by initiating repeated oral administration of 3-methylcholanthrene in suckling mice 7-9 days of age. Kelly and O'Gara<sup>2</sup> also noted that liver tumours were found in 6 of 11 male C3H mice after a single subcutaneous injection of 3-methylcholanthrene at birth.

Many tumours induced by chemical carcinogens are antigenic in the syngeneic and even autochthonous hosts<sup>3</sup>. This led to the concept, as noted by Miller *et al.*<sup>4</sup>, that "tumour progression might be possible only when the host's mechanism for homograft immunity is either poorly developed or depressed as a result of irradiation or of the chemical carcinogen itself". Depression of immun

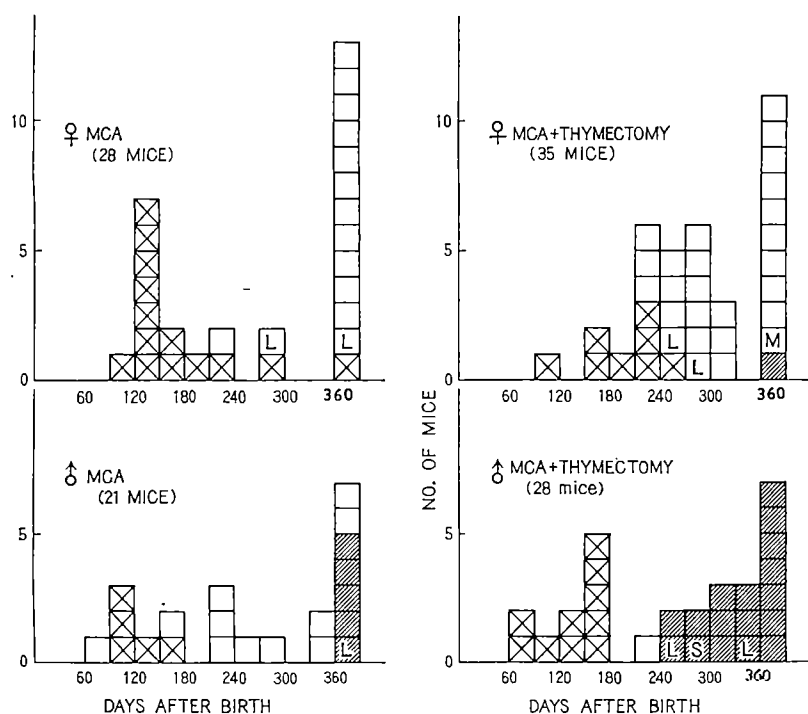


Fig. 1. Distribution of tumours in thymectomized and non-thymectomized mice injected with 20-methylcholanthrene at birth. Liver tumour; Subcutaneous sarcoma; Lymphoma; Mammary tumour; Liver tumour + lymphoma; Liver tumour + subcutaneous sarcoma.

sponse is noticed in animals exposed to carcinogenic chemicals<sup>8-9</sup>. The development of a normal immune system is dependent on the function of the thymus. The absence of the thymus during the postnatal period produces a depression of immunological reaction<sup>9-11</sup>. Because the thymus is involved in the immune reaction, it might be expected that the incidence and growth-rate of tumours would be changed in thymectomized animals. Miller *et al.*<sup>5</sup> reported that skin tumours induced by 4-benzopyrene appeared more frequently and rapidly, and became malignant in higher frequency, in mice that were thymectomized 3 days after birth than in sham-operated mice. Perri *et al.*<sup>12</sup> also demonstrated that transplantable Jensen sarcoma grew at a significantly lower rate in neonatally thymectomized rats than in normal rats. It was therefore decided to examine the effect of thymectomy on the induction of liver tumours by carcinogenic hydrocarbons.

A total of 112 new-born Swiss mice bred in this laboratory were used. Each mouse received one injection of 0.1 µg of 20-methylcholanthrene (MCA) within 24 h of birth. MCA, dissolved in 0.03 ml. of 1.0 per cent gelatine solution, was injected into the subcutaneous tissue of the terascapular area. The mice were weaned at 25 days and segregated according to sex. Sixty-three mice were

thymectomized at the age of 35–55 days. The remaining 49 mice were used as non-thymectomized controls. Another 79 mice without MCA-injection were used as non-treated controls. When animals were moribund from development of subcutaneous sarcoma at the site of injection or other causes, they were killed. Survivors were killed at 360–365 days of age. All the animals were autopsied and the different organs carefully examined for macroscopic tumours. The final diagnosis in every instance was based on microscopic examination. At autopsy, the liver was inspected for tumour nodules and the number of nodules visible on the surface was recorded. No tumour was included which did not measure at least 2 mm in diameter. The lungs, after fixation in formalin solution, were inspected for tumour nodules under a magnifying glass ( $\times 10$ ), and the number of surface nodules per lung was recorded. Then the lungs were cut into slices about 1 mm in thickness, and inspected for tumour nodules not detected on surface inspection.

Different types of tumours, including liver tumour, pulmonary adenoma, lymphoma, and subcutaneous sarcoma at the site of injection, were observed among the thymectomized and non-thymectomized mice treated with MCA at birth (Table 1 and Fig. 1).

Liver tumours developed in one male mouse among 79 non-treated control mice of both sexes, and in 5 among 21 non-thymectomized male mice killed at 360–365 days of age. In the thymectomized mice, liver tumours were observed in 17 of 28 males, and, in sharp contrast, only 1 of 35 females. All the males surviving more than 244 days of age bore liver tumours. None of 11 males that died or were killed before 225 days had liver tumours; 10 mice died of subcutaneous sarcomas, and one huge pulmonary adenomatosis. Liver tumour in one female mouse was solitary and rather small. In most instances liver tumours appearing in the males were multiple, an average of 5.3 per mouse. The fact that male mice are more susceptible to the induction of liver tumour than female mice has previously been reported in hepatotumorigenesis following exposure of infant and new-born mice to 3-methylcholanthrene<sup>1</sup>, urethan<sup>13,14</sup>, or 2-acetylaminofluorene<sup>15</sup>. This is true both in thymectomized and non-thymectomized groups after a single injection of MCA at birth. Among 17 males with liver tumours, 8 were killed or died, mostly due to weakness and dyspnoea, probably caused by the development of multiple pulmonary adenomas, 2 had liver tumours combined with lymphoma of node origin, and one bore subcutaneous sarcoma at the site of injection. The remaining 7 males were killed at 360–365 days of age and all had liver tumours. It seemed

Table 1. TUMOURS IN SWISS MICE GIVEN 20-METHYLCHOLANTHRENE (MCA) WITHIN 24 H OF BIRTH

Group	Sex	Total No. of mice	No. of mice with tumour					No. of mice without tumour
			Subcutaneous sarcoma	Liver tumour	Pulmonary adenoma	Lymphoma	Mammary tumour	
Non-injected control	M	36	0	1 (1.0)*	14 (1.7)†	3	0	19
	F	43	0	0	24 (1.6)	6	4	12
MCA-injected	M	21	0	5 (2.2)	21 (28.3)	1	0	0
	F	28	14	0	27 (37.2)	2	0	1
MCA-injected + thymectomy	M	28	11	17 (5.3)	28 (50.4)	2	0	0
	F	35	8	1 (1.0)	35 (59.9)	2	1	0

\* Mean tumour count (total number of liver tumours/total number of mice bearing liver tumour).

† Mean nodule count (total number of pulmonary adenomas/total number of mice bearing pulmonary adenoma).



clear that all males after combined treatment (MCA-injection plus thymectomy) developed liver tumours unless they died of subcutaneous sarcoma or pulmonary adenomatosis. The gross and microscopic appearance of liver tumours was similar to spontaneous liver tumours usually described as hepatomas, which appeared sporadically in many strains of mice<sup>16</sup>. Metastatic foci of liver tumours have not so far been observed. Transplantation was not attempted.

Pulmonary adenoma is one of the tumours most frequently induced in mice following neonatal administration of carcinogenic hydrocarbons<sup>1,2</sup>. In the present investigation, all the mice treated with MCA had multiple pulmonary adenomas. The incidence did not differ between the thymectomized and non-thymectomized groups. However, the number of adenomas was larger in the thymectomized group than in the non-thymectomized group. Because the number of adenomas is related to age<sup>2</sup>, more extensive data are needed to show whether thymectomy has a promoting effect on the development of induced pulmonary adenomas. No suggestion concerning the promoting effect of thymectomy on the development of either subcutaneous sarcoma or lymphoma has so far been obtained.

The evidence that tumours possess tumour specific antigenicity has been reported from other laboratories<sup>3,4</sup>. The data reported by Prehn<sup>6</sup> show that MCA, when given to animals in the usual way for tumour production, can indeed interfere with a weak immune reaction. Thymectomy performed shortly after birth is followed by a marked depression of immunological reaction during the postnatal period, including the skin homograft rejection mechanism<sup>9-11</sup>, and morphologically, by a severe depletion of the development of lymphatic tissue<sup>9-11,17</sup>. The regression of lymphatic tissue is also produced in mice thymectomized at 4-6 weeks of age<sup>18</sup> and at 35-45 days<sup>19</sup>. Therefore, it may be assumed that the combined treatment, MCA-treatment plus thymectomy, exhibits synergism in depressing the immune response which, in turn, facilitates the early and frequent development of spontaneous liver tumours. It may also be possible that the latent tumour cells produced by an action of MCA grow faster and form visible tumours in the host with a weakly depressed immune response resulting from thymectomy. It may be that interference with the immune mechanism plays an important part in tumorigenesis.

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## BIOLOGY

### Annual Cycle in the Size of the Gut of the Purple Sea Urchin, *Strongylocentrotus purpuratus* (Stimpson)

SEVERAL species of sea urchins undergo an annual reproductive cycle with a seasonal enlargement and shrinkage of the gonads<sup>1</sup>. In connexion with investigations on the annual reproductive cycle of the purple sea urchin, *Strongylocentrotus purpuratus*, it was found that the gut of the urchin also undergoes a seasonal cycle in size.

Twenty urchins were collected monthly from a tide pool near Yankee Point, California (36° 33' N., 121° 57' W.). The gut, exclusive of the pharynx, was dissected from each urchin, washed free of its contents, drained of free water and weighed. The gonads were also removed, drained and weighed. Both organs were then dried and reweighed to determine their water content. The relative sizes of the gut and gonads to the entire urchin were expressed as the gut index ( $\frac{\text{wet weight of gut}}{\text{wet body-weight}} \times 100$ ) and gonad index ( $\frac{\text{wet weight of gonad}}{\text{wet body-weight}} \times 100$ ) respectively.

The entire cycle in the size of the gut can be seen in Fig. 1. In both sexes the gut index increases in the summer, reaching a maximum in the autumn, and then decreases, reaching a minimum in the winter. This change did not result from changes in the water content of the gut tissue as the percentage of water remained constant throughout the year (Table 1).

The variation in the gut index can possibly be correlated with the amount of food ingested by the urchin. A seasonal change in the feeding behaviour of the urchin *Strongylocentrotus intermedius*, which consumes more food during the spring, has been described by Fujii<sup>2</sup>. Seasonal fluctuations in the amount, kind, or composition of marine algae available to the urchins in the intertidal region may also be factors.

Supporting the interpretation that the food consumption of the urchin affects the size of the gut are the data in Fig. 1, which show that urchins starved in the laboratory have low gut indices, and that continuous feeding of previously starved animals on the brown alga *Macrocyst pyriformis* can stop the decline in the size of the gut.

The histogram (Fig. 2) demonstrates that the decrease in the gut indices of starved animals is due to a decrease

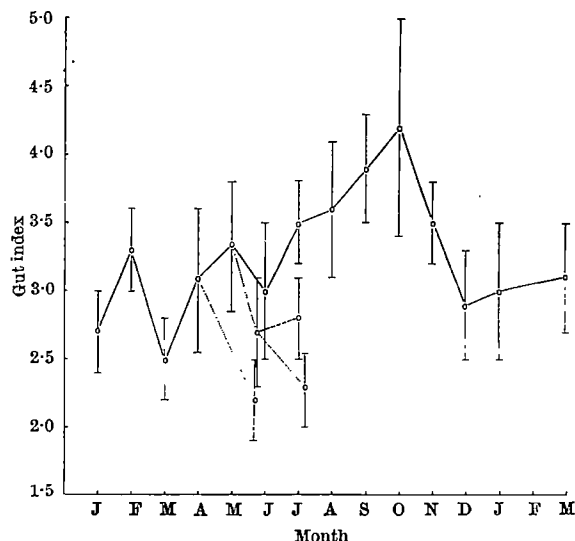


Fig. 1. Seasonal variation (January 1963 to March 1964) and effect of starvation and feeding on the gut index of *S. purpuratus*. The points and vertical lines represent mean and  $\pm 1$  S.D. —, Field animals; . . . . ., starved animals; - - - - -, fed animals

Table 1. PERCENTAGE WATER CONTENT OF THE GUT TISSUE OF *S. purpuratus* FROM JANUARY 1963 TO JANUARY 1964 AND OF THE GROUP THAT WAS STARVED FROM MAY TO JUNE 1963

Month	Mean % water content of gut	$\pm 1$ S.D.
J (1963)	75.2	0.5
F	76.9	1.7
M	76.5	1.7
A	77.7	2.3
M	77.5	1.9
J	78.4	1.7
J	77.6	1.9
A	77.4	1.4
S	77.5	1.5
O	78.2	1.7
N	77.2	1.3
D	76.6	1.7
J (1964)	78.6	3.3
Urchins starved May to June (1963)	77.9	1.0

1 the thickness of the epithelial layer lining all major regions of the digestive tract. Fuji<sup>3</sup> concluded from histochemical evidence that the inner epithelium of the stomach and intestine of *S. intermedius* possesses reserves of lipid and glycogen which decrease appreciably after starvation or five weeks. The seasonal cycle in the gut index is also probably due to fluctuations in the thickness of the epithelium lining all regions of the digestive tract.

These results support the contention that the gut, in addition to its digestive and absorptive functions, acts as a storage organ, a suggestion made by previous workers<sup>1,3,4</sup>. Whether the gut plays a part in storing nutrients for transfer to the gonads for gametogenic purposes, as has been indicated for the digestive gland in sea stars<sup>5-7</sup>, is

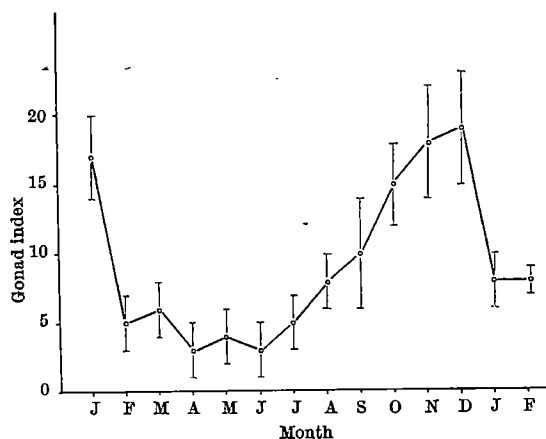


Fig. 3. Seasonal variation (January 1963 until March 1964) of the gonad index of *S. purpuratus*. The points and vertical lines represent means and  $\pm 1$  S.D.

undetermined, but is perhaps suggested by the manner in which the gonad cycle (Fig. 3) lags behind that of the gut.

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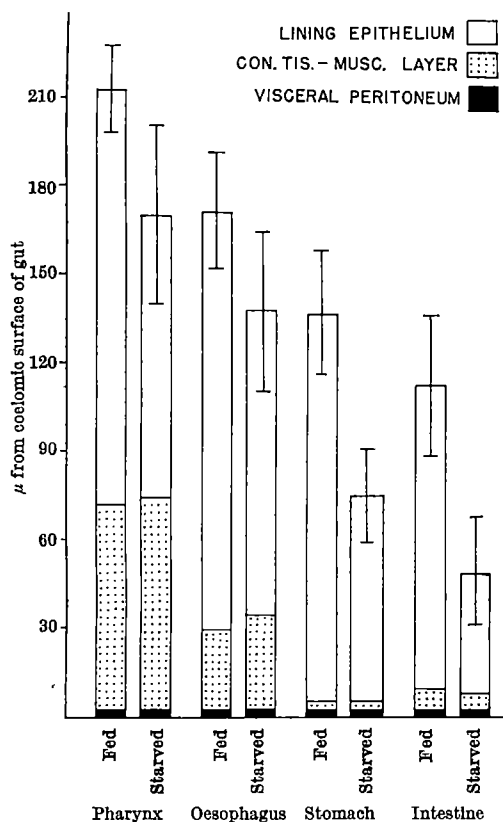


Fig. 2. The effect of starvation and feeding on the thickness of the gut wall of the *S. purpuratus*. The bar on the left for the tall interradial sectors of pharynx, the oesophagus, the first festoon of the stomach, and the third festoon of the intestine represents the average thickness of the gut wall for 10 sea urchins fed *Macrocyrtis* continuously from March 22, 1963, until June 3, 1963; that on the right, the average for 10 sea urchins starved from April 12, 1963, until August 27, 1963. The average weight of the fed and starved urchins was 25.3 and 26.5 g, respectively. Measurements were made on 7  $\mu$  cross-sections of gut fixed in sea-water Boulin's and stained with periodic acid Schiff-haematoxylin. The difference between the fed and the starved group in the thickness of the lining epithelium (shown  $\pm 1$  S.D.) is significant at the 0.01 degree level for all gut regions. There is no significant difference between the fed and the starved groups in the thickness of the connective tissue-muscle layer or visceral peritoneum in any of the gut regions.

## A Beaked Whale New to the Western Hemisphere

A 16-ft. female beaked whale of the genus *Mesoplodon*, family Ziphiidae, drifted ashore at Delmar, about 15 miles north of San Diego, California, on June 10, 1954. One of us (R. M. G.) made body measurements, drawings, and photographs of it, and preserved the skull and jaw. The specimen was presented to the United States National Museum where it is catalogued as No. 298237, but was temporarily retained for examination. Because the specimen could not then be identified to species, this material was laid aside and, in the pressure of other affairs, forgotten. After one of us (J. C. M.), in order to revise the genus *Mesoplodon*, had completed a tour of most museums of the world known to possess specimens, he was invited to investigate this Delmar specimen to include data from it in his revision.

Nishiwaki and Kamiya<sup>1</sup> described a new species of *Mesoplodon* from a specimen stranded on Oiso beach near Tokyo, Japan, on September 22, 1957, naming it *Mesoplodon ginkgodens*. Although those authors<sup>1,2</sup> splendidly documented their specimen with measurements and photographs, they had only one skull of an adult specimen and principally photographs and published measurements to compare it with, and were able to offer no diagnosis. Moore<sup>3</sup> reviewed at length the evidence that invalidates

the first dichotomy of the key that Nishiwaki and Kamiya<sup>2</sup> did present, and has since<sup>4</sup> produced a key to the seven species of *Mesoplodon* inhabiting the northern hemisphere based on original study of 42 specimens in North American museums. The characters of that key which distinguish the species *M. ginkgodens* identify the Delmar specimen as *M. ginkgodens*. (1) When the skull is upright with the long axis of the beak horizontal, the anterior view shows at least one of the pair of premaxillary foramina to open on or below a 'horizontal' plane (paralleling the long axis of the beak) that transects the centres of the maxillary foramina. (2) The length of vomer visible on the palate is greater than 100 mm. (3) In lateral view of the antorbital tubercle, the lacrimal is relatively thick (Pl. III, Nishiwaki and Kamiya<sup>2</sup>) and does not curl up around the anterior end of the frontal bone (Fig. 13, Raven<sup>5</sup>).

This is the first record of *Mesoplodon ginkgodens* for the eastern North Pacific, for North America, and for the western hemisphere. It is the third species of *Mesoplodon* to be known for the western coast of North America, and the seventh species to be known from the coasts of the North American continent<sup>4</sup>.

Deraniyagala<sup>6,7</sup> described a specimen of *Mesoplodon* stranded at Ratmalana, Ceylon, on January 26, 1963, and gave it a new name, *M. hotaula*. He did not compare it with the excellently illustrated details of the type of *M. ginkgodens* published by Nishiwaki and Kamiya<sup>1,2</sup>. Moore has since measured and studied in great detail both the Ratmalana specimen and the type of *ginkgodens*, and finds that the former is like the latter in virtually every feature of the skull except those commonly distinguishing between the sexes in this genus. The foregoing three enumerated characters of *ginkgodens* distinguish the type of *M. hotaula* Deraniyagala, 1963, as another adult female of the species *ginkgodens*.

Since this Ratmalana specimen is from deep in the tropics, the species *ginkgodens* may yet be found in the southern hemisphere, and further diagnosis is needed to distinguish *ginkgodens* from the *Mesoplodon* species of the southern hemisphere. Without especially depleting the revisionary work to come, it seems advisable to provide here the criteria needed for identification of further specimens of *M. ginkgodens* that may be found before the revision becomes available, and we are pleased to provide for *M. ginkgodens* this more complete taxonomic description: (4) The greatest length of the right nasal bone on the vertex of the skull (69, 76 and 81 mm) is more than twice the least distance between premaxillary foramina (30, 35 and 36 mm respectively). (5) The greatest transverse span of the combined premaxillary bones at the midpoint in the length of the beak (45–49 mm) is greater than 40 but less than 60 mm. (6) The greatest width of temporal fossa approximately perpendicular to the long axis of the temporal fossa is less than 60 mm.

**Diagnosis.** The foregoing enumerated six characteristics of *Mesoplodon ginkgodens* distinguish it from all the eleven other species of the genus *Mesoplodon* as follows: from *bidens* by 1, 4 and 5; *densirostris* by 1 and 4; *europaeus* by 2 and 4; *layardi* by 6; *hectori* by 4; *grayi* by 4 and 5; *stejnegeri* by 1, 4 and 5; *bowdoini* by 6; *mirus* by 3 and 5; *pacificus* by 6; and *carlhubbsi* by 1 and 4.

It is surprising that examination by one of us of perhaps 90 per cent of the specimens of *Mesoplodon* known to be in the world's museums in 1963 has revealed no skulls of *ginkgodens* evidently taken earlier than this Delmar specimen, but this note may alert zoologists of the Pacific and Indian Oceans to the probability that more specimens will be found on other shores. Evidence has been shown (Fig. 16, Moore<sup>8</sup>) that two other North Pacific species of the genus *Mesoplodon* appear to occupy partially allopatric ranges, *stejnegeri* from about 60° to 40° north latitude and *carlhubbsi* from about 50° to 30°. Another species, *M. densirostris*, has now been reported for the North Pacific by Galbreath<sup>9</sup> from one immature and one adult female stranded on Midway Island. This species, *densirostris*,

is earlier known<sup>10</sup> from the North Atlantic, the South Pacific, and Indian Oceans, however, and it is the only one of the (twelve) *Mesoplodon* species which is now well established as an inhabitant of temperate and warm areas of oceans on both sides of the equator. Since the species ranges of which are best known, *M. bidens*, *M. europaeus*, *M. stejnegeri*, and perhaps *M. mirus*, from charts Moore<sup>4,8</sup> seem to have ranges restricted to parts of single oceans, some interest now attaches to future occurrence of *ginkgodens* on other shores.

We thank Drs. Masaharu Nishiwaki of the Whal Research Institute, Tokyo, and P. E. P. Deraniyagala, director emeritus of the National Museum of Ceylon, Colombo, for permission and generous facilities to study their type specimens in those institutions, and the U. S. National Science Foundation for grant GB 507 to one of us (J. C. M.), which enabled him to visit Colombo and Tokyo to examine the foregoing type specimens.

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## Gestation Period and Neonatal Weight of the Hyrax

THE literature contains only one record of the gestation period of hyrax, namely, that of Murray<sup>1</sup>, which was for *Procavia capensis* in South Africa. Copulation was observed on April 3 and the nine-year-old female gave birth to a single young on November 21, a gestation period of 7.5 months (232 days), a little longer than a previous estimate of 6–7 months<sup>2</sup>. A recent observation on Mount Kenya hyrax (*Procavia johnstoni mackinderi* Thomas) provides confirmatory evidence. A mature female, which had been in captivity in the company of two males for nine months, showed a rise in rectal temperature at the end of March (1963), reaching a peak of 38.9° on April 6. On April 4 what appeared to be seminal fluid was present at the vulva. The female gave birth to a pair of male young on November 3, giving a gestation period of more than seven months (214 days). This suggests that there is little difference between the gestation periods of *P. capensis* and *P. johnstoni*, especially when the possibility of parturition being delayed in Murray's animal, due to it being a very old primipara, is borne in mind. The probability that the two gestation periods are the same is consistent with the anatomical evidence that all *Procavia* are races of one species<sup>3</sup>.

It is interesting to note that while the date of the birth agrees with Coe's<sup>4</sup> estimate of the breeding season of *P. johnstoni mackinderi* as between August and November the number of young does not fit in with his statement that "no animal apparently ever produces more than one young at a time". It is, however, in line with Van de Horst's experience<sup>5</sup> and my own<sup>6</sup>, for we have both stated that hyrax bears 2 or 3 young per litter. A probable explanation of Coe's observations is that they were made on a part of Mount Kenya where a very high population density reduced the litter size.



Table 1

Wt. of mother	Wt. of litter	Litter wt. $\times 100$		
		Actual	Predicted	Deviation
2,718 g	680 g	25	14.2	10.8

Although the large size of the new-born hyrax has often been commented on<sup>1,6</sup>, there are few records of birth weights. Leitch *et al.*<sup>7</sup> compared pre-mating weights of young adult females with the total weight of the newly born in 114 species of mammals and found that the larger the species the smaller (relatively) the total weight of offspring. They produced the following equation for the prediction of the weight of the young from the weight of a mother:  $N = 0.5408 M^{0.8923}$ , where  $N$  is the neonatal weight and  $M$  the maternal weight. Actual and predicted litter weights are compared with the maternal weight (average for the 6 months before mating) for the *P. johnstoni* female under consideration in Table 1. It will be seen that the actual litter weight is 10.8 per cent (of the maternal weight) greater than predicted, using the foregoing equation. The only wild mammals in the survey of Leitch *et al.* giving a greater positive deviation from the predicted value were two voles (*Microtus oeconomus timmingsi* and *M. arvalis*). The long gestation period (longer than for any other mammal of its size) and high neonatal weight of the hyrax support the palaeontological evidence that the ancestors of the modern hyrax were much larger animals<sup>8</sup>.

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### Absence of Growth Effects of Thalidomide on Higher Plants

THE teratogenic effects of thalidomide (TH),  $\alpha$ -N-phthalimido-glutarimide, are well recognized and have been recorded for a number of animals<sup>2</sup>. The critical period has been found to correspond to the time of morphogenesis<sup>2,3</sup>. These drastic effects in animals raised the question of possible effects on plants. Some positive evidence for effects on lower plants was indicated by the work of Boney<sup>1</sup>, who noted that high concentrations of TH not only inhibited growth but also induced abnormalities in sporangia of a marine red alga, *Callithamnion tetricum* Ag. Giacomello *et al.*<sup>4</sup> tested the activity of high concentrations of TH in embryonic vegetal tissues of the common onion, *Allium cepa* L., and reported alterations in cell mitosis as well as the occurrence of chromosomal aberrations.

A number of naturally occurring phthalide substances bearing structural resemblances to TH are known to act as growth inhibitors of plants<sup>5</sup>. These substances, isolated from *Levisticum officinale* Koch, have inhibited germination and growth of *Lepidium*, rhizoid growth of *Marchantia* and germination of pollen of *Impatiens*. The purpose of the present work was to investigate possible growth-inhibitory effects of TH on a number of higher plants. In order to ascertain the true biological action of TH, the massive doses used by the foregoing workers were avoided.

Table 1. EFFECT OF THALIDOMIDE (TH) AND L-GLUTAMINE ON ELONGATION OF ROOTS OR EXCISED ROOTS OF *Linum*

	Controls (mm)	L-Glutamine $6 \times 10^{-3}$ M (mm)	TH $6 \times 10^{-3}$ M (mm)	L-Glutamine + TH (mm)	L.S.D. 1%
Roots of seedlings	43.9	43.1	15.5	14.3	2.94
Excised roots	12.3	12.7	6.6	6.2	1.46

Table 2. EFFECT OF THALIDOMIDE (TH) AND INDOLYL-3-ACETIC ACID (IAA) ON THE ELONGATION OF OAT COLEOPTILE (mm)

IAA $\mu$ g/ml.	0	0.04	0.04	0.04	0	0	0
TH $\mu$ g/ml.	0	0	0.50	2.50	0.55	2.58	12.90
Length of coleoptile	5.93	6.87	6.81	6.99	5.96	6.09	6.03
L.S.D. 1% 0.51							

Tomato, *Lycopersicon esculentum* Mill., barley, *Hordeum vulgare* L., and soybean, *Glycine max* Merr., seeds were germinated in pots containing quartz sand. The seedlings were thinned and triplicate pots were treated with Hoagland's solution containing TH in the following concentrations: (1) 0; (2)  $1 \times 10^{-5}$  M; (3)  $1 \times 10^{-4}$  M; and (4)  $1 \times 10^{-3}$  M. After daily treatments for 15 days the growth effects were observed and compared.

There was no noticeable response to the treatments by soybeans and very little response by barley except for a slight yellowing of leaf tips in the highest treatments. The most concentrated treatments also produced a slight effect in tomato (see Fig. 1); the plants were generally smaller, the leaves were somewhat mottled and leaf margins were curled.

Because of the known effect of TH on animal embryonic tissue, its action was investigated on tomato flowers in all stages of development and on the fruit. Spray-treatment was continued for three consecutive days with a 1:1 water:acetone solution of TH and each plant received 100 mg of TH per day. Regardless of the stage of flower development when treated, TH did not affect fruit development. In addition, the seeds from TH-treated tomato plants germinated normally.

Seeds of *Lepidium* and *Linum* were placed on filter papers in Petri dishes, moistened with several concentrations of aqueous TH and set aside to germinate. For both species the seeds germinated in the same length of time except in the case of the highest concentration of TH ( $6 \times 10^{-3}$  M). For the latter it was found that germination was delayed for a period of one day, but the percentage of seeds that germinated was unaffected.

The effect of TH on root growth of flax was tested on seedlings which had previously been germinated in water and afterwards treated with aqueous solutions of TH. After 2 days the following root-lengths (average of 4 replicates) were found: control, 40.4 mm;  $1 \times 10^{-4}$  M, 39.9 mm;  $1 \times 10^{-3}$  M, 38.6 mm;  $6 \times 10^{-3}$  M, 19.2 mm. Since the least significant difference (L.S.D.) at the 1 per cent level was 4.3, the  $6 \times 10^{-3}$  M concentration caused a significant retardation of root elongation.

The effects of TH in inhibiting the elongation of oat coleoptile were checked by co-treatment with indolyl-3-acetic acid which is known to cause such elongation. Even when the relative concentration of TH:indolyl-3-acetic acid was as high as 40:1 such inhibition was not observed (Table 2).



Fig. 1. Effect of thalidomide (TH) on growth of tomato seedlings. From left to right: control,  $1 \times 10^{-5}$  M,  $1 \times 10^{-4}$  M and  $1 \times 10^{-3}$  M of TH



Boyle *et al.*<sup>2</sup> reported that L-glutamine gave complete protection against abnormalities in chick embryos caused by certain derivatives of TH. The results in Table 1 show that co-treatment with L-glutamine produced no similar effects on seedlings or excised roots of *Linum*.

The work reported here demonstrates that TH generally does not affect the growth of higher plants. Although it is possible that it causes some slight threshold effects, the concentrations of TH required for this are about two orders of magnitude higher than in the case of certain structurally related naturally occurring phthalides.

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## ENTOMOLOGY

### Detection of Lipids in the Honeydew of an Aphid

NUMEROUS analyses have been reported concerning the chemical composition of honeydew produced by many homopterous insects, especially members of the family Aphididae. Such compounds as fructose, glucose, sucrose and free amino-acids seem to be universally present in honeydew. Various other mono-, di-, and oligo-saccharides are occasionally found in honeydew. Proteins are apparently absent, although peptides have been reported. Miscellaneous compounds found in honeydew include several organic acids, inorganic ions, sugar alcohols and auxins, such as indolyl-3-acetic acid<sup>1</sup>. With one exception<sup>2</sup>, no reports have been located making reference to the presence of lipid material in honeydew, although it is well known that certain lipids (for example, sterols) are of great physiological importance to insects.

Between 150 and 200 mg of honeydew were collected by placing glass plates (which had been sprayed with 'Merthiolate') for 24 h under colonies of *Myzus persicae* (Sulzer) feeding on Chinese cabbage, *Brassica campestris*. The honeydew was washed from the plates with tepid water and extracted from the aqueous solution with chloroform. After evaporation of the solvent under reduced pressure and purifying the crude extract<sup>3</sup>, the amount of lipid material present in honeydew on a dry-weight basis was found to be 0.60 per cent. The purified extract was chromatographed on a silicic acid column<sup>4</sup> and the neutral lipid fraction purified by chromatography on a 'Florisil' column<sup>5</sup>. Tentative identification of the lipid classes was made by comparing the emergence of unknowns from the 'Florisil' column with known standards, and by co-chromatography of the 'Florisil' eluates with known compounds on thin-layers of silica-gel G in various solvent systems<sup>6</sup>. Free sterols and sterol esters were identified by the *L-B* reaction and by spraying the thin-layer plates with stannous chloride<sup>6</sup>. None of the thin-layer chromatography reported here was quantitated, although visual estimates of the relative amounts of the different classes of lipids were made. No work was carried out on the polar lipids eluted from the silicic acid column.

Using the foregoing methods, the major class of lipids in the honeydew samples was found to be the free fatty-acids. Gas-liquid chromatography of these free fatty-acids<sup>2</sup> revealed that palmitic acid accounted for 44 per cent of

the total. Free sterols were readily detectable in honeydew, although sterol esters were absent. Triglycerides were present, but only in minor amounts. Mono- and diglycerides could not be identified with certainty. Hydrocarbons and pigments were abundant and squalene was present in trace amounts. Two major unidentified fractions and numerous minor ones were present in all samples. The major fractions had chromatographic properties similar to hydroxy fatty acids.

The origin of the lipids found in the honeydew is not known, but it is suspected that at least some of them come directly from the host plant. A distinct correlation existed between the physiological condition of the host and the honeydew lipids. Aphids feeding on a senescent plant produced honeydew rich in hydrocarbons and pigments, but containing little or no free sterols; the reverse was true for aphids feeding on young plants.

I thank Prof. H. J. Vonk for allowing me to carry out this investigation in the Laboratory of Comparative Physiology, University of Utrecht, the Netherlands.

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## VIROLOGY

### Further Observations on the Effect of 6-Aminonicotinamide on Chick Embryo Tissue Cultures infected with Vaccinia and other Viruses

I HAVE recently described the inhibitory effect of 6-aminonicotinamide (6-AN) on the growth of vaccinia virus in the chick embryo fibroblast (CEF) host cell system<sup>1</sup>. When CEF cultures were treated with 6-AN at a dose of 5 µg/ml. for an interval of 8 h at 36° C, it was found that 6-AN rendered the cells refractory to infection with vaccinia virus. This effect increased as the time of exposure to 6-AN lengthened up to 8 or 10 h before virus infection. Evidence was also obtained to show that the inhibitory effect produced by 5 µg/ml. of 6-AN could be reversed by the simultaneous addition of 5–50 µg/ml. of nicotinamide, but not if it was added at a later time. The drug did not seem to exert a direct lethal effect on the virus.

Details of techniques, including the propagation of vaccinia virus, preparation of CEF cultures and a simple fluid-overlay plaque assay technique employed throughout the experiment have been described previously<sup>1</sup>. Influenza A virus (strain 'MEL') and influenza B virus (strain 'GL') were grown in the allantoic cavity of embryonated eggs. The [strain of herpes simplex virus was one originally isolated in primary human amnion cell cultures derived from a recent case of fatal encephalitis and was propagated in HA-FL cells. Sindbis virus was grown in CEF cultures.

6-AN when diluted in Hanks's balanced salt solution (BSS) to yield a 5 µg/ml. concentration was introduced to CEF cultures grown in 19 × 25 mm Leighton tubes for periods of 6, 4, 3, 2 and 1 h before infection with vaccinia virus; and simultaneously with addition of virus; and 6, 4, 3, 2 and 1 h after addition of virus. 6-AN was also added to cultures 6 and 8 h after virus adsorption up to termination of the experiment. The cultures were washed three times with BSS before and after the addition of 6-AN

Table 1. EFFECT OF ADDITION OF 6-AN AT TIME INTERVALS, ON VACCINIA GROWTH ON CEF CULTURES

Time before infection (h)					Infection (h) *	Time after infection (h)								Virus titre at 20 h P.F.U./ml.	% of control
-6	-4	-3	-2	-1		1	2	3	4	6	8				
M	M	M	M	M	Vaccinia	M	M	M	M	M	M	M	M	$1.3 \times 10^4$	100.0
6-AN	6-AN	6-AN	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	M	$1.0 \times 10^4$	0.77
M	6-AN	6-AN	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	M	$1.5 \times 10^4$	1.1
M	M	6-AN	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	M	$2.0 \times 10^4$	1.5
M	M	M	6-AN	6-AN	Vaccinia	M	M	M	M	M	M	M	M	$2.6 \times 10^4$	2.0
M	M	M	M	6-AN	Vaccinia	M	M	M	M	M	M	M	M	$1.0 \times 10^4$	7.7
M	M	M	M	M	Vaccinia + 6-AN	M	M	M	M	M	M	M	M	$2.0 \times 10^4$	15.4
M	M	M	M	M	Vaccinia	6-AN	M	M	M	M	M	M	M	$2.0 \times 10^4$	15.4
M	M	M	M	M	Vaccinia	6-AN	6-AN	M	M	M	M	M	M	$2.9 \times 10^4$	2.2
M	M	M	M	M	Vaccinia	6-AN	6-AN	6-AN	M	M	M	M	M	$2.5 \times 10^4$	1.9
M	M	M	M	M	Vaccinia	6-AN	6-AN	6-AN	6-AN	M	M	M	M	$1.6 \times 10^4$	1.2
M	M	M	M	M	Vaccinia	6-AN	6-AN	6-AN	6-AN	6-AN	M	M	M	$1.5 \times 10^4$	1.2
M	M	M	M	M	Vaccinia	M	M	M	M	6-AN	6-AN	6-AN	6-AN	$9.1 \times 10^3$	70.0
M	M	M	M	M	Vaccinia	M	M	M	M	M	6-AN	6-AN	6-AN	$1.1 \times 10^4$	84.6

\* Immediately after exposure to virus for 1.5 h with a virus input of 2-10 P.F.U./cell at 36° C, the cell cultures were washed and re-fed with the corresponding medium. Samples were taken from each group and assayed for the amount of virus adsorbed. Results showed that  $1.0-1.2 \times 10^4$  P.F.U./ml. of virus had been adsorbed per culture.

M = Hanks's BSS.

at each time interval as well as after virus adsorption. At the end of the experiments, the corresponding cultures were collected. Infectivity was determined by plaque-forming units (P.F.U.) on CEF cultures set up in screw-capped tubes, using a fluid-overlay plaque assay technique. Table 1 demonstrates that the 6-AN does not affect virus adsorption, or prevent the release of virus. It is interesting to note that 6-AN displays only a slight difference in the extent of inhibition on growth of vaccinia virus in the CEF cultures, when the latter were exposed to 6-AN for the corresponding period of time before and after a period of 1.5 h of virus adsorption. 6-AN appears to have no marked demonstrable effect when added 6-8 h after virus adsorption.

Single-cycle growth curve experiments were performed in 6-AN-treated (5  $\mu$ g/ml. in BSS for 8 h at 36° C) and untreated CEF cultures. The cultures were infected with stock vaccinia virus with an input multiplicity of 2-10 P.F.U./cell. After 2 h adsorption, the cultures were washed three times and re-fed with BSS. At time intervals, two cultures were removed from the incubator and the content of intracellular virus was measured by P.F.U. as before. The data, as shown in Fig. 1, reveal that the vaccinia virus multiplies at different rates in the two types of cul-

tures. It would seem that in treated cultures the eclipse phase was not as marked as that observed in untreated control cultures. This result suggests that only a small proportion of the adsorbed virus may participate in the eclipse period and so replicate in the treated cultures. By using fluorescence microscopy and acridine orange staining techniques<sup>2</sup>, it was also demonstrated that the development of vaccinia virus intracytoplasmic inclusions was markedly suppressed. The mode of action of 6-AN on the growth of vaccinia virus in CEF cultures is obscure and awaits further investigation.

Additional investigations on the effect of 6-AN on other viruses in CEF cultures indicated that 6-AN also exerted a delaying effect on the growth of influenza B virus but not the influenza A virus. Cultures were observed microscopically for cytopathic effect and assayed by standard haemagglutination procedures. Similarly, no evidence of significant inhibitory effect was revealed in the case of herpes simplex and Sindbis viruses.

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<sup>1</sup> Lee, S. H. S., *J. Bacteriol.*, **88**, 885 (1964).

<sup>2</sup> Armstrong, J. A., *Exp. Cell Res.*, **11**, 640 (1956).

### Relationship between Guanidine-dependence and Neurovirulence of Poliovirus

GUANIDINE-resistance<sup>1</sup> and guanidine-dependence can be induced *in vitro* in poliovirus<sup>2,3</sup>. We have recently demonstrated that both characters can be easily conferred and withdrawn in *in vitro* experiments<sup>4,5</sup>. Moreover, we have demonstrated that guanidine-dependent viruses are not neurovirulent for monkeys<sup>6</sup>. In the present report we show that neurovirulence (of the type producing paralysis) for monkeys and guanidine-dependence *in vitro* are two strictly related viral properties.

Indeed, although guanidine-dependent poliovirus is not neurovirulent for monkeys, it reacquires this character when guanidine-dependence is reversed. The techniques used are described in ref. 7. A strain of virulent Mahoney poliovirus isolated from the spinal cord of a monkey was cloned twice and was made guanidine-dependent *in vitro* by sub-culturing it in the presence of increasing concentrations of guanidine. When this strain had acquired a high degree of guanidine-dependence, it was sub-cultured in decreasing concentrations of guanidine and finally in the absence of guanidine. The original viral strain and the two strains obtained with the foregoing procedures were injected intramuscularly into cercopithecus velvet monkeys at a dosage of  $10^6$  cytopathic units (C.P.U.) per animal. The infected monkeys were observed for signs of paralysis up to 100 days.

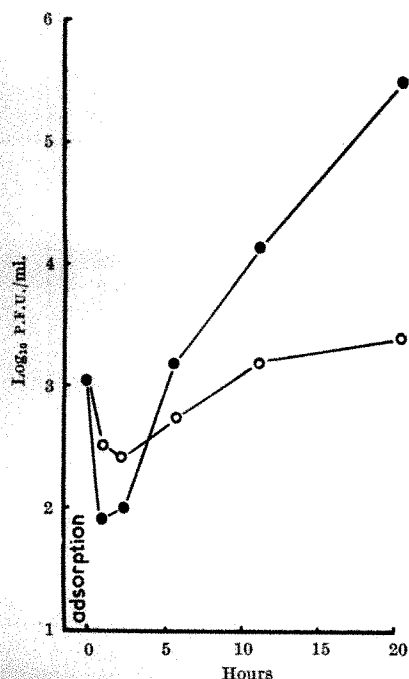


Fig. 1. Growth curve of vaccinia virus in 6-AN treated and untreated CEF cultures. ●, Virus grown in untreated cultures; ○, virus grown in treated cultures

Table 1. RELATIONSHIP BETWEEN *in vitro* SENSITIVITY OF POLIOVIRUS TO GUANIDINE AND ITS NEUROVIRULENCE FOR MONKEYS

Viral strain	Guanidine HCl $\mu\text{g/ml.}$ of culture medium		Neurovirulence for the monkeys		Range of onset of paralysis (days after injection)
	0	1,000	C.P.U. injected intramuscularly	No. monkeys with paralysis/No. monkeys injected	
	C.P.U. detected after 5 days at 37° C*				
CVM†	$3 \times 10^7$	$10^8$	$10^8$	5/5	4-11
CVM 70 G‡	$2.5 \times 10^5$	$7.5 \times 10^6$	$10^8$	2/2	7-8
CVM 70 G-20§	$1.2 \times 10^7$	$2.5 \times 10^5$	$10^8$	0/12	—
				6/6	5-10

\* End point method.

† CVM = Virulent Mahoney isolated from the spinal cord of a monkey paralysed after the injection of Mahoney polio I and cloned twice.

‡ CVM 70 G = CVM propagated 70 times in human amnion cells (Mascoli-line) in the presence of the following guanidine HCl concentrations ( $\mu\text{g/ml.}$ ): once, 16; once, 64; twice, 250; twice, 500; twice, 1,000; and 62 times, 2,000.§ CVM 70 G-20 = CVM 70 G propagated 20 times in human amnion cells (Mascoli-line) in the presence of the following guanidine HCl concentrations ( $\mu\text{g/ml.}$ ): twice, 1,000; twice, 250; once, 125; once, 64; once, 16; and 13 times without guanidine.

Table 1 compares the sensitivity to guanidine *in vitro* with the relative neurovirulence of the different strains used. It appears that the regression of guanidine-dependence *in vitro* is accompanied by a return of the neurovirulence *in vivo*. It is interesting to notice that guanidine-dependent poliovirus retains the cytopathogenic activity *in vitro* provided guanidine is present in the medium.

We may conclude that the guanidine-dependent poliovirus is non-pathogenic for monkeys since the concentration of guanidine required for its growth is much higher than that present in mammalian cells and fluids.

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\* Loddo, B., *Boll. Soc. It. Biol. Sperim.*, **38**, 1043 (1962).\* Loddo, B., *Boll. Soc. It. Biol. Sperim.*, **38**, 1047 (1962).\* Loddo, B., Ferrari, W., Spanedda, A., and Brotzu, G., *Experientia*, **18**, 518 (1962).\* Loddo, B., Brotzu, G., Spanedda, A., and Ferrari, W., *Boll. Soc. It. Biol. Sperim.*, **39**, 1005 (1963).\* Loddo, B., Spanedda, A., Brotzu, G., and Ferrari, W., *Life Sciences*, **10**, 739 (1963).\* Loddo, B., Brotzu, G., Spanedda, A., Gessa, G. L., and Ferrari, W., *Science*, **145**, 945 (1964).\* Loddo, B., and Zanda, C. E., *Arch. int. Pharmacodyn.*, **133**, 1 (1961).

### Posterior Paralysis of Hamsters with Herpes simplex Infection of the Cervix

LITTLE has been reported about specific virus infections of the female genital tract. Most text-books on gynaecology make sparse or no reference to virus infections of the tract except for lymphogranuloma venereum in human beings. As part of an investigation of the role of 'ordinary' viruses in neoplasia of the cervical tract, an attempt was made to develop an experimental model which would enable the interrelationships of viruses and carcinogens to be studied. Herpes simplex virus was selected for initial investigation because of the relatively frequent occurrence of vesicles in the tract of women and because of the latency aspect of its pathogenesis.

The D.J. strain of herpes virus (isolated by Dr. G. Waddell from a child with stomatitis) was used in the investigation. Virus pools were prepared in primary rabbit cell cultures. Hamsters (40-60 g) were infected as follows: Vaginal tracts were first swabbed with cotton pledgets on tooth picks. A second swab which had been soaked in the virus fluid ( $10^7$  ID<sub>50</sub> per ml.) was introduced through a paediatric otoscope speculum and applied directly to the cervix.

Animals were kept under observation for viral growth in the vaginal tract and for clinical and pathological effects. Vaginal exudates were collected daily from the

tract using a swab soaked in Hanks's solution. The swabs were placed in vials containing 1 ml. of Eagle's solution with 10 per cent skimmed milk, and then quick-froze and stored until all samples could be assayed for virus at the same time. Daily samples from pairs of hamsters were pooled and each was titrated in primary rabbit kidney cells using typical cytopathic effects as a measure of the infection.

Six experiments involving 117 infected animals were carried out. Infection in most animals was characterized clinically by a moderate to heavy vaginal exudate which had appeared usually by the third day. At about the fifth to the seventh day many of the hamsters showed posterior paralysis involving both hind limbs. Such animals appeared alert and continued to eat for several days. A few died and some even recovered, but most were killed for further investigations: in all 48 hamsters (41 per cent) showed paralysis. The results of several experiments are summarized in Table 1.

Table 1. EFFECTS ON HAMSTERS OF HERPES SIMPLEX VIRUS ADMINISTERED BY WAY OF THE VAGINAL TRACT

Exp. No.	No. hamsters	No. with vaginocervicitis	No. with posterior paralysis	No. dead
6	20	19	6	8
22	12	11	8	8
23	10	10	6	5
Cont.*	26	3†	0	0

\* Controls were inoculated with Eagle's medium.

† Inflammatory response probably resulted from inoculation technique.

Preliminary histopathological findings include a cervicitis and vaginitis characterized by mononuclear cell thickenings of the sub-epithelial layers. In some animals epithelial microabscesses with some cells containing intranuclear inclusions were noted. Spinal cords of the few paralysed animals examined had lesions of viral myelitis involving primarily the grey matter. Lesions were not found in the brains of these animals.

Assays of vaginal washings indicated a period of low yield of virus shortly after inoculation with increasing amounts until day 5 when maximum yields of about  $10^4$  TCD<sub>50</sub> per ml. washing fluid were obtained. This was followed by a tapering off until day 12 when virus could no longer be demonstrated. Virus was also isolated specifically from the cervix of some animals by explanting tissues and growing them on 'Millipore' filters; however, the results of this will be presented in a separate report.

The observation of special interest was the development of posterior paralysis in many of the animals with vaginocervicitis. This raises the question of how the virus reached the central nervous system from the tract. The posterior involvement suggests that spread of virus was by means of local neural routes. Paralysed animals did not show signs of encephalitis. It is likely, however, that once the virus had reached the spinal cord, it spread throughout the central nervous system, thus accounting for the death. As yet investigations of nervous tissue for virus growth and spread have not been made.

Johnson<sup>1</sup> recently studied virus pathways to the nervous system of mice given herpes by several non-neural routes. His work indicated that, depending on the route of administration, the virus can enter the central nervous system by either haematogenous or neural routes. Mice given virus subcutaneously or by way of the foot pad developed posterior paralysis. As determined by immunofluorescence techniques, the virus apparently spread along nerves by infection of endoneurial cells (Schwann cells and fibroblasts). A similar pathway is suggested in the present investigation.

This work was supported by grant C-4967 from the U.S. Public Health Service.

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<sup>1</sup> Johnson, R. T., *J. Exp. Med.*, **119**, 343 (1964).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, March 22

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Mr. A. J. Steeds: "Some Considerations Affecting the Selection of Risks".

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (in the Main Lecture Theatre, Department of Electrical Engineering, Exhibition Road, London, S.W.7), at 5.30 p.m.—Mr. A. Wickens: "Riding of Railway Vehicles".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Report on the Conference on Education and Training, held at Imperial College on 14/15 September, 1964" introduced by Prof. S. R. Sparkes.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. A. P. Willmore: "Experimental Instrumentation in Scientific Satellites".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "New Teaching Techniques in Engineering Laboratories".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"Films of the Far East" (Colour films).

## Monday, March 22—Friday, March 26

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Great Hall of Alexandra Palace, London, N.22)—Seventeenth Technical Exhibition.

## Tuesday, March 23

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Meeting on "Statistics".

INSTITUTION OF CHEMICAL ENGINEERS, SOUTH EASTERN BRANCH (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 2 p.m.—Symposium on "Hydrogen for Industry".

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Trends in Machining Techniques and Processes".

ROYAL AERONAUTICAL SOCIETY (joint meeting with the Institution of Electrical Engineers, at Savoy Place, London, W.C.2), at 6 p.m.—Discussion meeting on "The Problem of Aerial Installation and Siting in Modern Aircraft" opened by Mr. R. A. Burberry and Mr. H. Hill.

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (jointly with the Paper and Textile Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. B. Ranby: "Paper Fibre—Synthetic Polymer Combinations".

OFFICE OF HEALTH ECONOMICS (at the Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1), at 8.30 p.m.—Dr. M. E. Beesley: "Where Does the Public Interest Lie?"

## Tuesday, March 23—Thursday, March 25

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1)—Spring Meeting.

## Wednesday, March 24

PLASTICS INSTITUTE, REINFORCED PLASTICS GROUP (at the Connaught Rooms, Great Queen Street, London, W.C.2), at 9 a.m.—Conference on "Research Projects".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. P. J. Lawther: "Air Pollution and the Public Health".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at the School of Pharmacy, University of London, Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. Arthur Stoll, F.R.S.: "Ergot—a Treasure House for Drugs" (Hanbury Memorial Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Institutions of Civil and of Mechanical Engineers, at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. J. C. Duckworth: "Incentives to Invention and Innovation" (Eleventh Graham Clark Lecture).

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP AND FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. G. D. Rosen: "Food Industrial Developments in Oilseed Proteins and Allied Products".

ROYAL AERONAUTICAL SOCIETY, GRADUATES' AND STUDENTS' SECTION (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Annual General Meeting and Film Show.

## Thursday, March 25

CHEMICAL SOCIETY AND THE ROYAL INSTITUTE OF CHEMISTRY (at the Middlesex Hospital Medical School, Cleveland Street, London, W.1), at 2 p.m.—Review Symposium on "Organometallic Chemistry".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. C. R. Burch, F.R.S.: "University Physics—The Challenge of Technology" (Review Lecture).

INSTITUTION OF CIVIL ENGINEERS, TRANSPORTATION ENGINEERING GROUP (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Cargo Containers for All Kinds of Transportation" introduced by Mr. E. S. Tooth.

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Colloquium on "Critical Path Analysis".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Polytechnic Chemical and Physical Societies, at the Polytechnic, Regent Street, London, W.1), at 6 p.m.—Symposium on "Surface Reactions Using Ultra High Vacuum Techniques".

## Thursday, March 25—Friday, March 26

INSTITUTION OF MECHANICAL ENGINEERS, PROCESS ENGINEERING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1)—Conference on "Vacuum Technology".

## Friday, March 26

ROYAL AERONAUTICAL SOCIETY, ROTOCRAFT SECTION (at 4 Hamilton Place, London, W.1), at 2.15 p.m.—Mr. G. J. Hunt: "Canadian Autogyro Development"; Mr. N. O. Brantly: "The Brantly Helicopter".

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Insulating Thin Films".

## Monday, March 29

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 10 a.m., 2.30 p.m. and 5.30 p.m.—Colloquium on "Earth Loop Impedance Testing".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. Knight, Mr. R. E. Davies and Dr. R. G. Manton: "Vertical Radiation Patterns of h.f. Curtain Arrays on Plateau Sites".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. J. W. Davidson: "Plastics in Building".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. Michael P. Ward: "In Unknown Bhutan".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

COMPUTING ASSISTANT (with a good honours degree in mathematics or in a pure or applied science, and preferably some experience with large-scale computing machinery) IN THE ELECTRONIC COMPUTING LABORATORY—The Registrar, The University, Leeds, 2 (March 26).

LECTURER (with good academic qualifications and teaching experience) IN EDUCATION with special reference to the teaching of mathematics—The Secretary, University of London Institute of Education, Malet Street, London, W.C.1 (March 27).

LECTURER (well qualified in chemistry and experience in teaching, research or industry) IN INORGANIC CHEMISTRY—The Vice-Principal, Kingston College of Technology, Penrhyn Road, Kingston upon Thames, Surrey (March 29).

CHAIR IN BIOLOGY; CHAIR IN CHEMICAL ENGINEERING; CHAIR IN PHYSICS; CHAIR IN MECHANICAL ENGINEERING; and CHAIR IN PHYSICAL CHEMISTRY—The Secretary, Royal College of Advanced Technology, Salford, 5, Lancashire (March 31).

D.S.I.R. RESEARCH ASSISTANT (graduate in geology, interested in palaeozoic stratigraphy and palynology, and preferably some knowledge of botany and chemistry) IN THE DEPARTMENT OF GEOLOGY—Prof. J. H. Taylor, Head of the Geology Department, King's College (University of London), Strand, London, W.C.2 (March 31).

RESEARCH ASSISTANT (graduate in biology or biochemistry) IN THE DEPARTMENT OF IMMUNOLOGY, to assist in a study of the *in vitro* synthesis of antibodies—The Secretary, St. Mary's Hospital Medical School (University of London), Paddington, London, W.2 (March 31).

LECTURERS OR ASSISTANT LECTURERS IN PHILOSOPHY at Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (April 2).

READER IN APPLIED MATHEMATICS at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 2).

LECTURER IN GEOLOGY—The Registrar, Magee University College, Londonderry, Northern Ireland (April 3).

LECTURER (with qualifications in social psychology) IN EDUCATION—The Registrar, The University, Manchester, 13, quoting Ref. 41/65 (April 3).

SENIOR LECTURERS (2) IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Sheffield (April 3).

CHAIR OF CIVIL ENGINEERING SCIENCE—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (April 5).

RESEARCH ASSISTANT OR SENIOR RESEARCH ASSISTANT (with an honours degree, G.I.L.C. or Dip. Tech., with suitable postgraduate experience or a higher degree) IN THE DEPARTMENT OF CHEMISTRY, for work involving the study of fluorinated heterocyclic compounds—The Registrar, and Secretary, University of Durham, Old Shire Hall, Durham (April 5).

SENIOR LECTURER and a LECTURER IN THE DEPARTMENT OF BACTERIOLOGY—The Secretary, The Welsh National School of Medicine, 34 Newport Road, Cardiff (April 7).

MASTER to teach Physics to university scholarship level—The Warden, St. Edward's School, Oxford (April 9).

HEAD (preferably with a knowledge of the agricultural industry) OF THE APPLIED NUTRITION DEPARTMENT (the work has special reference to determining the nutrient requirements of farm livestock)—The Secretary, The Rowett Research Institute, Bucksburn, Aberdeen (April 10).

LECTURER OR ASSISTANT LECTURER IN MATHEMATICAL STATISTICS, to work with an existing group of five members of staff—The Registrar, The University, Manchester, 13, quoting Ref. 48/65 (April 10).

LECTURER OR ASSISTANT LECTURER (preferably with special interests in applied mathematics) IN MATHEMATICS at Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (April 10).

LECTURER (preferably with previous experience in histopathology and morbid anatomy) IN VETERINARY PATHOLOGY at the Royal (Dick) School of Veterinary Studies—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (April 10).

JUNIOR LECTURER IN PSYCHOLOGY, and a JUNIOR LECTURER IN ZOOLOGY—The Secretary, Trinity College (University of Dublin), Dublin, Republic of Ireland (April 12).

LECTURERS (2) IN BOTANY (one in plant physiology, and the other preferably in morphology or taxonomy) at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (April 15).

READER IN THEORETICAL PHYSICS, and a LECTURER IN THEORETICAL PHYSICS—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 15).



**LECTURER IN APPLIED MATHEMATICS**—The Reader in Biometry, Unit of Biometry, University of Oxford, 7 Keble Road, Oxford (April 17).

**LECTURER** (with a special interest in the investigation of biochemical problems in plant pathology) in **BOTANY**—The Registrar, The University, Manchester, 13, quoting Ref. 47/65 (April 20).

**RESEARCH FELLOW** (medical graduate with an interest in biochemistry and child nutrition) in the **DEPARTMENT OF BIOCHEMISTRY**, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (April 20).

**ASSISTANT LECTURER IN MATHEMATICS**—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 23).

**CHAIR OF EDUCATION** at the University College, Nairobi (University of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (April 23).

**READER IN PURE MATHEMATICS**—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 23).

**SENIOR LECTURER IN THE DEPARTMENT OF ORGANIC CHEMISTRY**, University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 24).

**LECTURER** (with postgraduate qualifications and research interests in experimental psychology) in **EXPERIMENTAL PSYCHOLOGY**, at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, April 30).

**RESEARCH FELLOWS IN COMPUTING: FLUID DYNAMICS AND HEAT TRANSFER; NUCLEONIC INSTRUMENTATION; REACTOR CONTROL AND KINETICS; AND REACTOR PHYSICS**—The Secretary, Winfrith Fellowship Board, A.E.E., Winfrith, Dorchester, Dorset (April 30).

**LECTURER IN EDUCATION** at the University College, Nairobi (University of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (May 23).

**GOLDSMITHS' CHAIR OF METALLURGY**—The Registrar, University Registry, The Old Schools, Cambridge (May 29).

**READER IN MINERAL INDUSTRY** at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 31).

**SENIOR LECTURER OR LECTURER IN TROPICAL MICROBIOLOGY AND IMMUNOLOGY; A SENIOR LECTURER OR LECTURER IN TROPICAL SOCIAL AND PREVENTIVE MEDICINE; and a LECTURER IN TROPICAL MEDICINE** (Technical Assistance Lectureships)—The Secretary, The University, Edinburgh (May 31).

**MASTER** to teach Chemistry throughout the School—The Headmaster, Merchant Taylors' School, Crosby, Lancashire.

**MASTER** to teach Mathematics throughout the School, including the Sixth Form—The Headmaster, Bristol Grammar School, Bristol, 8.

**POST-DOCTORAL INVESTIGATOR** for work with Dr. S. G. Waley on the structural chemistry of lens proteins—Dr. Waley, Nuffield Laboratory of Ophthalmology (University of Oxford), Walton Street, Oxford.

**SENIOR RESEARCH WORKER** (post-doctoral or equivalent) for work sponsored by the Ministry of Defence on the chemical engineering aspects of two-stage oil combustion processes with intermittent heat exchange—R. P. Fraser, O.B.E., Chemical Engineering and Chemical Technology Department, Imperial College, London, S.W.7.

**SENIOR FELLOW IN THE BOTANY DEPARTMENT**, 68 Half Moon Lane, S.E.24, for duties which will include assisting in research and teaching—The Secretary, Ref. 6604, King's College, Strand, London, W.C.2.

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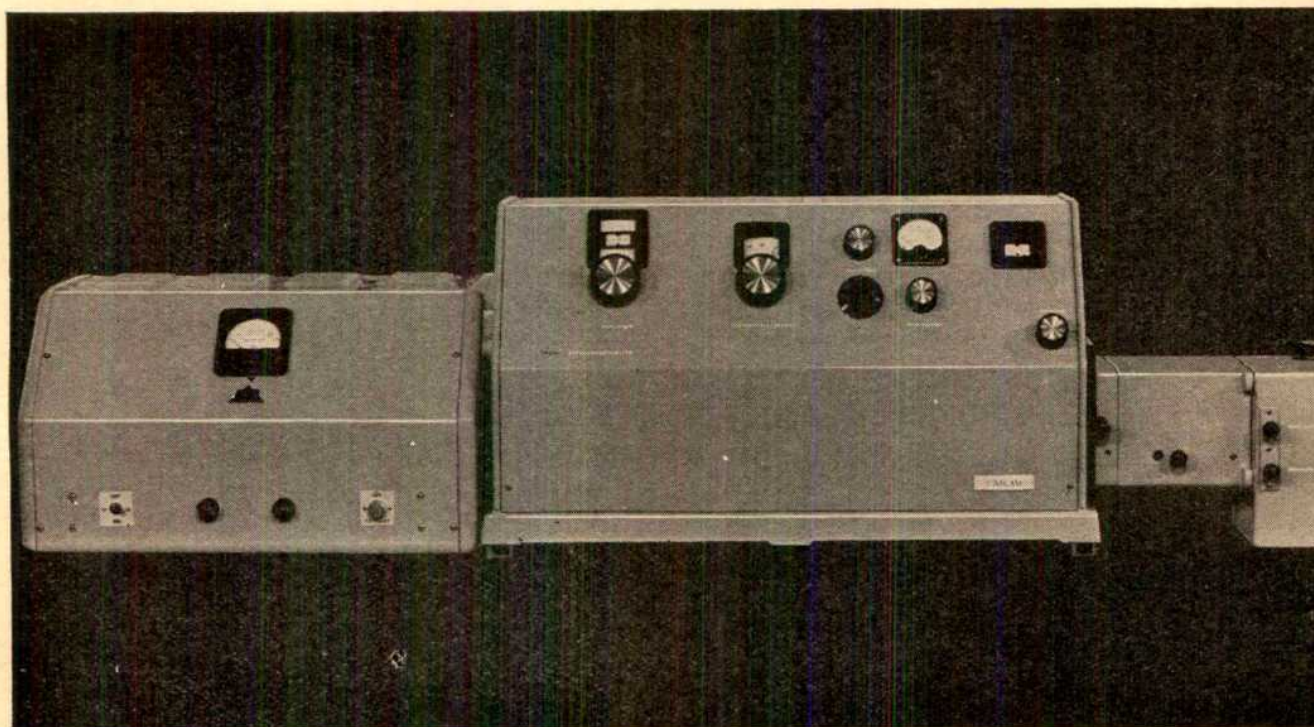
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## FOUNDATIONS OF SCIENTIFIC POLICY

THE lecture on science and Government which Dr. S. Toulmin gave in October 1964, and which has now been published in the January and February 1965 issues of *State Service* (the journal of the Institution of Professional Civil Servants), makes particularly interesting reading in the light of subsequent changes in the organization of civil science in Britain and of two recent books. Both Mr. S. Brittan's *The Treasury under the Tories: 1951-1964\**, and Mr. N. H. Brasher's *Studies in British Government†* were written before the change of Government, and Dr. Toulmin's lecture was delivered before the election, but all three gain rather than lose in pertinence through the consequent detachment, and by and large the differences in emphasis and in points of view are suggestive for the scientist. Moreover, Dr. Toulmin does much more than repeat the arguments of his earlier paper printed for private circulation, also *Nature*, 200, 503, 1030; 1963), and if the title "Foundations of Scientific Policy" could be equally appropriate for this lecture, the whole structure of his argument is adapted to the wider and perhaps less-informed audience he addressed in October.

He begins by drawing a three-fold distinction. Science can be, first, the servant of the formation of policy, as when technical advance contributes to decisions regarding defence or the administration of prisons. Secondly, it can itself be the subject of policy, as when the University Grants Committee or the Advisory Council on Scientific Policy advises on the allocation of central funds for the support of scientific research. Thirdly, it can be an instrument in the formation of policy, as when the analytical work of a Buchanan or a Moser contributes to the solution of political problems by elucidating what the actual choices are for those responsible for taking decisions. This last-mentioned distinction is the newest of the three modes of interaction between science and policy, and it was a main concern of Prof. G. A. Miller's Granada Lecture, "Computers, Communication, and Cognition", in October 1964, which likewise has now been published (*The Advancement of Science*, 21, 417; 1965).

Dr. Toulmin points out that traditionally the structure of Government in Britain has been specifically adapted to deal with the first of these roles, both as regards advice and administration. As one consequence, many scientists have come to be prejudiced against becoming involved with Government and administration, whereas in the present situation the second and third roles have become a vital element in public affairs. This is now recognized by all political parties and is emphasized repeatedly in the difficulties experienced in reaching sound decisions in such matters as aircraft and weapons policy, apart from all questions of party policy. Aloofness and prejudice on the part of the scientist and technologist can to-day be a real danger to public welfare.

In analysing this situation, Dr. Toulmin turns first to the question of the structure for advice, stating the problem as follows: "When questions arise about the bearing of science and technology on Government policy,

what sorts of outside advice should Ministers seek, and through what channels?". He answers this question by referring to the way in which, in the past thirty years or so, when the advice of the merchants and bankers in questions of trade, taxation, money and employment proved ineffective the Government turned to the professional economists. Dr. Toulmin suggests that here the formation in 1940 of the Economic and Statistical Section of the War Cabinet Secretariat, which has since developed into the Economic Section of the Treasury, was the crucial step. While he does not claim too much for the intervention of academic economists, he suggests that since 1940 the nations of the West have succeeded, by methods based on the analyses of economic theorists, in avoiding any recession comparable with those which, throughout the previous 75 years, had seemed unavoidable. Moreover, he points out that even the humblest voter is now expected to master some of the basic concepts of economics, and that nobody would now seriously propose to abandon the attempt to base economic decisions on an explicit and thorough analysis of the problems of the day using the best concepts and theories available, and return to relying on unanalysed hunches.

Dr. Toulmin is obviously simplifying, and his suggestions should be considered in the light of the much fuller discussion of economic policy contained in Mr. Brittan's book. Leaving that aspect for the moment, Dr. Toulmin uses the transition in the formation of economic policy to illustrate his main thesis that the corresponding transition has not taken place in the arrangements for obtaining expert advice about the issues of scientific policy. What the Government should seek here in its advisers is something much more than profound knowledge within science proper: rather an understanding of scientific affairs is needed, of that whole complex network of processes by which the activities of scientists and technologists interact with the rest of the national life, and of the economic and political consequences of these interactions. This requires a much deeper study and understanding of the interactions between science and other areas of public concern—a fresh grasp of the social roles of scientific research and technological development, their potential contributions to public affairs, and the problems to which they give rise, comparable with the grasp we have gained from economics of the roles, problems and potentialities of money, trade, industrial production and employment.

While Dr. Toulmin believes that this need is well enough recognized in the international agencies, for example by the Directorate of Scientific Affairs of the Organization for Economic Co-operation and Development, he questions whether scientific affairs have any standing in Britain as a field of serious study. He is critical that the one really promising programme of research on the economics of research and development under Mr. C. Freeman at the National Institute for Economic and Social Research should be financed not from any British source but under contract from the Organization for Economic Co-operation and Development. It is difficult to point to anything in the pattern of recent changes which could offset the frustration which Dr. Toulmin himself has experienced or remove the obstacles he describes. The essential

\* *The Treasury under the Tories, 1951-1964*. By Samuel Brittan. (Pelican Book A 722.) Pp. 375. (London: Penguin Books, Ltd., 1964.) 6s.

† *Studies in British Government*. By N. H. Brasher. Pp. vii + 177. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1965.) 18s. net.

studies on scientific affairs, he believes, are being frustrated by the continued dominance within the administration of men whose training excludes all first-hand knowledge of scientific subjects. Further, this situation is maintained by the basic structure of the Civil Service and the pattern of the educational system feeding it.

In arriving at the question of the mechanism of administration, Dr. Toulmin reaches a conclusion that Sir Henry Tizard arrived at long ago: we must recruit our future administrators of the first rank from young men and women who are at ease with science and scientific affairs, and the universities must shape their curricula to permit such an education, combining, for example, the analytical training of the Greats course, with an emphasis on cogency, literacy and intellectual discrimination, with a general grasp both of scientific ideas and of scientific affairs. An administrator to-day has inescapably to experiment and innovate in every field of public affairs and, for sound judgment, discrimination, balance and knowledge, particularly new knowledge, must be added. In all its branches Government will be forced increasingly to sponsor and apply the results of deliberate social research.

Anomalies, such as Dr. Toulmin notes in this lecture, do not appear to have been removed in the new structure set up by the present Government, nor does it appear that the barriers and salary-differentiates between the higher administrative grades and the professional grades have as yet been dismantled as Dr. Toulmin suggests. Beyond all this, however, one further essential step has to be taken: the creation of a central 'research and intelligence' section to serve the Ministries responsible for science policy, having a status comparable with that of the Economic Section of the Treasury. Such a section would also undertake *ad hoc* enquiries about scientific manpower, priorities and economics. Moreover, it would have the right and the duty to sponsor parallel enquiries in universities and other outside organizations, with the view of promoting long-term thought about the problems of scientific policy. In so doing, it would foster the informed public opinion necessary for the intelligent discussion of the politics of science.

It is for its contribution to the understanding of the working of the British Government at the present time and more particularly for the light it throws on the threats to traditional freedoms latent in present concepts of Government that Mr. Brasher's *Studies in British Government* is of interest to the scientist and technologist. Mr. Brasher's title is not well chosen, for although it is intended to stimulate discussion it lacks the depth that such a title would suggest. While it ranges more widely than either Mr. Brittan's *The Treasury under the Tories* or Mr. B. Crick's *The Reform of Parliament*, it is too penetrating to be fairly described as discursive. Mr. Brasher gives readable accounts of the place and functions of the Monarchy, the Cabinet and the Prime Minister, and Ministers and Civil Servants. He interpolates a chapter on the redress of grievances before proceeding to discuss, successively, the House of Commons and its procedural problems, the implications of different electoral systems, the House of Lords and some problems in the administration of justice. Before finally attempting to assess the present strength and weakness of the British constitution he glances briefly at some recent and prospective changes in local government and at Britain's relations with the Commonwealth, more especially at the problems presented in granting independence.

On the powers of the Prime Minister and the Cabinet as against the House of Commons, Mr. Brasher is as penetrating as Mr. Crick if somewhat less forthright. While he believes that, so far as the Civil Service is concerned, the doctrine of Ministerial responsibility give reasonable safeguards for the ordinary citizen, he considers that new forms of protection are required for the freedom of the citizen since the traditional safeguards are losing their effectiveness. He sees in the House of Lords one means by which more time could be secured for the Commons to consider the increased volume of legislation but when it comes to the problem of how to secure stronger Parliamentary control over matters which specialization to-day has made intelligible only to the expert, he never mentions the question of scientific and technological advice. He is rightly critical of the way in which time is wasted in the House of Commons by all parties, but on this central issue in the relation of science and technology to public policy he gives no further hint that its existence is recognized.

Mr. Brasher disclaims, it is true, any suggestion that his book is comprehensive, and in the context of this question of science and Government it should be noted that most of the book was written in 1962, well before both political Parties gave the issue its present prominence. None the less, it is perhaps significant that substantially the same criticism could be made against Mr. Brittan's book for all its concern with the mechanism and effectiveness of advice in economic and financial matters. Admirably as Mr. Brasher has set forth certain trends, such as the growth of central direction and the nature of the ultimate restraints on Government authority it is vital that this omission should be remembered and its implications receive due consideration.

Much of the first part of Mr. Brittan's book complements or duplicates Lord Bridges's recent book on *The Treasury* (Nature, 203, 555; 1964), but the second part on "Economic Steering" is highly relevant to the relations between the Treasury and the Ministry of Economic Affairs established by the new Government, and to any stress or strain that may develop between these two Departments, and even between them and others such as the Board of Trade or the Ministry of Technology. Added to this, Mr. Brittan is now one of Mr. G. Brown's expert advisers in the Ministry of Economic Affairs for which his book argues. Mr. Brittan holds that, at least so far as economic policy is concerned, the traditional relation between Ministers and Civil Servants has broken down under the sheer complexity of the issues. The Civil Service is not a ruling bureaucracy and the system can only work successfully if the guiding lines of policy come from the Ministers themselves. Mr. Brittan does not appear to favour the practice of asking distinguished outsiders to pronounce on essentially political issues: on the contrary, he challenges the whole idea of impartial advisers analysing 'means' and politicians choosing 'ends', and suggests the creation of a new class of adviser, distinct from the main Civil Service, with both political and technical responsibilities.

These advisers would be selected in accordance with the personal preferences of Ministers and would move freely in or out of the academic world, industry and the professions, tending to change with Governments but not necessarily so. The system would, above all, be flexible and take account of the fact that many of the real divisions of opinion and of personal compatibility cut across Party lines. These advisers differ appreciably from those which Lord Bridges rejected as undesirable in a



broadcast talk last June: it is possible that they are similar to what the present Prime Minister has in mind. Nor does Mr. Brittan think that such a scheme would adversely affect recruitment to the permanent Civil Service: it is a bad and not a good feature of the present system that high officials should be able to opt out to the present extent from the political consequences of their advice. Furthermore, he visualizes some administrative advantages in the scheme as well as a decisive encouragement to mobility and interchange between the Civil Service, private or nationalized industry, the universities and the professional world generally.

Mr. Brittan is careful not to claim too much for the greater use of professional experts in this way. Undoubtedly the process could itself help to promote informed criticism and, as he remarks, the quality of advice might well be improved if officials were more often required to defend it in public against expert criticism: there is no reason why a democracy should acquiesce in conventions designed to save the Government any embarrassment in all circumstances. Such criticism and the public explanation of Whitehall thinking would be assisted by establishment of the specialized House of Commons committees which have often been advocated, and by providing better working conditions for Members of Parliament. In the last resort, however, no weaknesses in either the Civil Service or the House of Commons can excuse the Government from its own responsibilities.

Mr. Brittan, no more than Dr. Toulmin, suggests that institutional arrangements can be a substitute for right decisions on policy. They may increase the chances of a right decision, and his concluding chapter on the choice of priorities is particularly pertinent in relation to the changes which have now been made with regard to the National Economic Development Council. The real significance of establishing that Council, he suggests, was that it was a potential pressure group for growth, and this should apply to any new Ministry that takes over its function. Such a planning organization does not need powers to ensure that its forecasts are fulfilled. Its value lies in spotlighting the need for regional policy, for training and redundancy schemes, tax reforms and numerous ways of improving efficiency as well as in putting forward ideas for policy, and especially in ensuring that Government policy is held firm to ensure the achievement of that target of growth to which the Government has committed itself.

Mr. Brittan suggests that the confidence with which both sides of industry have treated the National Economic Development Council is due largely to their belief that it is their own organization and not a Government Department—an advantage that cannot be overstressed. Nor does he favour the establishment of a new Economic Ministry to determine strategy, leaving the Treasury to determine tactics. He would start with the existing Treasury as a base, but transfer control of Government expenditure to a separate Expenditure Bureau. If the control of the Civil Service went to an enlarged Civil Service Commission the Treasury would be rid of some of the main psychological impediments to its acting as a proper Economics Ministry and could become specifically responsible for advising on spending projects conducive to growth. The residue could also enlarge its functions, take a more detailed interest in industry and hold a wide brief to think about economic policy as a whole.

Mr. Brittan's proposals obviously differ considerably—and suggestively—from the new structure which has

emerged with the establishment of a Ministry for Economic Affairs, even if the details are as yet by no means clear. The alternative centre of power which he desiderates now exists, and it remains to be seen whether the influence of the Bank of England or traditional preoccupation with candle-ends continues to hinder economic growth. Beyond this, however, there are even wider issues on which his book throws some light, and if, regrettably, he himself does not touch on this question of science and Government and the structure or character of scientific advice for those alive to such issues his book is scarcely less suggestive than Dr. Toulmin's lecture.

## LANGUAGE OF THE SOCIAL SCIENCES

### A Dictionary of the Social Sciences

Edited by Julius Gould and William L. Kolb. (Compiled under the auspices of the United Nations Educational, Scientific, and Cultural Organization.) Pp. xvi+761. (London: Tavistock Publications (1959), Ltd., 1964.) 136s. 6d. net.

THE publication of *A Dictionary of the Social Sciences* represents the achievement of a great success for the Department of Social Sciences of Unesco. The object of its compilers has been to present "a general introduction to the main problems and developments" in the social sciences, and "to provide for specialists in other disciplines a source of understanding of the main concepts used in fields outside their own". This undertaking is a formidable one, because the social sciences are still far from sharing a common outlook, methodology, or even language. The barriers that separate sociology from political science and economics still remain more or less apart. But there is a tendency for sociology, anthropology, and (in some degree) psychology to come together on a common front, and there is evidence in the *Dictionary* that a certain mutual interest is developing. The effort that has gone into its compilation cannot but stimulate its further development.

It is a remarkable thing, then, that the *Dictionary* has been compiled at all. It is much more remarkable that the results are so impressive, and that the difficulties mentioned here have been overcome so satisfactorily. It achieves both its special and its general purposes outstandingly well. It is a genuine 'must' to all practising social scientists, who should all have access to it, and the nearer they have it within reach, the better for them. In general, the authors have proved themselves to be well informed, conscientious, and fair-minded, even when they tackle the more awkward aspects of highly controversial subjects. Moreover, their writing is usually clear and precise, which is not true of the common run of social science texts.

So far as content is concerned, the articles by Americans outnumber those by British Commonwealth authors by two to one. As might be expected, this creates a certain bias towards American ways of looking at things in the social sciences, but this is nothing like so serious or as intellectually misleading as the fact itself would lead one to expect. Bias is sometimes apparent, as in the articles on race and racism, but the fault is one of proportion and balance rather than in fundamental approach or method. Furthermore, there is the problem that is general in the social sciences of the significance of what an American author has called "intendedly rational behavior" as a subject of study, for social scientists "have come to be suspicious" of the assumption that social behaviour can be regarded by them as autonomous and purposeful. Social scientists are much more at their ease when they deal with the influences of social environment than when



they seek to examine individual choice and intention, or processes of planning and administration. It is to be regretted that there is no article on, for example, so-called applied sociology, which might have dealt with what is so often termed 'problem-centred' work. There is, however, one on applied anthropology by Dr. Margaret Mead, in which the issue is briefly mentioned, but the articles on welfare and social problems do not go to the root of the matter.

These are no more than blemishes, however, and are fully redeemed by the value of the work as a whole for general use. Some words, such as 'anomy', may be understandable as terms of art by the social scientist; they involve esoteric mysteries to the uninitiated. Their use is becoming more common as they refer to universal problems in the modern world, and clarification of their meaning is thus welcome. Others, such as 'value', have both general and special meanings. In so far as they may have a limited, but not at all precise, significance when used in connexion with sociological theory, they again require clarification with some degree of urgency. Finally, many new words, such as 'scientism' or 'meta-sociology' (the latter dealt with in the *Dictionary* under the umbrella of that inelegant word 'metaanthropological'), are coming into common circulation, and the social scientist finds himself having to do too much explaining about their uses. Here, again, the publication of the *Dictionary* is helpful, as a labour-saving device.

T. S. SIMEY

## QUID HOC NOMEN GENERICUM ORCHIDEUM SIBI VULT?

### Generic Names of Orchids

Their Origin and Meaning. By Richard Evans Schultes and Arthur Stanley Pease. Pp. xiii+331. (New York and London: Academic Press, 1963.) 86s.

THE Latin names of plants, until the middle of the eighteenth century, were usually brief phrases which described the plant. From 1753 onwards the binominal system of Linnaeus has been increasingly used and to-day there is an international code of nomenclature governing the formation and application of these binominals, which may refer to a feature of the plant or to its habitat or locality or may commemorate its founder or a famous person. The majority of early botanists were classical scholars well versed in ancient Greek or Latin and to-day a certain number of mainly older workers have a wide knowledge of these languages. To most of us, however, Latin, if we learnt it at all, probably ended in the fifth form and consequently the wealth of information contained in the Latin names of organisms remains untapped.

The orchids, because of their combination of peculiar botanical, horticultural and aesthetic qualities, have always aroused a special interest in botanists, gardeners and flower lovers. This interest, plus the fact that the orchids comprise the largest family of flowering plants, obviously suggested to two Harvard scholars that an etymological dictionary of orchid generic names would be an essential as well as a fascinating book to taxonomists and non-taxonomists alike.

Thus a taxonomic botanist, Dr. Schultes, and a classical scholar, Prof. Pease, joined forces to produce *Generic Names of Orchids: Their Origin and Meaning*, which alphabetically lists some 1,250 names. In addition to their etymology, the place of first publication of each genus is given together with a summary of its geographical distribution and its position in the standard work on the classification of the family. Genera with interesting and unusual features are illustrated by excellent line drawings of one or more of their species. Some of these are rather fanciful but serve well to illustrate their point.

Others, for example those showing orchids in cathedral settings, are perhaps a little out of keeping in a scientific text-book. However, this emphasizes the fact that systematic botany, a complete appreciation of which necessitates knowledge of modern taxonomic methods as well as of literature up to more than two hundred years old, is not so narrowly specialized as many other scientific disciplines.

For those who have little knowledge of the orchids Dr. Schultes has added excellent introductory sections on the Orchidaceae, their morphological features and economic uses together with a summary of their classification, which is basically that of Schlechter. For those who are not taxonomists, there are introductory articles on vernacular and taxonomical nomenclature and an account of the International Code which governs the latter.

It is unfortunate that the work is incomplete in that many genera are not mentioned, inaccurate in that there are rather too many obvious typographical errors, and incorrect in the stated and supposed meaning and origin of several of the generic names. For example, *Baskerville* is not in honour of the engraver John Baskerville, but is almost certainly dedicated to the young student and great friend of Prof. Lindley, Thomas Baskerville, who died at an early age. *Osyricera* surely has no direct relationship with the Egyptian god of the underworld as stated by Schultes and Pease but was almost certainly likened by its author, Blume, to *Osyris*, a genus of the Santalaceae which was well known at the time the orchid genus was described.

However much these rather too numerous errors of fact and omission may offend and distract the working botanist, the reader must realize the enormous amount of work that has gone into the preparation of this book and the authors must be congratulated on conceiving and producing such a useful and fascinating volume. It must be remembered that most of the information contained therein is not to be found in any other scientific publication and it is a good example of a work in which the art of compilation has been successfully combined with genuine research and scholarship.

P. FRANCIS HUNT

## MORPHOLOGY OF INFLORESCENCES

### Die Infloreszenzen

Typologie und Stellung im Aufbau des Vegetationskörpers. Erster Band. Von Dr. Wilhelm Troll. Pp. xvi+615. (Jena: Gustav Fischer Verlag, 1964.) 112.50 D.M. 192s. 8d.

MORPHOLOGISTS concerned with reproductive structures of higher plants have usually directed more attention to flowers than to the axes that bear them. Nevertheless, the inflorescence deserves study as a whole. As a branch system it is often clearly related, but with subtle variations in the distribution of growth, to that of the vegetative phase from which it arises. Functionally, the order of its development and the interrelation of its parts often contribute substantially, by determining the spatial and temporal pattern of the opening of the flowers, to the effectiveness of the reproductive phase. Dr. Troll believes, with justice, that this neglect of the study of the inflorescence follows from two related deficiencies; first of a satisfactory natural system of classification of inflorescences, and second of a philosophical framework enabling the investigator to reduce to manageable proportions the multiplicity of detail involved.

Troll's approach to the morphology of inflorescences is that of an unrepentant, indeed enthusiastic, typologist. This will come strange to many contemporary botanists for whom 'type' and 'typology' have become almost improper words. This undoubtedly reflects failings in

the teaching of morphology, because typology, used with understanding, is a very effective method of organizing information, and facilitating discussion and comparison of complex biological material. This first volume of what is to be a four-volume treatise does in fact go far to vindicate, by the clarity of the reasoning and presentation, the author's philosophical position.

Two of the nine sections promised are contained in *Die Infloreszenzen: Typologie und Stellung im Aufbau des Vegetationskörpers*. The first, devoted to a general account of flower-bearing systems, introduces a scheme of classification based largely on Eichler. Simple inflorescences, in which the branching consists entirely of the production of lateral flowers, are distinguished from complex, in which partial inflorescences stand in the place of the flowers of simple inflorescences. Dr. Troll maintains that the ground plan of all inflorescences is monopodial, and on this reasoning the cyme cannot be accepted as a simple inflorescence, but as a complex derived (conceptually) from the thyrsus by suppression. The section concludes with a discussion of the various forms of fusion and unequal growth which so modify the appearance of many inflorescences. The second section, which initiates the typology of inflorescences, begins with a series of rigorous definitions. The compound inflorescence becomes technically a *Synfloreszenz*, terminating in a *Hauptfloreszenz*, and bearing below *Paracladien*, each terminating in a *Cofloreszenz*. The remainder (some two-thirds) of the book consists of illustrations of the concepts defined, the species concerned representing a wide, but largely herbaceous, range of higher plants.

*Die Infloreszenzen* is certainly outstanding. It has all the care and penetrating observation that have distinguished Dr. Troll's earlier work, and, within the framework chosen, it is intellectually stimulating and of enviable scholarship. There are abundant drawings and many photographs of a remarkably high standard, on which the artists and printers should also be congratulated. Provided they are interested at all in the growth of higher plants, botanists generally, even those of an entirely empirical temperament, will find an acquaintance with *Die Infloreszenzen* a rewarding experience. P. R. BELL

## ELECTRONICS: ADVANCES AND EXPANSION

**Advances in Electronics and Electron Physics**  
Vol. 18. Edited by L. Marton. Pp. x+342. (New York and London: Academic Press, 1963.) 89s. 6d.; 12.50 dollars.

NO detailed definition of 'electronics' would survive long enough to be of more than historical value, and any attempt to set a limit to the subject's coverage would meet much disapproval. But a measure of the price one must pay for a carefree approach to the question "What is electronics?" is given in this volume of the series of reviews of progress made in electronics and electron physics. Five subjects are surveyed, spread over a very wide field; one of them is indisputably electron physics and a second almost emphatically so even though it is largely applications of known principles to new instrumentation. A third subject is electronics as many users of electronic devices understand it. By contrast a fourth might have appeared equally well under other omnibus headings, such as spectroscopy, or the Earth's atmosphere; electron physics has to stretch itself somewhat to encompass it. The fifth, on computer organization, has negligible electronics content to warrant inclusion. Such wide coverage may discourage sale other than to libraries, or may call for publication of the articles separately.

The first article, on the nightglow, is short and is largely a collection of observations. The spectral line due

to atomic oxygen at 5577 Å figures prominently; much speculation persists concerning the conditions applying in the regions of the Earth's atmosphere where the emission originates. The next, and even shorter, article is on computer logical organization. The cost of using modern digital computers has been as much reduced by attention to programming as to design principles and reliability. Among the improvements discussed and illustrated here are time-sharing and real-time multiprogramming, content-addressed memories, multi-level memories and the fixed-plus-variable structures which afford added flexibility.

A longer article then looks at a subject with much history—collisions of low-energy electrons and ions with atoms and molecules. It remains of great interest because of the intrinsic simplicity of the systems being studied. The new data provided by refining old experimental techniques and introducing new methods, such as using the microwave conductivity of a discharge, have made necessary reassessment of the relative importance of the processes responsible for the transfer of energy and charge.

A second long article examines how far the electrical performance of junction transistors and diodes conforms to expectations based on the structures and the physical theories of the effects at work. The objective is very worthy but the author fails at times to prevent the diversity of effects from confusing his approach. Direct current and transient properties receive most attention. Despite several references to work published since 1960, the review reflects much more nearly the position in the late 'fifties.

The final review is of electron emission microscopy, a subject which has received much attention in Europe. It shows promise of competing with or complementing transmission microscopy, though the best resolution obtained so far is only of the order of 100 Å. The three main methods described use photo-emission by ultra-violet irradiation, secondary emission by electron bombardment and secondary emission by ion bombardment. With the second method, the primary beam can scan the specimen, and the intensity of the secondary electrons produced can be synchronously presented on a television tube for easy viewing and photography.

J. R. TILLMAN

## BONE AND TOOTH SCIENCE

### Bone and Tooth

Edited by H. J. J. Blackwood. (Proceedings of the First Symposium held at Somerville College, Oxford, April 1963.) Pp. xvii+425. (London and New York: Pergamon Press, 1964.) 100s. net.

THE first European Bone and Tooth Symposium was held in Oxford in April 1963. The title of this book and of the Symposium derives from the Bone and Tooth Society, formed in London in 1950 to bring together all those involved in research provoked by an interest in bones, teeth and calcium metabolism.

Many countries and disciplines were represented at the Symposium. As a live pulsating thing it was adjudged a success: this book forms the permanent trace of its existence. Forty short papers and four longer lectures were read. Three of the lectures are now presented in brief summary form. For one of the short papers, only an abstract was available in time for publication. All the contributions are presented in English and there are useful references at the end of most papers. A bibliographical index is included at the end of the book. Since many of the contributors vanished to the four corners of Europe and beyond within minutes of the end of the Symposium, and since a certain amount of opposition to publication of the proceedings was democratically expressed during

the Symposium, the editor's task was clearly not an easy one. It has been well and carefully done.

Since the formation of the Bone and Tooth Society in 1950, a variety of research tools has provided a wealth of data for analysis and digestion. Microradiography, electron microscopy, histochemistry, bone biopsy, tetracycline labelling, radio-isotope tracer studies, not to mention spectacular advances in biochemistry have revolutionized thinking about the dynamic behaviour of bone. For the treatment of patients with bone diseases, a much better model of bone behaviour is now available. These techniques and some of the advances they have produced are well represented in this Symposium, at which the balance between experimental and purely laboratory work on one hand, and clinical and closely allied investigations on the other, seems to have been a reasonable one.

There is a group of four papers related to the use of tetracycline as a marker. There are two papers devoted primarily to studies of bone turnover using microradiographic techniques. There are two papers on the role of the adrenals in the control of calcium-levels in plasma, one paper on hypercalcaemia associated with carcinoma of the breast and a prescient article on calcitonin, based on a consideration of Copp's work, of control systems in general, and of experimental evidence relating magnesium and parathormone.

The interest in kinetic studies is reflected in the considerable number of papers in which calcium or strontium isotopes were the fundamental tools. The hazards of nuclear fission and fusion and terrestrial contamination have brought as a useful by-product a great deal of fundamental work on bone-seeking isotopes with studies on their location in bones and teeth.

A vastly increased interest in bone matrix and its components is manifest in this Symposium, and no fewer than nine papers can be said to be related to this topic. By contrast, fewer than half this number of papers is concerned with inorganic and physical chemical investigations of bone mineral and related compounds.

Two clinical papers are devoted specifically to studies in human osteoporosis. There is a very detailed and precisely documented account of 42 cases of idiopathic osteoporosis in men younger than fifty-five years, with comments on treatment by oestrogens and calcium supplements. The other paper is concerned with the effect of calcium supplements on the spinal density of patients with osteoporosis, the spinal density being estimated by a densitometric analysis of lateral tomograms of the lumbar spine and intervertebral disks.

There are single papers on many other topics—organ culture, enzymes of cells in bone, the structure of dental enamel, the tensile strength of human compact bone, parallel chemical and microradiographic studies of osteoporotic, osteomalacic and normal human bone, a palaeontologist's account of the origin of bone, etc.

This Symposium offers a useful selection of the contributions of European workers to the advancement of knowledge of living bone up to April 1963.

F. H. DOYLE

## A PROBLEM OF AGE

### Ageing

*The Biology of Senescence.* By Dr. Alex Comfort. Revised and re-set edition. Pp. xvi+365. (London: Routledge and Kegan Paul, Ltd., 1964.) 42s. net.

TEN to fifteen years ago, Dr. Korenchevsky, who may be regarded as the father of gerontology as an organized, internationally recognized research discipline, often recalled the fact that his early years in ageing research had not been easy. Often he came up against official criticism of his plans for studying the processes of ageing. The young research worker intending to embark on a

career aimed at the study of the diseases of the elderly or the processes which bring about changes in ageing organisms, will not to-day be met by such lack of interest. Gerontology has become a scientifically and socially acceptable subject. In Britain the universal interest in the 'ageing problem' has been exemplified by the numerous popular articles and radio and television programmes which, over the past few years, have brought the layman into contact with the research worker. One of the foremost exponents of this popularization of gerontology has been Dr. Alex Comfort, but proof, if such is needed, that the scientific knowledge on which the popular story is based has also been developing apace can be obtained from the fact that Dr. Comfort's earlier book *The Biology of Senescence* has had to be republished in a completely revised form within eight years.

It is not fair to judge new editions of books solely on the basis of statistics, but in a relatively new field it is comforting for practitioners to be able to point to the advancement of their subject by a more than 40 per cent increase in the size of the new edition.

During the past ten or eleven years Dr. Comfort has been in the forefront of one particular aspect of ageing research, namely, the application of analytical procedures to the study of a variety of parameters which alter with age. His work has, in the main, been concerned with non-human populations, and it is not surprising that the bulk of the material presented represents an assessment of his own findings in the light of the observations of other workers throughout the world. Although this preoccupation with survival tables is understandable, it might give a rather lop-sided view of the progress of gerontology over the past decade to any newcomer to the field who treated the sub-title of the book in its broadest sense and looked for a fuller coverage of the work of those groups which have been considering biological changes in ageing in the present-day fashion at a molecular level.

Although this type of approach is mentioned in passing the references are more in the nature of an annotated bibliography than a true review of the situation, and it is significant that such references appear, in the main, in the introductory chapters.

One interesting inclusion in the new edition is a chapter on the effect of ionizing radiations. Here the reader is introduced to the beginnings of what will obviously provide a basis for much future work, but its inclusion does demonstrate one of the marked changes which have occurred in the approach to ageing since the first edition was published.

To many readers the section of the book which immediately attracts attention is the chapter on the mechanism of senescence, if only because the theoretical background to a subject always excites most interest. It is therefore a little discouraging that only one new sub-section has been introduced in the new edition. This apparent dearth of new ideas is not, however, so pronounced as would appear from the retention of the old headings. New material on the concepts of somatic mutation, cellular turnover and death, and hormonal control of ageing are discussed at some length, but again the possibilities of the prime lesion being at a molecular level are dismissed in a page.

Ageing can be studied at a variety of levels, dealing respectively with populations, the intact organism, the hormones, the cell, the gene, the enzyme and the molecule. Dr. Comfort's book deals in the main with the first half of this list, but although workers in other fields may regret the lack of coverage of the latter half, the excellence of the author's presentation does much to justify his more limited approach and should act as a stimulus to other writers to provide a review of the relevant material. Gerontology, or geratology as Dr. Comfort would like to see it called, has come of age. No longer can one author do it justice.

D. A. HALL

## SCIENCE AND ENGINEERING

In his inaugural address as president of the Institution of Electrical Engineers, on October 8, 1964, Mr. O. W. Humphreys spoke from long experience of industrial research and organization.

Describing electrical engineering as a science-based industry, Mr. Humphreys observed that the early power engineers, with little but basic scientific principles to guide them and exploiting their own inventive genius, were not infrequently ahead of scientific investigation. In electronic engineering, however, scientific and technological development had from the beginning gone hand in hand and now throughout the whole field interdependence was complete.

Research could, very broadly, be divided into that designed to be, either directly or indirectly, of benefit to industry and that concerned with the study of environment from which no return, other than that of deeper understanding, is demanded. This fundamental research increasingly required the use of very costly tools, and a country's capacity for advancing this type of knowledge may well depend as much on financial resources as on the skill of its scientists. Fundamental research is international and its results are, in the main, freely published.

Research of importance to industry may again be subdivided into 'background' research, designed to increase the general pool of knowledge, and 'project' research, which supports a specific development programme. Background research is usually published and thus made available to industry at large, while project research, necessarily closely integrated with development, may well be withheld from publication.

Background research is carried on in universities, in Government laboratories and in industry, and is subject to relatively little co-ordination. Leaving for a moment the special considerations which apply to research associations and accepting that, for this type of research, central planning is impracticable, the fact remains that lack of co-ordination results in a substantial loss of industrially useful output. Each industrial organization determines its research programme very carefully in relation to its own needs, but there is undoubtedly far more overlapping of the areas of activity of different companies with common technological interests than would be dictated by sound considerations of commercial security.

Collaboration in research involves, so far as considerations of national and commercial security permit, exchanges of information as to what is being done or planned elsewhere, knowledge of the broad trends of industrial development and of the nature of the information which industry requires. There is no easy means of devising machinery to serve these purposes, but fairly recently the electronics industry has made some progress in the matter. Eight trade associations, five concerned with electronic equipment, the other three with electronic components, jointly formed an organization, termed the 'Conference of the Electronic Industry', for the purpose of dealing with matters of common interest and concern to the branches of industry represented. One of those interests being research, the Conference gave immediate support to the drive for the creation of a National Electronics Research Council. This Council, incorporated in July 1964, has on its General Committee representatives of the Ministries of Aviation, Defence, Education and Science, the Board of Trade, the Post Office, the universities and the electronics industry. Twelve of the industry's representatives are nominated by the Confer-

ence. The Institution of Electrical Engineers is linked through its Research Committee with the Council's activities. The expressed intention of the Council is to endeavour in the first instance to increase the yield of the existing electronics research effort.

Research associations developed following the establishment of the Department of Scientific and Industrial Research in 1915. There are, to-day, fifty research associations with a total annual expenditure of some £9,500,000, of which in 1963 £7,400,000 came from industry and £2,100,000 from the Government. The Department of Scientific and Industrial Research, in fostering the growth of research associations, expressed its aim as that of developing within industry an appreciation of the benefits to be derived from scientific research. In trying to form some assessment of the effectiveness of research associations it is necessary to recognize that they are varied, not only in their specializations but also in the type of industry which they serve and the kinds of service which they provide. They can, in fact, be divided into four groups. First, there are those serving industries which are still mainly craft based, such as the Baking, Cutlery, File and Furniture Associations. Such associations can be a great source of strength to industries which formerly were almost wholly dependent on the individual skill of their workers and the experience of their management. They are, in some instances, handicapped by their small size. The Iron and Steel and the Ship Research Associations are at the other end of the scale, serving, as they do, very compact industries closely knit in their scientific interests and keenly aware of the vital part which research has to play. These are large associations and their members entrust to them a very substantial part of their research effort.

Another group is typified by the Welding and Production Engineering Research Associations. These associations deal with technologies having, in one form or another, almost universal application in the engineering industry. Their special knowledge and skills are of such a character as would be difficult for an individual company, even a very large one, to acquire. The fourth is a numerically small group of associations which serve scientifically based but rather diversified industries. Of these the Electrical Research Association is a notable example. The electrical industry has among its members some of great scientific strength, whose research resources considerably exceed those of the Association, but, at the other end of the scale, there are small companies which, individually, may show little or no research activity. This presents the problem of reconciling the needs of the scientifically weak and of the scientifically strong members.

The large companies, scientifically strong, are sensible of the advantages of collaboration but find it difficult to decide what form of collaboration is appropriate and the extent to which they can entrust work which is of common interest to outside bodies. Project research, being allied to development, is not usually amenable to collaboration. To an increasing extent background research is becoming more akin to project research. For example, research on semiconductors and development of new manufacturing processes may be carried out by closely integrated research and development teams working in the same laboratory. Work of this kind must be carried out in industry. Notwithstanding these difficulties there is still much research which can be entrusted by a group of manufacturers to a competent research organization. An excellent example of this is the employment, a few years ago, of one of the well-known sponsored research organ-



izations in the United States, by a group of the largest semiconductor manufacturers, to search for new semiconducting materials.

The real need of the scientifically strong companies is for a highly efficient sponsored research organization operated co-operatively by industry and capable of carrying out specified researches which would be paid for by the group of members requiring the investigations. If the sponsors of such research were willing that the results should be published, the Department of Scientific and Industrial Research would contribute to the cost, as the information would be available to industry at large. There has been a growing tendency for industry to share joint programmes among their own research laboratories, and the Department of Scientific and Industrial Research has been devising means whereby this form of collaboration can be encouraged.

Turning from research to engineering development and design, Mr. Humphreys said that three of the main causes of loss of efficiency in the use of engineering and design effort were abortive projects, faulty planning of development and inadequate attention to priorities in design. The biggest single source of wasted effort is, undoubtedly, the abortive project. With a complex project such as the development of a computer, a nuclear power plant or an electronic telephone exchange, where there are alternative technical approaches, it is seldom possible, in the first instance, to say which approach will be the most rewarding. The right course is to proceed by successive elimination, first on the basis of paper studies and then by experimental investigation. Assessments should be made by an independent team, not too directly involved in the studies themselves, and should be made at frequent intervals. When from the process of elimination one technical approach has been selected, assessment must be continued so that changing circumstances can be taken into account and the continuing technical and commercial viability of the project confirmed.

Development is usually related to a marketing date, and whether demanded by the marketing date or not, it is desirable that development and engineering should be compressed into as short a time as practicable. This not only saves cost in itself but also allows the incorporation of the latest design improvements available. However complex a project may be, it can always be broken down into a number, possibly many thousands, of operations to each of which a time can be assigned. There will be a sequence of identifiable stages within which many of

these operations can be performed in parallel. The identification of the sequence of operations which determines the minimum time within which the whole project can be completed—the 'critical path'—is a highly skilled professional operation. Such analysis demands the attention of a team of highly specialized engineers fully backed by computer facilities.

Design is, as a rule, something of a compromise among considerations of performance, reliability, appearance and cost. The ever-present important factor of cost has to be taken into account along with the other parameters which in different projects will have different priorities. In the television and motor-car industries, for example cost is paramount, whereas in the design of a nuclear power station or a telephone exchange, performance and reliability are dominant factors.

Minimum cost or maximum value for money is a study which must be made by a special team of engineers who will study every detail of design with the view of pointing out redundancies which could be eliminated. These engineers should have no responsibilities for anything but cost. This procedure, commonly known as 'value engineering', does call for some measure of special training. These techniques, possessing something of the qualities of 'applied common sense', are finding wider acceptance in industry and can influence substantially the efficiency of utilization of engineering effort.

Mr. Humphreys's review was completed by observations on two further problems of very general interest—the 'information explosion' and education in relation to career prospects in research and engineering management. In regard to the problem of information retrieval he observed: "Difficult though it may be for many of us to understand, the probability that computers may soon be used to analyse, store, select and deliver information demanded of them is very real. . . . It is highly probable that, within the life-time of many of us here to-night, the preparation of literature surveys will no longer require the laborious and very time-consuming searching of the abstract journals, followed by the reading of many papers of which but a few prove to be important. Instead, the research worker will present a description of the information he requires to a computer in language which it can understand, and will forthwith be presented with a list of the papers he should study. In all probability the computer will be maintained and its store of information kept up to date at an information centre connected by permanent data links to its more frequent users".

J. GREIG

## SIGNIFICANCE OF MAJOR MOORLAND FIRES

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IT has been believed for many years that the fires which rage on Britain's moors in dry years have profound effects on those moors. Exactly how frequent, extensive and effective these fires are has never been considered. The extent to which they remove vegetation and the underlying peat has never been analysed. The following article is an examination of one area, which lies to the east of the Derbyshire Derwent, between Penistone and Matlock, in which an attempt is made to assess the number, frequency and extent of fires, and to examine the vegetation and peat in order to establish the extent to which fires totally destroy them, thus contributing to the erosion of the area and changes in the appearance of the landscape.

Since 1920 some of the fires have been very fierce but none has equalled the 1959 fires in extent and effect.

The fires have caused a large-scale retreat of the edge of the blanket of peat and stimulated a tremendous amount of erosion on the burnt moors, particularly since 1959.

It is impossible to know whether all the fires that have occurred in the area have been noted, and so the evidence must be considered incomplete, but sufficient has been revealed to suggest that fires have not been an important feature throughout history.

### The Peat Cover

The peat, of very variable thickness, covers most of the southern Pennine hills and descends in some places as low as 800 ft. O.D. Generally, the peat consists of a series of beds of brown, acidic, partially humified vegetation of varied compaction. The peat is 'sealed' by a

fairly continuous and interwoven mat of vegetation roots. Within the peat, the water-table fluctuates with precipitation; normally it is close to the surface, but occasionally the peat may dry to a depth of 4-5 ft. During these dry periods the peat, which on most moors is less than 6 ft. deep, is preserved from mechanical erosion by the network of roots. Fires appear to be extremely important as a cause of erosion, particularly on the periphery of the peat cover, where, during dry spells when the shrubs on the surface are tinder dry, intense burning may devour the plants and shallower peat and effectively reduce the area of blanket bog.

### The Cause of Fires

One can rarely be sure of the exact cause of any particular fire. Before 1900 fires were linked with pipe-smokers, but opposed to this is the care taken by the moorland's occupants, and the precious nature of the minute quantities of tobacco used in the 'fairy pipes' and other clay pipes. The recording of moor fires by the Press has tended to be more colourful than exact; a cause has always been suggested, often without any supporting evidence.

Tobacco from pipes and home-made cigarettes rarely remains alight when discarded. 'Tailored' cigarettes, however, can remain alight for a considerable time and are the probable cause of many fires in the fifty years since they became fashionable and the moors became more accessible. (This is well recognized in Canadian National Forests. 'Tailored' cigarettes burn much longer when discarded than home-made ones or pipe ash, and are a much greater fire hazard.)

It is not certain that glass bottles or lightning have caused fires. Sparks from steam trains appear to be unimportant, but some deliberate arson on certain moors appears probable.

### Frequency of Fires before 1900

There have been many hundred fires in this area, but there are several reasons for believing that there were few major fires before 1900. Even to-day fires are poorly recorded, but there have been hundreds; for example, 700 fires were reported to Derbyshire Fire Brigade during September 1-11, 1959 (*S.T.*, September 12, 1959). In this article, a major fire is defined as one which burned for at least two days; it is thought that this is long enough for the fire to penetrate the peat and destroy the root zone; observation has shown that a fire can burn for more than a day and still be confined to the surface litter.

Prior to Parliamentary enclosure of the moors (1760-1855), the whole area was utilized by tenants of the adjacent farm-land. Sheep were 'staf-hearded' on 'sheep-walks' on the commons and wastes by flock-masters. The customary sheep-walks are noted in thirteenth century deeds for Midhope, Moscar, and elsewhere<sup>1</sup>; there is a map of Bleaklow and adjacent areas which shows the whole moor as a series of walks<sup>2</sup>. Cattle were stinted on customary beastgates, sometimes called 'summerings', and women milked cows and ewes on the moors<sup>3</sup>. Goats and horses were also kept<sup>4</sup>. Bracken, rushes, heather, second-growth timber, grass sods, bilberries, millstone, and other stone materials were carefully utilized. The whole was so carefully protected by intricate manorial rights and regulations that fires would be extremely rare. Small fires were specifically forbidden in some parishes to minimize the risk of uncontrolled fires which one might expect from time to time. One verdict of Sheffield Manorial Court, 4.10.1733, forbade the burning of bracken, which had several economic uses, such as bedding animals down. Elsewhere the firing of turf, sods, soil, and heather is specifically forbidden. Rights were known as Fernary, Furbary, etc.<sup>5</sup>.

Table 1. THE FREQUENCY OF FIRES

Date	Location	Source*	Remarks
1762	Broomhead Moors	Hunter (450)	Several thousand acres reduced
1826	Broomhead Moors	<i>S.I.</i> , 5.7.1826	—
1868	Hallam Moors Moscar Moors	Hunter (460) Hunter (460)	3,000 acres Burnt for more than two weeks
1887	Midhope Moors	Wood (109)	Burnt for several weeks, but could not have been extensive
1921	Big Moor Hallam Moors Houndkirk Moor	<i>S.C.R.</i> , 1922 (122) <i>S.C.R.</i> , 1922 (122) <i>S.C.R.</i> , 1922 (122)	Froggatt Edge Wyming Brook area —
1933	Leash Fen	<i>S.C.R.</i> , 1934 (96)	East of Gardoms Edge, 100 acres
1935	Baslow East Moor	<i>S.C.R.</i> , 1936 (147)	Gardoms Edge area, 300 acres. Three days burning
1938	Big Moor	<i>S.C.R.</i> , 1939 (171-3)	Nearly all the moor
	Hathersage Moor	<i>S.T.</i> , 9.5.38	"2,000 acres"
1939	Pike Lowe	Broomhead Keeper	Near the summit
1947	Broomhead Moor	Broomhead Keeper	Burned for 10 weeks
1948	Big Moor	<i>S.C.R.</i> , 1950 (181)	800 acres burnt below White Edge
	Hallam Moors	<i>S.C.R.</i> , 1949 (161)	Below Stanage Edge
	Hathersage Moor	<i>S.C.R.</i> , 1949 (161)	Near Carl Wark, 0.5 sq. mile
	Houndkirk Moor	<i>S.C.R.</i> , 1949 (161)	—
	Totley Moor	<i>S.C.R.</i> , 1949 (161)	Brown Edge, 300 acres
1949	Totley Moor	<i>S.C.R.</i> , 1950 (181)	800 acres burnt
1953	Leash Fen	<i>S.C.R.</i> , 1954 (110)	East of Gardoms Edge, 300 acres
1959	Baslow East Moor	Observation	Much of the moor
	Beeley Moor	Observation	Whole moor
	Brampton East Moor	Observation	A few acres
	Burbage Moor	Observation	Whole moor
	Hathersage Moor	Observation	Almost all the moor
	Moscar Moor	<i>S.T.</i> , 28.8.59	Upper Rivelin, south side
	Totley Moor	<i>S.T.</i> , 15.9.59	Almost all the moor

\* Full titles for books occur in the text. G. H. B. Ward's *Sheffield Clarion Ramblers Handbook* (*S.C.R.*) is the best single record and copies are kept in Sheffield City Library. Newspapers quoted are the *Sheffield Independent* (*S.I.*), *Sheffield Telegraph* (*S.T.*), and *The Star* (*S.S.*). Pages in parentheses.

Enclosure of the moors replaced this communal system with a different economy based on private grouse breeding. On such areas which are still private, being owned by water-boards or individuals, there are still no recorded fires; for example, on most of the Langsett, Hallam, and Derwent Moors, and the Chatsworth Estate.

The introduction of 'moor-management' by game-keepers led to the exclusion of both human and animal life from the moors in favour of game birds. 'Muirburn', introduced around 1800, is the seasonal burning of patches or strips of heather, but this burns only the stems and foliage, and not the root system or the peat. These fires no doubt eliminated some plants from the flora, but their most important effect has been to deposit ash on the upper layer of the peat.

The economic exploitation of the moors before 1800, and the privacy imposed up to and after 1900, thus kept the fires to a minimum. There are three known exceptions, which are listed in Table 1 and below.

The first discovered record is that of the 1762 devastation of Ewden and Broomhead Moors (*SK.* 2295, \*2395):

"1762 was the driest summer since 1723. Everything burnt up, and the moors being on fire from Ewden to this (Broomhead) common and consumed several hundred acres. Cowel was for the most part on fire and almost all the moors in England and Wales"<sup>6</sup>.

1826 was a very dry year, and fires were widespread. They included a 20-mile wide fire in Aberdeenshire, blazes on many of the moors of southern Scotland and the Pennines, all the way into Derbyshire. A "scorched and arid plain" was made of much of the Broomhead Moors, burning several thousand acres. This latter fire was attributed by some to a bilberry gatherer knocking out her pipe on the dry peat<sup>7</sup>. Of these fires a reporter wrote:

"The fires have not only spread over a wide extent of surface, but burnt to a great depth, consuming not only the moss, but the peat underneath. In some places, where they reach a soft

sub-stratum, they run to a great length under ground, and break out at the surface at other spots<sup>18</sup>.

Hunter believed the Broomhead fire to have been an extremely rare occurrence. In 1868 there was a large fire to the west of Sheffield. J. Wood says it was on Bradfield Moors<sup>9</sup>, but it is probable that the 3,000 burnt acres lay:

"... betwixt Moscar and Hollow Meadows, Redmires, and the Wyming Brook... The conflagration lasted about a fortnight, the smoke and flames being visible at a great distance; and the smell of the burnt peat extended much farther"<sup>10</sup>.

This fire was attributed by some to bilberry gatherers taking revenge for being ejected from their traditional picking grounds. In 1887 there were fires on Midhope and Strines Moors, but details are lacking<sup>11</sup>. These are the only fires for which evidence has been discovered in nineteenth-century documents, but it is probable that there were lesser ones.

### Fires in the Twentieth Century

From 1921, the number of recorded fires has steadily increased. In 1921, fires occurred around Wyming Brook, below Stanage Edge, on Froggatt Edge, and on Houndkirk Moor. On the latter moor, the fire is believed to have been started by "a spark from a steam-engine at 5.30 a.m."<sup>12</sup>. In May 1938, 1,700 acres of Big Moor and 500 acres of Hathersage Moor were burnt in what is probably the largest single fire known in the area. It consumed nearly all the peat on Big Moor, and the smoke blackened Buxton, 12 miles to the west<sup>13</sup>. In 1939, according to Mr. H. Hemsley (head gamekeeper, Broomhead Estates), a fierce fire burnt for several weeks on Pike Lowe, Yorkshire. Across the valley, parts of Broomhead Moors were burnt by a fire which raged and smouldered for more than ten weeks in 1947. (Began September 19, and was finally put out in the new year; information supplied by Mr. H. Hemsley.) In May 1948, 300 acres of Totley Moor were devastated<sup>14</sup>, and in the following year 800 acres below White Edge were reburnt<sup>15</sup>; perhaps Ramsley Moor was burnt at this time.

A careful examination of the ground shows that there have been serious fires on other moors for which there is no documentary evidence, but these areas are not extensive. Some of the Chatsworth Estate (on Brampton East Moor, and immediately east of Harland Edge) has no peat under the heather cover in locations where one might expect to find it. Partly-healed patches on Hingliff Hill, Langsett, and elsewhere, show that the foregoing record is not complete. However, the information on the effect of fires which can be collected for 1959 more than compensates for this.

### The Fires of 1959

Derbyshire was Britain's driest county in 1959, and, after six dry months, fires swept the moors in September. At Redmires Dam, May–September was the driest period since records began in 1842. Light anticyclonic winds gave Sheffield 78 per cent (23.96 in.) of its average precipitation, and only 10 per cent of its September average total. The mean maximum temperature exceeded 60° F in six consecutive months, during which time there was only 6.58 in. of rain. The area missed all the main thunderstorms affecting the rest of Britain (*Meteorological Office Monthly Reports*, 76–7; 1959–60). The variable winds complicated the fire-fighting, and in some cases it was noted that the fires advanced both with and against the prevailing wind. Totley Moor burned for 6 days; Barlow East Moor for more than 10 days; Beeley Moor more than 4 days; and Burbage–Hathersage Moors for at least 4 days<sup>16</sup>. People attracted by the novel sight of parched reservoirs along with other tourists probably dropped cigarette ends which were the cause of the fires. They destroyed more moorland and consumed more peat than in any preceding year.

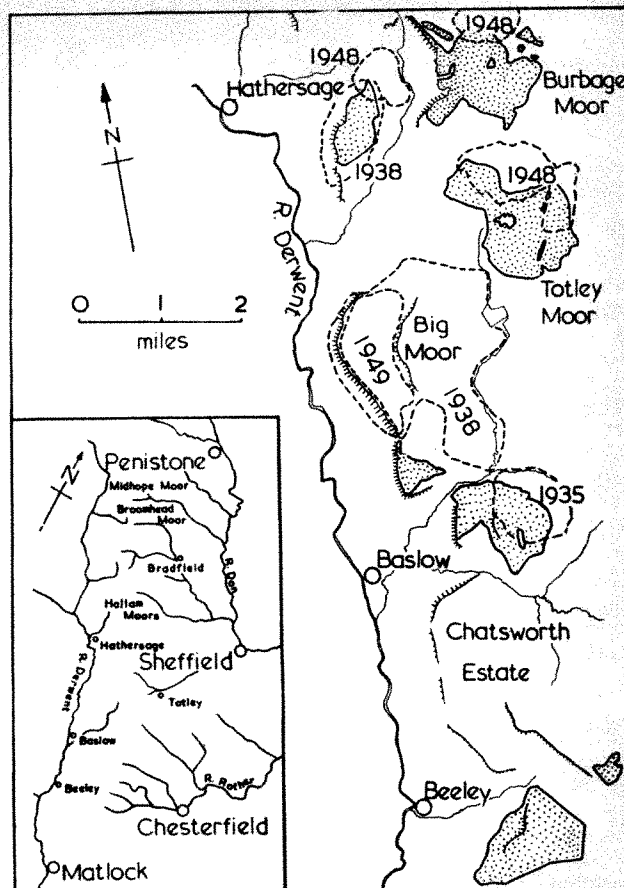


Fig. 1. Location map. The area of burning in 1959 is stippled

The burnt areas were mapped in 1961 at a scale of 2.5 in. : 1 mile. The information has been reduced and is shown in Fig. 1, which represents the approximate area devoid of blanket peat, where once there were considerable depths. It can be seen that these fires have an overall importance, and that they are relatively recent. The removal of the peat has had profound effects which can be observed at the present time on some of the moor burnt in 1959.

### Distribution of Intensity of Burning

Moss, Tansley and Pearsall directed attention to the botanical and geographical distinction between 'moor' on one hand, and 'moss' or 'bog', on the other<sup>17</sup>. In this article 'moorland' is used to denote a blanket bog which has lost its *Sphagnum* in favour of a heather or mixed vegetation; the peat is usually 3–5 ft. thick and is confined to slopes and ridges of the wet highlands of Britain. In contrast, a 'moss' is technically a true bog permanently waterlogged with acid peat often in excess of 20 ft. in thickness. In the investigation area, the toponymics 'moor' and 'moss' generally form a distinct demarcation of land above 1,000 ft. Fires normally occur on the moors and not on the mosses. There appears to have been a general upper limit to the burning of the order of 1,300–1,500 ft., which is perhaps a climatic limit. Most of the moors occupy ridges to the east and south of the main crest-line of Derwent Edge. The mosses have been fire-free because they occur in waterlogged swales which are either cut in shale, or choked with solifluction material which impedes the drainage and gives rise to bog conditions.

In the higher land at the latitude of Bradfield, there are mosses at 1,700 ft., and moors descending to 900 ft. below Broomhead Moors. At the latitude of Hathersage

the hills are lower, and White Path and Middle Mosses occur at 1,250–1,400 ft., while the burnt Burbage and Hathersage Moors are chiefly below 1,250 ft. At the altitude of Great Rowsley, there are no mosses and all the moors up to the 1,100 ft. summit are liable to be burnt. The mosses survive the burning, and are important in that they preserve some peat to regulate partially the run-off into adjacent reservoirs, and also preserve zones of peat in the lower areas which are otherwise denuded of peat.

The fires before 1920 were all north of Ringinglow, whereas the fires since 1920 have all been south of Ringinglow, which is probably a reflexion of the change in habits of Sheffield holiday-makers. Bradfield used to be a favourite 'resort' in the days of the wagonette; to-day the public moors around Hathersage are a greater attraction, with appropriately better public transport from Sheffield and cars using the A.623, A.624 and A.625. In both areas the fires are confined to the periphery of the peat.

The individual moors were burnt with varying degrees of thoroughness. Hathersage Moor was completely stripped; burning on Beeley Moor was patchy; and elsewhere holes and tunnels were burnt into otherwise untouched peat. The burning appears to have been limited only by the extent of the dry peat. Roads, natural rock outcrops, and even ploughed strips were not always effective limits to the burning.

#### Immediate Effect on the Flora

Even though special investigations are being made of the ecology of such burnt areas by botanists, it is necessary to note here the rapid rate of 'healing', which must be partially controlled by the completeness of the removal of the vegetation and peat. It is difficult to believe from the appearance of the vegetation cover that Big Moor was burnt in 1949, or that many parts of Pike Lowe's peat and vegetation cover were burnt in 1939.

There is a considerable range in the time taken for the moors to regain a reasonable cover. On Beeley Moor, *Eriophorum* spp. has spread over huge areas, to the general exclusion of other species. This rapid extension has been both by seeding and vegetative reproduction. Elsewhere, *Calluna vulgaris* is the main colonist using both methods of regeneration. On Birchen Edge there are several species involved in the production of an almost complete ground cover:

*Salix atrocinerea*  
*Betula pubescens*  
*Sorbus aucuparia*

*Calluna vulgaris*  
*Deschampsia flexuosa*  
*Agrostis tenuis*  
*Holcus mollis*

*Polytrichum commune*  
*Polytrichum piliferum*  
*Pohlia albicans*  
*Ceratodon purpureus*

*Chamenerion angustifolium*

The trees occur in areas not occupied by moss and grass, often adjacent to relict patches of woodland, while the mosses and grasses form large attractive splashes of varied greens across most of the dark, burnt moors.

Areas at a similar height on Hathersage Moor are still bare. On Big Moor, a close examination of the heather cover reveals that, after 15 years, the cover is fairly complete but still rather sparse in places. On Pike Lowe, considerable areas have been recolonized by heather and grasses, but the summit is still bare owing to its exposed nature. Similarly, near Broomhead Shooting Lodge, grass and heather, both sown and planted, and rows of straw-bales have been introduced to reduce the continuing erosion of the regolith, but to no avail. Apparently the rate of healing varies considerably from place to place, but it can be said that within 3–5 years most areas have a shrub or herbaceous cover.

When the colonizing is complete the rather monotonous sweep of each moor takes on a superficial appearance of uniformity, but firing creates one obvious but vital difference: beneath unburnt moors there is a peat layer,

while beneath burnt moors there is not. At the junction of these zones, the peat may remain in isolated mounds, revealing the depth of the former peat blanket. The past 50 years has seen a considerable retreat of the peat fringe in the areas which have been thoroughly burnt. (It seems probable that the peat fringe extended to lower altitudes prior to the advance of agriculture towards the moors, but much work remains to be done on this aspect.)

#### Changes on the Mineral Surface revealed by the 1959 Fires

The effects of fires prior to 1959 have escaped investigation, but the effects of the abnormally fierce and extensive 1959 fires can be taken as illustrative of the changes which can be made. The extensive desolate tracts are the most obvious short-term scenic change, and their barrenness has permitted several forms of erosion and deposition to develop.

Little load is removed by streams flowing from peat-covered hills, while burnt areas lose considerable detritus in the form of ash, peat and sand. Removal of the peat reduces the water-storage capacity of a moor, and an accurate record of the moorlands which have lost their peat would probably reveal catchment areas with changed run-off characteristics. (Similar findings were made by Conway, V. M., and Millar, A., "The Hydrology of Some Small Peat Covered Catchments in the Northern Pennines", *J. Inst. Water Eng.*, 14, No. 6; October 1960.)

There have been no landslides, mudflows, or any other large-scale surface movements in the survey area. Large, smooth slopes are grooved with hundreds of braided rills and gulleys. In three years, they have cut 12–15 in. on Burbage Moor, while after 15 years on Broomhead Moor, channels many hundred yards long have exceeded 3 ft. in depth. Rivulets have filled holes made by wallers and road-makers, choked hollow packhorse ways and boundary ditches. Culverts under roads, for example, at Hell Bank Top (SK. 287681), are repeatedly blocked. Sand has ponded behind walls, creating small deltas such as the one north-west of Owl Bar below Flask Edge (SK. 292784). It is not known whether this new stream-load has had any effect on the principal rivers.

More impressive is the effect of wind. The sand and ash on the exposed moors are being constantly removed by the prevailing west winds. Hard frosts heave and loosen the upper few centimetres of soil, and this is, in due course, blown away, scouring the surface and etching long parallel grooves. All the ash on exposed summits was removed within 2 years. On Burbage Moor, the wind has lowered the surface and created a shallow pebble 'pavement', resting on hard clay or sand. Localized sand-storms during very strong winds have blotted out visibility over extensive areas. Dust clouds, similar to the clouds of air-borne ash experienced during the fires, are carried beyond the moors into residential areas; in May and June 1960 reddish dust enveloped Sheffield for several days<sup>18</sup>, and as late as June 1962 considerable clouds of dust were being blown into Totley and Ashover. There is no evidence of wind abrasion on rocks.

There are impressive depositional effects. Large sand-drifts block Houndkirk Road (SK. 287827), and walls on Beeley, Burbage and Totley Moors are almost buried (Fig. 2). Surviving patches of cotton-grass act as sand traps, which often display such dune features as parallel ripples, horned spurs, and barchan-like crests.

The actual heat from the fires has baked some soils red and yellow and these are quite distinct from the effects of podsolization. Fine-grained sedimentary and crystalline pebbles were exploded. Blocks of gritstone, possibly weakened by weathering, have disintegrated since the fires. Extensive areas of partly burnt peat were hardened, or reduced to a coke-like ash. (This is best seen near the ventilation shaft, Totley Moor: SK. 276794.) In 1938, the fire caused several large rock-falls from Froggatt Edge<sup>18</sup>.





Fig. 2. Dune-like developments on Totley Moor

All these features are small in themselves but the total effect is considerable. It is also conceivable that they could affect future interpretations of the surface of the soil: the sand drifts look like some periglacial sand pockets; the pebble pavement and soil colour could be identified as products of pedogenic processes; the exploded pebbles could be due to frost action. Holes in the peat made by fire sometimes become pools, which could be attributed to normal bog development. Patchy burning, creating an uneven surface, could be related to tunnel collapse beneath the peat, and so on.

#### Exceptional Nature of the 1959 Fires

Previous fires removed peat and stimulated erosion but there has been nothing so impressive as the 1959 fires.

An examination of pack-horse roads on Derbyshire moors in 1956-57 showed that the many miles of roads, which had cut down through the peat, were usually overgrown and had suffered little erosion or infilling<sup>20</sup>. Since most of these packways have been abandoned for more than 150 years, their condition must be indicative of little erosion during that period (Fig. 3), even though they cross moors, such as Totley Moor, which have been severely burnt prior to 1959. Since 1959, extensive lengths of packway have been choked with wind- and water-borne sand and ash. Following the snows of the 1962-63 winter, when snow still occupied the hollows, it was observed on Totley Moor that ash and sand, to a depth of 4 in., were washed in a uniform cover over the snow in a matter of days.

Below Brown Edge on Totley Moor a wall follows the 1,100 ft. contour for more than half a mile. It is a natural trap for all erosion on the hill which rises 150 ft. above it. Fig. 4 shows the result of a section cut through deposits trapped by the wall (Fig. 5) (SK. 291,3710).



Fig. 3. Blown sand in a pack-horse road on Beeley Moor

The original soil is a hard, iron-enriched, yellow compact sand. Above this, there is a mixed sand and stone deposit, 6-7 in. thick, which is probably wind and water transported. The zone is dark and confined by the ancient humus layer and the humus layer which has developed on the deposit, but above this there is 20 in. of loose, stratified, black and grey, wind-borne sand which has been deposited on both sides of the wall since 1959. From 1809, when the wall was erected, to 1959 about 7,000 ft.<sup>3</sup> of detritus had been trapped against the upper side of the wall; since 1959 more than 25,000 ft.<sup>3</sup> has been trapped excluding the material below the wall, and there are many miles of wall smothered in sand elsewhere. The erosion since 1959 has been phenomenal and the total effect really exceptional.

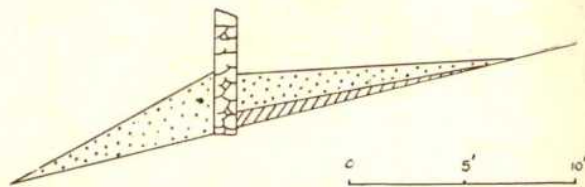


Fig. 4. Section through deposits below Brown Edge, Totley Moor. Dotted deposit is the 1959 wind-blown sand; the hatched zone is pre-1959 deposit

#### Revealed Periglacial Features

The removal of the peat permits an examination of the regolith on which the Pennine peat blankets rest. The barren landscape which is exposed must look, in some ways, as it did prior to the return of the flora in post-glacial times. We know almost nothing of highland conditions during the final phases of the Wurm period or up to the earliest discovered pollens at the base of the peat which date from perhaps 5,000 B.C.—a gap of more than 10,000 years—during which there appears to have been only a little rounding of the angular blocks which make up the chaotic periglacial landscape.

The block fields are the characteristic periglacial feature. Large areas of angular, frost-riven boulders have been revealed. They are concentrated on the upper parts of dipslopes and below the 'edges' (Fig. 6). On Beeley Moor, Baslow East Moor, Hathersage Moor, and Totley Moor there are whole hillsides strewn with blocks. It is difficult to conceive how much more blockfield remains beneath the Pennine Peat.

Some surfaces are free from boulders. Here bedrock may be at the surface (for example, east of Mother Cap, Hathersage Moor; above Wildmoorstones Edge; above Gardom's Edge), or there may be a considerable depth of regolith if it is developed on flaggy or shaley bedrock. The upland swales may have even greater depths of solifluction. It is evident that the considerable erosion



Fig. 5. Sand trapped against the 1809 enclosure wall on Totley Moor





Fig. 6. Typical burnt moorland, still bare after 5 years

during the Pleistocene was later arrested and replaced by processes of accumulation when the peat began to form.

### Conclusions

In the area there have been almost 30 fires which could be called disastrous. They have occurred mainly since 1920, on the fringes of the peat blanket. It is not possible to determine in detail how many acres suffered from these fires, but it is certain that there has been nothing to equal the 1959 fires. The depths of peat on most moors is imperfectly known and so it is not possible to quote amounts which have been removed.

The peat in many parts of Britain is being severely eroded by subaerial forces<sup>21</sup>, but the firing provides a method of erosion not previously emphasized. It removes whole tracts of peat and plant cover in a matter of days and permits intensive erosion for several years. Eventually the area is 're-vegetated' and the area usually resumes its previous appearance. However, where run-off and present-day erosion rates are used to establish the age of valleys, as in the case of Young's study of the Strines area<sup>22</sup>, great care must be taken to ascertain whether the area has been burnt; if it has, the rate of erosion might have been far from constant.

The rate at which plant life recolonizes the burnt moors varies, but even on the 1959 burnt areas the scars of firing are quickly being concealed. The removal of the acid peat should help the proposed afforestation of Big Moor, and it seems possible that it will permit more trees to grow

on moors protected by water authorities from browsing animals and further fires; already there is a considerable growth of birch and willows on parts of Ramsley Moor which is now owned by Chesterfield Water Board.

While general texts<sup>23</sup> scarcely mention the effects of major fires on the moors, it seems probable that the vegetation of the southern Pennine moors and elsewhere is, in part, pyrogenic, and it is equally probable that the role of fire will increase in importance, now that more and more moors are being opened to the public and week-end car excursions to the moors continue to increase in popularity.

A knowledge of moorland fires such as those discussed here should be of interest to ecologists, hydrologists, and geographers, and it is to be hoped that comparative studies will be made elsewhere.

<sup>1</sup> Ronsley MS. No. 1197, 1212 (Sheffield City Library).

<sup>2</sup> Fairbanks Collection, Hop 25L (Sheffield City Library).

<sup>3</sup> Ronsley MS. No. 1197; *Victoria County History, Derbyshire*, 1, 407 (1912).

<sup>4</sup> Farey, J., *A General View of the Agriculture . . . of Derbyshire*, 3, 149 (1811). The last wild goats were seen c. 1805.

<sup>5</sup> Ronsley MS. No. 4165.

<sup>6</sup> Ronsley MS. Nos. 9339, 9202.

<sup>7</sup> *Sheffield Independent*, July 15, Aug. 5 (1826). Hunter, J., *History of Hallamshire*, edit. by Gatty, 459. One report describes the scene: "the rain descended in torrents and the ground for miles around in flame!" . . . "amidst thunder and lightning".

<sup>8</sup> *Sheffield Independent*, July 15 (1826).

<sup>9</sup> Wood, J., *Remarkable Occurrences and Interesting Dates . . .*, 108 (1890).

<sup>10</sup> Hunter, J., *op. cit.*, 460.

<sup>11</sup> Wood, J., *op. cit.*, 109.

<sup>12</sup> *S.C.R.*, 122 (1922). "There were scores of them [that is, fires] in England and Scotland".

<sup>13</sup> *S.C.R.*, 171 (1939). Where acreages are available they should be treated with caution; the popular Press quoted areas of 4-10,000 acres for the Big Moor fire—it is only about 2,000 acres. Hathersage Moor had "2,000 acres" of its 500 acres burnt. Ward's figures are reliable, and are taken, where possible, in preference to others.

<sup>14</sup> *S.C.R.*, 161 (1949).

<sup>15</sup> *S.C.R.*, 181 (1950).

<sup>16</sup> *S.T.*, September 9, 12, 15, 24, 26, 28, 29, 30 and October 2 (1959). Before the Burbage fire, there was 8 ft. of peat in places (*Geol. Mem. Sheffield*, 162; 1957).

<sup>17</sup> Moss, C. E., *Vegetation of the Peak District*, 173 (1913); Tansley, A. G., *British Isles and their Vegetation*, 672 (1939); Pearsall, W. H., *Mountains and Moorlands*, 65, 152 (1950).

<sup>18</sup> *S.T.*, August 6 (1960). *S.S.*, June 15 (1960).

<sup>19</sup> *S.T.*, May 7 (1938).

<sup>20</sup> Radley, J., "Peak District Roads prior to the Turnpike Era", *Derbs. Arch. J.*, 83, 39 (1963).

<sup>21</sup> See, for example, Bower, M. M., "The Cause of Erosion in Blanket Peat Bogs", *Scot. Geog. Mag.*, 18, 33 (1962); Radley, J., "Peat Erosion on the High Moors of Derbyshire and West Yorkshire", *East Mid. Geog.*, No. 17, 40 (1962).

<sup>22</sup> Young, A., "A Record of the Rate of Erosion on Millstone Grit", *Proc. Yorks. Geol. Soc.*, 31, 149 (1958). (It is probable that part of the moor to the south of Strines Inn has been burnt, but its extent is not known.)

<sup>23</sup> See, for example, Pearsall, W. H., *Mountains and Moorlands* (1950); he notes the contribution of fires to changes in bog vegetation (155-6) but nothing more.

## HIGH-RESOLUTION OBSERVATIONS OF THE RADIO SOURCES IN CYGNUS AND CASSIOPEIA

By PROF. M. RYLE, F.R.S., B. ELSMORE and ANN C. NEVILLE

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Cambridge

DURING the initial testing of the new Cambridge radio telescope<sup>1</sup> some observations have been made of the radio sources Cygnus A and Cassiopeia A at a wavelength of 21.3 cm. Though the observations of the former source do not add greatly to the information which has already been obtained<sup>2-4</sup>, by fitting simple models to the observed amplitude-spacing curves, the results for Cassiopeia A are of considerable interest because the complexity of this source cannot be established without the adoption of a full mapping procedure.

The instrument consists of one rail-mounted and two fixed 60-ft. paraboloids along an east-west axis; by

combining the signals from the movable aerial with those from each of the two fixed ones two interferometric systems are obtained simultaneously. If the observations made of the same region of sky over a 12-h period are combined, two rings of a large equivalent aerial can be synthesized<sup>1,5</sup>. By repeating the observations with different positions of the movable aerial, all the rings can be synthesized to build up an elliptical aperture approximately 1 mile in diameter.

When observations are made of a source which is small compared with the reception pattern of each individual aerial, it is unnecessary to use all the spacings and a

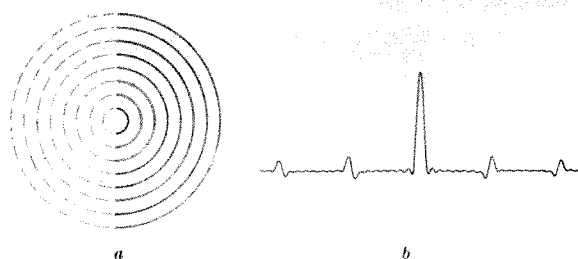


Fig. 1. *a*, Equivalent grating aerial; *b*, section across the centre of the corresponding reception pattern with an aperture grading which falls to 30 per cent for the outermost ring (spacing = 7,050  $\lambda$ )

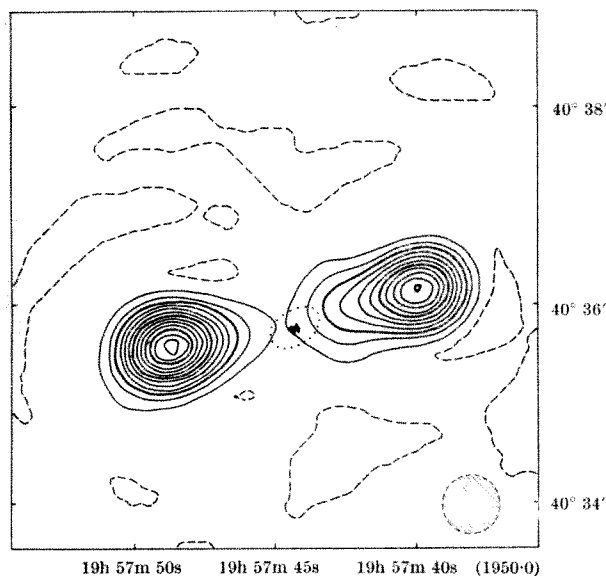


Fig. 2. Contour map of Cygnus A. The contour interval is 26,000° K. The beam to half power is shown in the corner of the map. The limits of the optical source are shown by the dotted ellipses

circular grating aerial may be synthesized as in Fig. 1*a*.

The reception pattern of such an aerial has circular grating responses surrounding the main beam, but these introduce no difficulties provided that the angular radius of the first of these responses is appreciably greater than the angular extent of the source under investigation, and that other sources in the vicinity are considerably weaker.

In the observations recorded here the two sources are contained within regions having diameters of approximately 1.8 and 5.0 min of arc, respectively, and measurements using 8 and 16 different aerial separations are adequate; four 12-h runs for Cygnus A and eight 12-h runs for Cassiopeia A were therefore made. The dipoles were orientated with position angle = 0°. The observations for each source were combined in *Edsac II* in a manner analogous to that used in an earlier survey of an area around the North Pole<sup>5</sup>, and the output maps were drawn automatically on a curve plotter attached to the computer. The maps shown in Figs. 2, 4 and 5 were obtained using a grading function which fell to 30 per cent at the edge of the aperture; this grading gives a reception pattern the width of which to half intensity is 23 sec of arc in right ascension and 23 cosec 8 sec of arc in declination with a first sidelobe response of

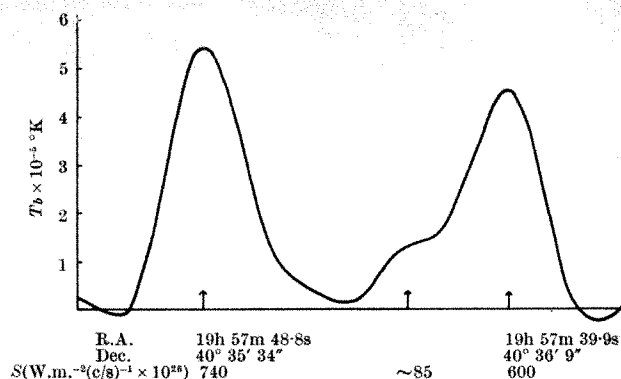


Fig. 3. Section along the major axis of Cygnus A. The co-ordinates (1950-0) of the peaks of maximum brightness are indicated, together with the integrated flux density from the different components. The separation of the major peaks is  $108 \pm 5$  sec of arc

5 per cent. The observations were also combined with uniform grading in order to provide slightly greater resolution so that an investigation could be made of structure which was barely resolved.

*Cygnus A*. The contour map is shown in Fig. 2, which also indicates the position of the optical galaxy; the relative positions of optical and radio sources are believed to be accurate to within  $\pm 5$  sec of arc in each coordinate. The section along the major axis (position angle = 109°) of the map obtained with uniform grading is shown in Fig. 3.

An examination of the distribution perpendicular to the major axis shows that for both the main components the distribution can be fitted by a Gaussian source which falls to half intensity at a distance of  $7.5 \pm 2.5$  sec of arc either side of the centre. The gradient of the outer edge of each component also corresponds to a similar distribution. As can be seen from Fig. 3, the inner gradients are less steep, and the central intensity falls to less than 5 per cent of that at the peaks. There is, however, evidence for a third component at  $\alpha = 19h 57m 43s$  which contributes about 6 per cent of the total flux density.

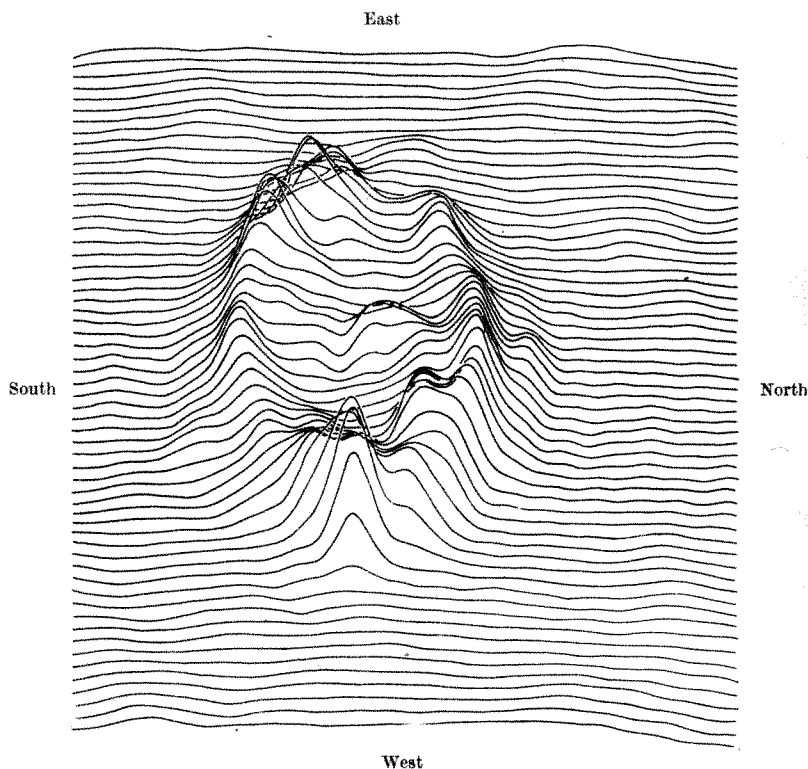


Fig. 4. Profiles of Cassiopeia A as presented by the automatic curve-plotter



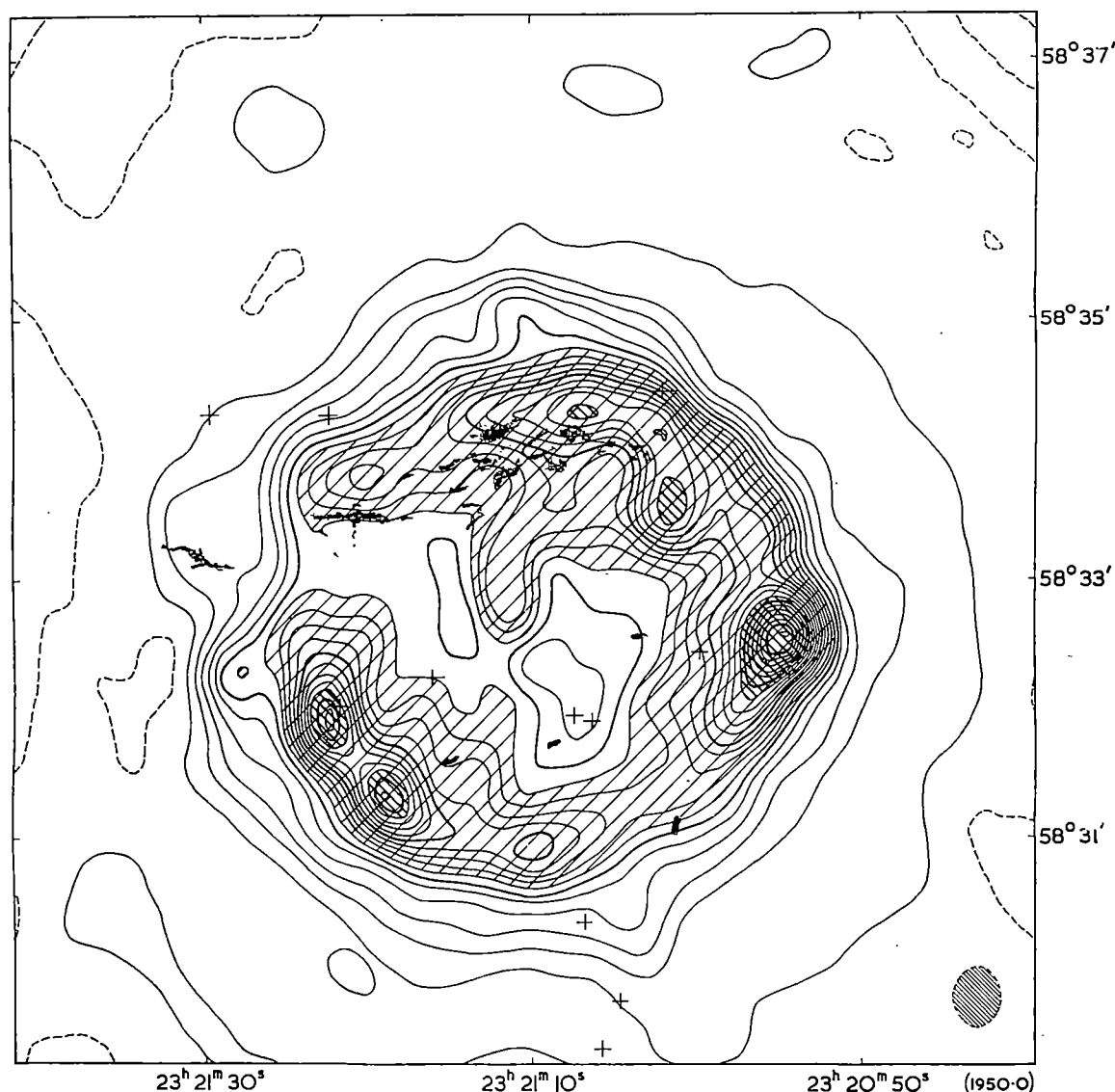


Fig. 5. Contour map of Cassiopeia A showing also the main optical filaments. The contour interval is 3,000° K. The positions of some of the brighter stars in the field are indicated by crosses

*Cassiopeia A*. The map derived for the source in Cassiopeia is presented as cuts across the two-dimensional contour map in Fig. 4, which shows that the main feature of the source is a remarkable ring of emission containing localized peaks of enhanced surface brightness.

The results are also shown as a contour map in Fig. 5. An interesting feature of the source is the gap in the ring diametrically opposite to the most intense peak. A comparison has been made between the structure which is revealed in the radio map and the optical filaments in the source. The area shows considerable and uneven obscuration, but a number of filaments are distinguishable; the positions of a number of these were measured from a print of Baade's original plate and were corrected for radial expansion. It can be seen from Fig. 5 that the intense peaks of the radio emission do not coincide with any optical feature; the most prominent optical filaments which are visible in the northern half of the source lie just within the ring of enhanced emission, although one falls in the gap in the ring.

Although it is evident that the source shows considerable non-uniformity, 90 per cent of the radio emission can be accounted for by a spherical shell of uniform and

isotropic emissivity, having a shell thickness  $\delta r$ , where  $\delta r/r = 0.25$ . This thickness is greater than that of the region containing the optical filaments<sup>4</sup>. Superimposed on any such simple model there must be local regions of greater volume emissivity to account for the large peaks in the observed distribution. It seems likely that the region of enhanced emission seen near the centre of the source ( $\alpha = 23^{\text{h}} 21^{\text{m}} 11^{\text{s}}$ ;  $\delta = 58^{\circ} 33'$ ) is similar to those seen in projection around the edge of the source. The volume emissivity within these localized regions must be considerably greater than the average emissivity of the shell; in the case of the most intense region ( $\alpha = 23^{\text{h}} 20^{\text{m}} 55^{\text{s}}$ ;  $\delta = 58^{\circ} 32'.6$ ) this enhancement is approximately 20.

Theories of the emission of radio waves from the shells of supernova remnants have been proposed by van der Laan<sup>7,8</sup>, based on the compression of the surrounding interstellar medium by the expanding material. The observation that Cassiopeia A is a shell source the outer limits of which are rather accurately circular supports van der Laan's model, but the large shell thickness implies that only a small compression of the original interstellar magnetic field has occurred. The very great emissivity of the shell (some  $10^8$  times that of the inter-



stellar medium) can then only be explained if most of the high-energy electrons responsible for the emission originate within the supernova, as proposed by van der Laan for the early stages of the expansion.

There appear to be a total of some 10–12 localized regions distributed around the shell which have an even greater volume emissivity; these sources, which have typical dimensions of 0.2–0.3 parsec, might be associated with regions of greater magnetic field strength.

We thank the Director of the University Mathematical Laboratory for the use of *Edsac II*.

<sup>1</sup> Ryle, M., *Nature*, **194**, 517 (1962).

<sup>2</sup> Lequeux, J., *Ann. d'Astrophys.*, **25**, 221 (1962).

<sup>3</sup> Maltby, P., and Moffet, A. T., *Astrophys. J.*, Supp., **7**, 141 (1962).

<sup>4</sup> Moffet, A. T., *Science*, **146**, 764 (1964).

<sup>5</sup> Ryle, M., and Neville, A. C., *Mon. Not. Roy. Astro. Soc.*, **125**, 39 (1962).

<sup>6</sup> Minkowski, R., *IAU/URSI Symp. Radio Astronomy*, Paris, 315 (1958).

<sup>7</sup> Laan, H. van der, *Mon. Not. Roy. Astro. Soc.*, **124**, 125 (1962).

<sup>8</sup> Laan, H. van der, *Mon. Not. Roy. Astro. Soc.*, **124**, 179 (1962).

## OBITUARIES

### Prof. J. M. F. Drummond

WITH the death of James Montagu Frank Drummond, emeritus professor of botany in the University of Manchester, at his home in Exmouth at the age of eighty-three, British botany has lost an unusually well informed academic and practical exponent. He was born in 1881, the second son of J. R. Drummond of the Indian Civil Service. He is survived by his second wife, *née* Dorothy Maria Farrant. His first wife, *née* Agnes Marguerite Ives, died in 1923.

Drummond was educated at King's College, London, and at Gonville and Caius, Cambridge, where he had the distinction of holding the Frank Smart research studentship during 1904–6. His first appointment as lecturer in botany at Armstrong College, Newcastle upon Tyne (1906), was followed, in 1909, by his joining the staff of Prof. F. O. Bower in the University of Glasgow as lecturer in plant physiology. There he remained until 1921, his teaching on various branches of botany being much appreciated by his students. It was during this period that he produced his masterly translation and up-to-date annotation of an eminent—and extensive—German text, Dr. G. Haberlandt's *Physiologische Pflanzenanatomie* (*Physiological Plant Anatomy*), 1914. His academic work was interrupted by the First World War when he saw active service with the Highland Light Infantry in France and the Palestine campaign.

When, in 1921, the Scottish Plant Breeding Station was established at Corstorphine, Midlothian, Drummond was invited to become its first director. His botanical interests, always broadly based and liberal, now turned in the direction of genetics, with special emphasis on the improvement of cultivated plants. Under his care the new Station was staffed and organized and was soon known for its varied and progressive activities. His work in this new field is recorded in the annual reports of the Station and in various public addresses. But still further opportunities were to come his way. In 1925, he was appointed Regius professor of botany in the University of Glasgow, and in 1930 he became George Harrison professor of botany in the University of Manchester, a post which he held until his retirement in 1946. During this period the trustees of the former Manchester Royal Botanical Society made a considerable bequest to the University to promote interest in horticulture in the region. On the funds thus made available, Prof. Drummond purchased and developed in Cheshire what are now the Jodrell Bank Botanical Experimental Grounds, and he also established a University lectureship in horticulture and a system of training for student gardeners. All this was very close to his heart: he was a true lover of plants and gardens and possessed an unrivalled knowledge of species, both native and exotic.

Much of the effect of Prof. Drummond's work lay, not so much in what he achieved under his own name, but rather in what he did for others and enabled them to achieve. For example, he greatly assisted Prof. Bower in re-editing, and in incorporating new material in, the third edition of *Botany of the Living Plant*. To all, but especially to his younger colleagues and students, he was unstinting of his help and encouragement of their work. His outlook,

thinking and writing were of unusual clarity and perspicacity. He held firmly to the view that a university was not simply a seat of learning but essentially a place both for study and character formation. This led him, among many other activities, to continue his interest in military training, and from 1932 until 1945 he was officer-in-command of the University Officers' (Senior) Training Corps. During the Second World War this greatly enlarged unit, which he organized and commanded with distinction, functioned as the 61st (Lancs.) Battalion of the Home Guard.

C. W. WARDLAW

### Mrs. Dorothea Waley Singer

MRS. DOROTHEA SINGER, the widow of Prof. Charles Singer, the historian of medicine and science, died on June 24, 1964. She was well known in many countries especially for her work on early manuscripts dealing with science and medicine, and for her activities on various international bodies.

Dorothea Waley Singer, the eldest daughter of Nathaniel L. Cohen, and of Julia M. Waley, was born in London on December 17, 1882. In early womanhood she spent some years at Queen's College, London, and in 1910 she married Charles Singer, who was then engaged mainly in clinical and pathological work. Soon afterwards they both became interested in the history of medicine and science and in 1913 they presented a joint communication on the history of the *contagium vivum* at the International Medical Congress held in London in that year. In following years they wrote several joint papers, dealing mainly with medieval medicine.

Meanwhile, Mrs. Singer had been very actively engaged on studying the surviving medieval manuscripts in the British Isles which dealt with different aspects of science and medicine. In 1916 she presented before the History Section of the Royal Society of Medicine a very careful and detailed study of more than a hundred plague tractates of the fourteenth and fifteenth centuries. She had for some years been trying to catalogue and codify the manuscripts in Britain which bore in any way on science and medicine, and by 1919 she knew of more than 30,000 many of which were, of course, direct copies or variants. The full fruition of these investigations began to be seen in 1924, when the Union Académique Internationale published the first volume of her monumental catalogue of alchemical manuscripts in the British Isles. The first volume is a small work because it deals only with the comparatively few Greek manuscripts in Britain. But the three succeeding volumes (1928–31) deal with the vast number of Latin and vernacular manuscripts, and they form a catalogue of great complexity and scholarship which will remain a standard work.

Mrs. Singer had continued to work on plague tractates and in 1950 she published a definitive catalogue of Latin and vernacular works on plague which dated from before the sixteenth century and were extant in Great Britain. Her cumulative catalogue, dealing with all scientific subjects, forms a very large collection of cards, and microfilms of it are deposited in some of the great libraries of the world.

Apart from this work on manuscripts Mrs. Singer wrote several books and many papers in learned journals. In 1924 she published a very useful study of Ambroise Paré, together with a generous selection from his writings. Her important long paper on Sir John Pringle and his times gives much information which is not readily available elsewhere. For nearly twenty years she was intermittently engaged on a detailed examination of the works of Giordano Bruno, and in 1950 her *Giordano Bruno, his Life and Thought* was published. It is a very scholarly and illuminating investigation of the work of that complex personality. Her last book to be published was a translation from the German of Prof. Klemm's *History of Technology* (1959).

Mrs. Singer was also well known for her activities in various societies, British and international, and for her great interest in social work. She performed an important service in the placing of girl refugees from Nazi Germany as student nurses in British hospitals, and for many years she did much to stimulate the improvement of hearing-aids for the deaf. Along with her husband she for many years provided generous hospitality to numerous foreign scientists and scholars visiting Britain.

E. ASHWORTH UNDERWOOD

### Dr. I. J. Kříženecký

DR. I. JAROSLAV KŘÍŽENECKÝ, director of the Gregor Mendel Museum in Brno, died on December 21, 1964, at the age of sixty-eight. He was born and educated in Prague. Between the two World Wars his researches into animal breeding took him to the United States and to England. In 1921 he moved to Brno, where he was professor of animal breeding and genetics in the College of Agriculture until 1949. In the 1950's he won the admiration and friendship of many biologists both in Czechoslovakia and in other countries for his resolute defence of Mendelian genetics. When plans for a Mendel Museum were made in 1962, Dr. Kříženecký was chosen to direct the work. It is largely due to his untiring efforts that a Department of Genetics has been founded in the Moravian Museum and a Memorial Hall is being prepared for the forthcoming Mendel symposium this summer. Dr. Kříženecký worked very hard despite great and protracted suffering to make this symposium a worthy tribute to Mendel. It is sad that he has not lived to complete his work. Czechoslovak scientists have lost a dedicated scientist who, like Mendel, was a humble man and a great teacher.

R. C. OLBY

## NEW FELLOWS OF THE ROYAL SOCIETY

AT a meeting of the Royal Society on March 18, the following were elected to fellowship of the Society:

DR. G. D. H. BELL, director, Plant Breeding Institute, Cambridge, distinguished for his application of genetical science to the advancement of plant breeding.

PROF. R. E. BELL, Rutherford professor and director of the Foster Radiation Laboratory in McGill University, Montreal, Canada, distinguished for his contributions to nuclear physics, especially for his measurements of very short life-times and of the binding energy of the deuteron.

DR. S. BRENNER, member of the Scientific Staff of the Medical Research Council Laboratory of Molecular Biology, University Postgraduate Medical School, Cambridge, distinguished for his contributions to molecular biology, especially in the study of the genetic material and its control of protein synthesis.

DR. G. S. BRINDLEY, lecturer in physiology at the Department of Physiology, in the University of Cambridge, distinguished as a physiologist, especially for his contributions to the study of human colour vision.

PROF. B. N. BROCKHOUSE, professor of physics in McMaster University, Hamilton, Canada, distinguished for his pioneering work on slow neutron spectroscopy, and for the applications of this technique to lattice dynamics and spin-waves.

PROF. A. R. COLLAR, Sir George White professor of aeronautical engineering in the University of Bristol, distinguished for his contributions to aero-elastic theory, the engineering application of matrices, aerodynamics, and for his work on aircraft flutter and stability.

SIR EDWARD COLLINGWOOD, mathematician, Alnwick, Northumberland, distinguished for his contributions to the theory of functions, and in particular for his work on cluster sets of arbitrary functions.

DR. R. R. A. COOMBS, assistant director at the Department of Pathology in the University of Cambridge, distinguished for his contributions to the immunological study of cell surfaces, of congenitins and immunoglobulins, and of allergic phenomena.

PROF. K. G. DENBIGH, Courtauld professor of chemical engineering and director of the Chemical Engineering Laboratories at the Imperial College of Science and Tech-

nology, in the University of London, distinguished for his application of chemical thermodynamics and kinetics to the optimization and improvement of chemical reaction processes.

PROF. G. E. FOGG, professor of botany at Westfield College, in the University of London, distinguished for his work on the metabolism of the algae and especially on nitrogen fixation in the blue-green algae.

DR. C. E. FORD, head of the Cytogenetics Section, Medical Research Council Radiobiological Research Unit, Harwell, distinguished for his contributions to the cytogenetics of mammals, and for his studies of chromosomal abnormalities in man.

PROF. R. A. GREGORY, George Holt professor of physiology in the University of Liverpool, distinguished for his work on the mechanism of gastro-intestinal movement and gastric secretion.

PROF. DOROTHY HILL, research professor of geology in the University of Queensland, Brisbane, Australia, distinguished for her researches on Palaeozoic fossil corals, their stratigraphical implications and the geological significance of their distribution on a world scale.

PROF. A. W. JOHNSON, Sir Jesse Boot professor of organic chemistry and head of the Department of Chemistry in the University of Nottingham, distinguished for his work on the organic chemistry of natural products, especially that of vitamin B<sub>12</sub>.

PROF. R. V. JONES, professor of natural philosophy in the University of Aberdeen, distinguished for fundamental physical measurements by novel instruments of exceptional precision; and for application of scientific methods to defence.

PROF. C. KEMBALL, professor of physical chemistry in Queen's University of Belfast, distinguished for his application of modern physico-chemical methods to the study of adsorption and catalysis at metal surfaces.

DR. J. S. KENNEDY, senior principal scientific officer, Agricultural Research Council Unit of Insect Physiology, Department of Zoology, Cambridge, distinguished for his contributions to the problem of insect migration and for his studies of the physiology and behaviour of locusts and greenfly.

PROF. T. KILBURN, professor of computer engineering in the University of Manchester, distinguished for his contributions to the development, design and engineering of electronic digital computers and high-speed computer components.

PROF. H. L. KORNBERG, professor of biochemistry in the University of Leicester, distinguished for his work on the elucidation of metabolic pathways in micro-organisms and of the control mechanisms governing them.

DR. H. KRONBERGER, scientist-in-chief, Reactor Group, United Kingdom Atomic Energy Authority, Risley, Warrington, Lancashire, distinguished for his applications of physical science to all aspects of the United Kingdom programme for the development of nuclear power.

PROF. C. P. LEBLOND, professor of anatomy in McGill University, Montreal, Canada, distinguished for his work on the physiology of the thyroid gland, cell population kinetics and on the cytology of protein synthesis.

PROF. P. MAHESHWARI, professor of botany and head of the Department of Botany in the University of Delhi, India, distinguished for his outstanding services to the advancement of science in India and for his sustained contributions to the embryology of seed plants.

PROF. B. J. MASON, professor of cloud physics at the Imperial College of Science and Technology, in the University of London, distinguished for his experimental and theoretical investigations into the physics of clouds and the electrification of thunderstorms.

PROF. W. V. MAYNEORD, professor of physics applied to medicine, at the Chester Beatty Research Institute, in the University of London, distinguished for his applications of physics to medicine, especially in the identification of chemical carcinogens, the rationalization of radiation dosimetry and the assessment of radiation hazards.

SIR GILBERT ROBERTS, partner, Freeman Fox and Partners, London, distinguished for his contributions to

civil engineering by advancing the design of structures, particularly long-span bridges.

PROF. S. K. RUNCORN, professor of physics and director of the Department of Physics in the University of Newcastle upon Tyne, distinguished for his experimental and theoretical researches on the Earth's magnetic field and for his studies of the palaeomagnetic properties of rocks.

PROF. P. M. SHEPPARD, professor of genetics in the University of Liverpool, distinguished for his contributions to ecological genetics and the study of natural selection in animal populations.

PROF. K. STEWARTSON, Goldsmid professor of mathematics and joint head of the Department of Mathematics at University College, in the University of London, distinguished for his contributions to our knowledge of boundary layer flow associated with the movement of bodies through compressible and incompressible fluids.

PROF. J. M. THODAY, Arthur Balfour professor of genetics in the University of Cambridge, distinguished for his research on the action of radiations on chromosomes and for his experimental studies of the genetical effects of selection.

PROF. J. C. WARD, professor of physics in the Johns Hopkins University, Baltimore, distinguished for his work in quantum field theory, particularly with reference to the renormalization of electric charge.

PROF. G. B. WHITHAM, professor and head of the Department of Applied Mathematics in the Graduate Aeronautical Laboratories, California Institute of Technology, Pasadena, distinguished for his development of new theoretical methods of calculating shock waves and other related hydrodynamical phenomena.

PROF. G. WILKINSON, professor of inorganic chemistry at the Imperial College of Science and Technology, in the University of London, distinguished for his contributions to nuclear and inorganic chemistry, particularly in relation to the structure of organo-metallic complexes.

## NEWS and VIEWS

Chief Veterinary Officer of the Ministry of Agriculture, Fisheries and Food : Sir John Ritchie, C.B.

ON April 2 Sir John Ritchie retires from the post of chief veterinary officer of the Ministry of Agriculture, Fisheries and Food. His period of thirteen years in this office has been exceeded by only one of the previous eight holders of the Government's major veterinary appointment. John Neish Ritchie was born at Turriff, Aberdeenshire, in 1904, and obtained his professional qualifications at the Royal (Dick) Veterinary College and the University of Edinburgh. He held appointments with the City of Edinburgh, the County of Midlothian, and then in 1935 with the Department of Agriculture for Scotland in charge of the Tuberculosis Attested Herds Scheme. In 1938 Ritchie became a superintending veterinary inspector of the newly formed Animal Health Division of the Ministry of Agriculture and Fisheries; he was promoted in 1945 to deputy chief veterinary officer and in 1952 to chief veterinary officer. His name will always be remembered for his work in the eradication of bovine tuberculosis; thirty years ago 40 per cent of dairy cattle were affected with this disease, which has now virtually been eliminated. In the international field he has played a major part in keeping foot-and-mouth disease away from Great Britain, where there has been no case of this disease since 1962. In 1955 the Royal College of Veterinary Surgeons elected him a Fellow in recognition of special eminence in veterinary science, and in 1959 he became its president. He

was awarded the C.B. in 1955 and was made Knight Bachelor in 1961, and in that year, 1962 and 1965 he received honorary doctorates from the Universities of Liverpool, Toronto and Edinburgh. Sir John Ritchie is, in October 1965, to become Principal and Dean of the Royal Veterinary College of the University of London.

J. Reid

MR. JOHN REID, who is to succeed Sir John Ritchie as chief veterinary officer of the Ministry of Agriculture, Fisheries and Food, brings a ripe experience to the post. He was born in Berwickshire in 1906 of farming stock and was brought up in Perthshire, where he was educated at the McLaren High School, Callander. Having developed a keen interest in livestock, he entered the Royal (Dick) Veterinary College at Edinburgh, where he qualified as a member of the Royal College of Veterinary Surgeons in 1929. He gained the Diploma in Veterinary State Medicine in 1931. He worked successively with two local authorities, Midlothian and Cumberland, gaining wide experience with bovine tuberculosis and other diseases of the dairy herd, and with meat inspection techniques. In 1938, when the Animal Health Division was set up, Reid was appointed a divisional veterinary officer and as such worked in Breconshire, at the Ministry's headquarters in London and once more in Cumberland, where he was closely associated with the campaign against sheep scab—a disease which was eradicated from Great Britain in 1952. In

1952, on promotion to superintending veterinary officer, Reid returned to London. In the past twelve years he has taken increasing responsibility in connexion with the control of animal diseases, particularly those of poultry. In 1963 he was appointed as the first director of veterinary field services, a post parallel to the directorship of the veterinary laboratories. Reid's career has progressed steadily to the post of chief veterinary officer in which he will be professional head of some 550 Civil Service veterinarians in the field, the Veterinary Laboratories and the Veterinary Investigation Service.

#### Biochemistry in King's College, London:

Prof. H. R. V. Arnstein

DR. H. R. V. ARNSTEIN, a member of the scientific staff of the Medical Research Council at the National Institute for Medical Research, has been appointed to the chair of biochemistry in King's College, London, in succession to Prof. H. Harris, who has been appointed Galton professor of human genetics at University College, London (see *Nature*, 187, 286 (1960); 198, 1148 (1963)). Dr. Arnstein, a graduate of the Imperial College of Science and Technology, London, was one of the original team working on the structure of penicillin under Sir Ian Heilbron and Dr. A. H. Cook. After spending a year as a Post-doctoral Fellow at the University of Rochester, New York, he joined the Biochemistry Division of the National Institute for Medical Research at Hampstead in 1948 and collaborated with Dr. A. Neuberger on glycine metabolism. In his work on penicillin biosynthesis, and more recently on protein synthesis, he has been one of the leaders in the application of radioactive isotopes to biochemical problems. Dr. Arnstein has also been an active officer of the Biochemical Society, having served both as meetings secretary and as committee secretary, as well as being for several years a member of the Editorial Board of the *Biochemical Journal*.

#### Applied Physical Sciences in the University of Reading:

Prof. P. D. Dunn

MR. P. D. DUNN, principal scientific officer in the Applied Physics Group of the Atomic Energy Research Establishment, Harwell, has been appointed to a professorship of applied physical sciences in the University of Reading. Mr. Dunn gained B.Sc. degrees at Nottingham (1950) and London (1951). In 1950 he joined the Linear Accelerator Group of the Atomic Energy Research Establishment at Malvern, where he was concerned with various problems of electron linear accelerators and a design study of a linear magnetron. The Group moved to Harwell in 1953 to build a proton linear accelerator, and his particular interest was in systems of coupled resonators for acceleration from about 150-MeV upwards. In 1957 he was much involved in the design studies leading to the choice of a constant gradient proton synchrotron (*Nimrod*) for the National Institute for Research in Nuclear Science, and he set up a group to design, construct and commission the radiofrequency accelerating system. Concurrently, he started an entirely separate programme of work on the investigation of thermoelectric, thermionic and magnetohydrodynamic methods of producing electricity directly from heat, and the consideration of feasibility in relation to reactors. In 1960 he was promoted to a Band post (senior principal scientific officer equivalent) as group leader of the two groups. In September 1963, after the successful commissioning of *Nimrod*, he again became a full-time member of Harwell staff and the direct conversion studies were somewhat broadened in the Applied Physics Group which he led. The factors which have contributed to the undoubted success of so many activities are originality, a sound knowledge of physics and engineering, and the ability to direct and inspire others. Besides having a general interest in further education Mr. Dunn has been greatly concerned with the training of engineers at Harwell. He has frequently

lectured on engineering and allied topics at training courses and has directed particular attention to the training and supervision of young engineers who have worked in his group on various problems of engineering physics. He is an associate member of both the Institution of Electrical Engineers and the Institution of Mechanical Engineers.

#### The Linnean Gold Medal and the H. H. Bloomer Award

DR. J. HUTCHINSON, lately keeper of museums of botany, Royal Botanic Gardens, Kew, and Dr. J. Ramsbottom, lately keeper of botany, British Museum (Natural History), has been nominated for the award of the Linnean Gold Medal for 1965. The Linnean Gold Medal, the Society's highest award, is awarded annually in recognition of the recipient's services to science. Mr. E. C. Wallace has been awarded the H. H. Bloomer Award. This Award is given to an amateur naturalist in recognition of important contributions to biological knowledge. The presentations will be made at the anniversary meeting of the Linnean Society on May 24.

#### Relationship of Pharmaceutical Industry and the National Health Service

IN the House of Commons on March 3, the Minister of Health, Mr. K. Robinson, announced that he had decided to set up a Committee of Enquiry to examine the relation of the pharmaceutical industry in Great Britain with the National Health Service, having regard to the structure of the industry, to the commercial policies of the firms comprising it, to pricing and sales promotion practices, to the effects of patents, and to the relevance and value of research. The Committee would draw up recommendations based on its findings. In reply to questions, Mr. Robinson added that he thought it would be impossible to examine British pharmaceutical companies which were subsidiaries of American companies without examining the relation between the two; the establishment of the Committee had been welcomed by the industry, with which there had been informal consultations.

#### The Science and Technology Bill

AT the report stage of the Science and Technology Bill in the House of Lords on March 9, the Parliamentary Secretary to the Ministry of Technology, Lord Snow, said that, to meet certain objections raised by Lord Bridges, the Government was making suitable administrative changes. The Science Research Council would have delegated authority to approve projects for grants up to £100,000 and also power to authorize the creation of new posts in its laboratories to a level equivalent to senior principal scientific officer in the Civil Service. As regards the National Institute for Research in Nuclear Science, the Science Research Council would replace the Atomic Energy Authority and approval of the Department and of the Treasury would only be required for items of expenditure exceeding £100,000. Lord Snow also met Lord Bridges's views by moving an amendment giving effect to the Government's decision to authorize the Science Research Council to recruit new staff to the Rutherford Laboratory of the Institute on terms enabling them to be members of the Atomic Energy Authority's pension scheme if they so chose. It was not intended to apply this provision to the Daresbury Laboratory, and it was not intended to make any difference in terms of employment for those recruited under the Atomic Energy Authority's scheme if they went to other employment under the Science Research Council. At the third reading on March 11, Lord Bridges questioned the wisdom of excluding the 100 members of staff at Daresbury from the Atomic Energy Authority's pension scheme if they wished to join. Lord Snow replied that this was an administrative decision. The Bill, as amended, passed its third reading, and was returned to the House of Commons, where the Lords amendments were agreed on March 15.



### Desalination Research

IN reply to a question in the House of Lords on March 11 regarding progress in the problem of desalting water, Lord Snow said that the Water Research Association had made good progress in studying the economic factors underlying the provision of sweet water, and a mission was to leave shortly for the United States for discussions with the Office of Saline Water. The Committee on Desalination Research had considered a research programme in conjunction with industry, and the Atomic Energy Authority had undertaken work on the use of nuclear energy for a desalination plant. The Committee and the Authority had watched closely work in the United States and elsewhere. British industry had already established, primarily for arid areas, a commercially proved method for converting sea-water into fresh in the multi-stage flash evaporator which was regarded as the most efficient in use to-day. Over the past 10 years British firms had supplied some 40 installations to various parts of the world, representing more than 70 per cent of the land-based plants in operation. The Government recognized the need to support research to maintain Britain's lead in a potentially growing export market, and the Minister of Technology hoped to make a statement fairly soon on plans for increasing the scale of effort on desalination research. Lord Snow said he was aware of the design studies at the Oakridge National Laboratory of the Atomic Energy Commission in Tennessee for a desalting plant with a capacity of 20 million gallons a day, with a possible increase of up to 50-100 million gallons a day. Co-operation was continuing in basic research, but Britain's effort would have to be increased if she were to maintain her present strong position.

### British Space Research

IN written answers in the House of Commons on March 5, the Secretary of State for Education and Science, Mr. A. Crosland, stated that the 3 scientists and 4 engineers in the Space Research Management Unit of his Department were the only ones engaged on space research and directly responsible to him. They would be transferred to the Science Research Council when that Council was formed. The Convention establishing the European Space Research Organization came into operation in March, 1964, and a financial protocol provided for a programme of space research (with sounding rockets and satellites) over the 8 years up to 1972. The programme for 1965 provided for launching 33 experiments in 14 payloads with sounding rockets. Contracts were being placed for 27 *Centaure* rockets from Sud Aviation de France and 20 *Skylark* rockets from the British Aircraft Corporation, and a contract for a satellite to investigate solar X-rays and cosmic radiation (to be launched in the spring of 1967) was being negotiated with Hawker-Siddeley Dynamics. Preliminary design studies had been completed for a polar ionospheric satellite to be launched towards the end of 1967 and tenders for its development invited. A large satellite of stellar astronomy experiments was scheduled for launching in 1968-69 and preliminary proposals for payloads were being considered. The Organization had established a space technology centre in the Netherlands and was establishing a space laboratory nearby. A data centre was being established in Germany and a launching range at Kiruna in Sweden. The Organization awarded fellowships to enable space scientists from member states to study in other member states or, under an agreement with the National Aeronautics and Space Administration, in the United States.

### Co-operation in Pharmacology between Milan and Buffalo

ARRANGEMENTS have been concluded for a close working relationship between the Institute of Pharmacology of the University of Milan (director, Prof. E. Trabucchi)

and the Department of Biochemical Pharmacology of the State University of New York at Buffalo (chairman, Prof. J. F. Danielli). The arrangements were evolved after a visit by Prof. R. Paoletti to Buffalo, and a visit by Dean D. H. Murray of the School of Pharmacy at Buffalo, together with Prof. Danielli, to Milan. Collaboration between the Institute and the Department will involve exchange of staff, of research students and of technicians, the sharing of certain equipment, and the organization of a joint symposium annually. In 1965 the first symposium, on "Cell Permeability and Transport Phenomena", will be organized from Milan (secretary, Dr. L. Bolis, Via Alamanni, 19, Milan, Italy). The 1966 symposium will be held at Buffalo on "Drugs Affecting the Immune Response", and the 1967 symposium will be held at Milan on "Peptides".

### New Museum and Art Gallery, Doncaster

H.R.H. PRINCESS MARGARET, COUNTESS OF SNOWDON, accompanied by the Earl of Snowdon, officially opened the new Museum and Art Gallery, Doncaster, on October 30, 1964. The new building cost just more than £291,000 and replaces the old museum and art gallery, now demolished, which was housed in an eighteenth-century building for 55 years. The new building consists of a main exhibition block on two floors, the ground floor being Museum and the first floor Art Gallery, with a part used temporarily for costume and uniforms. About half the Art Gallery is air conditioned. Attached to the main block is a separate wing containing the administrative offices, laboratories, store-rooms and workshops.

### Western Australian Museum, Perth

THE Western Australian Museum has received many objects from the wrecks of the Dutch East Indiamen of the seventeenth century, now lying on the sea-bed at no great distance from the shore. The Museum has been kept fully informed of this interesting work, but there is no existing legal provision which would enable the State to control the recovery of the material from these ships, which antedates Captain Cook by more than 100 years, and is thus of great interest in early Australian history. The objects already handed over to the State collection include an astrolabe, two apothecary's mortars, a protractor and a pair of navigator's dividers. The annual report of the Museum for 1963-64 includes a very useful list of type specimens in the zoological collections (Pp. 46. Perth: Western Australian Museum, 1964).

### The Museums Journal

THE *Museums Journal* for December 1964 (3, No. 3) includes an interesting and useful paper on typography in museums, and emphasizes the importance of the visual impact made by a wise selection of printed display matter. The prehistoric collections at the National Museum in Prague are described especially from the point of view of modern display. The remainder of the issue gives an account of the 1964 annual conference at Nottingham and includes a verbatim report of the presidential address by Dr. Dilwyn John. The *Journal* is produced in the attractive format which it has used during the past few years.

### Man

THE journal *Man* for November-December 1964 contains a number of important articles. On the social anthropological side there are papers on "Networks in Indian Social Structure", by Prof. M. N. Srinivas and André Bételle, and on "New Guinea Highland Models and Descent Theory", by Dr. R. F. Salisbury. Two accounts of iron smelting, one in north-east Nigeria and the other in Zambia not far from Livingstone, are also of special interest. In Nigeria the ore is magnetic iron ore, said by Przeworski not to be suitable for smelting, though David Livingstone described cases where it was

used as long ago as 1874. The magnetite is mixed with charcoal in a special furnace and duly fired. When trade started with western Europe the local smelting nearly ceased, but the imported steel was of such a poor quality that it has all started up again. The Zambia example from a site at Mr. Strydom's farm some 8 miles from Livingstone is not dissimilar. But no mention of the kind of iron ore used is given. Smelting has been observed (Burkitt, M. C., unpublished) at Serenje, also in Zambia, but a good way north of Livingstone. Here the ore was a carbonate and hence much more easily smelted. There is plenty of interesting material in this number of *Man*, but the attention of all anthropologists should be especially directed to the long and sympathetic obituary of the late Lord Raglan by Prof. C. Daryll Forde.

### Archaeological Excavations

A MEMORANDUM on the subject of safety precautions in archaeological excavations has been prepared by the Council of British Archaeology. It will act as an *aide-mémoire* to site directors and supervisors of archaeological excavations and also give valuable information to societies for whom they may be working. Copies (6d. plus postage) can be obtained from the Council of British Archaeology, 10 Bolton Gardens, London, S.W.5.

### The Acorn Lectures

VOLUME 2 of the Acorn Lectures, delivered at the A. D. Little Research Institute, Inveresk, contains abridged versions of four lectures, namely those of Dr. C. B. Amphlett on "Inorganic Ion Exchanges"; Dr. D. W. Pashley on "Electron Microscope Studies of Thin Films"; Prof. C. E. H. Bawn on "Stereoscopic Forces in Free Radical and Ionic Polymerization"; and of Prof. R. H. Thomson on "Black Pigments" (Pp. v+51. Inveresk: Arthur D. Little Research Institute, 1964). The lectures are not dated and the volume is edited by W. Banks.

### Metal-to-Metal Bonds

THE number of compounds in which metal-to-metal bonds have been shown to exist has greatly increased during the past few years. Such compounds may be divided into various classes, depending on the kind of interaction between the metal atoms which, for transition metals, may be direct or indirect, involving interaction via other atoms. Compounds of the first type show weak interaction or strong co-valent bonding, depending on the metal chosen, the oxidation state, and the attached ligands. Bonds between dissimilar transition metal atoms are less common than those between similar metal atoms, but this appears to be due to lack of purposeful effort to obtain such derivatives until recently. The extent to which co-valent, ionic, multi-centre, and indirect interaction occurs in these compounds has been discussed by Prof. J. Lewis of the University of Manchester and Prof. R. S. Nyholm of University College, London, in *Science Progress* (52, No. 208; 1964). The role of physical methods in assessing this is also reviewed.

### Soil Fertility and Elements of Rural Sociology in Africa South of the Sahara

IN its participation in the campaign against hunger, the Centre de Documentation Economique et Sociale Africaine has produced a bibliography entitled *Fertilité des Sols et Eléments de Sociologie Rurale en Afrique au Sud du Sahara*, by J. Lebrun and P. C. Lefevre (Pp. xvii+182. Bruxelles: Centre de Documentation Economique et Sociale Africaine, 1964. 500 francs). The object has been to collect as many as possible of the publications dealing with the soils and their fertility, especially as related to social problems, in order to assist in finding remedies for the problems concerning food production. There are 1,413 references to authors in alphabetical order, with the titles of their work, and a list of the periodicals

cited. These references have also been summarized by number under various heads such as agricultural development, soils, erosion, productivity, fertilizers and silviculture. There is a geographical index.

### Announcements

DR. W. C. ELMORE, chairman of the Department of Physics and professor of physics at Swarthmore College, Dr. F. Verbrugge, associate Dean of the Institute of Technology and professor of physics at the University of Minnesota, and Dr. L. R. Weber, department head and professor of physics at Colorado State University, have received distinguished service citations from the American Association of Physics Teachers, for their outstanding contributions to the teaching of physics in colleges and schools.

IN No. 789 in the Science Library Bibliographical Series some references to hovercraft from 1959 to 1964 are collected, both from books and periodicals, as well as reports and British patents specifications (Pp. 13. London: The Science Museum, 1964).

A SYMPOSIUM on "Pump Design, Testing and Operation" will be held at the National Engineering Laboratory during April 12-14. Further information can be obtained from the Organizing Secretary, Pump Symposium, National Engineering Laboratory, East Kilbride, Glasgow.

A GENERAL discussion of the Faraday Society on "The Kinetics of Proton Transfer Processes" will be held in the University of Newcastle upon Tyne during April 12-14. Further information can be obtained from the Assistant Secretary, the Faraday Society, 6 Gray's Inn Square, London, W.C.1.

THE first meeting of the Environmental Group will be held in the Imperial College of Science and Technology on April 9. Dr. Chrenko will address the meeting on the subject of "Freshness". Further information can be obtained from the Secretary, the Environmental Group, 16 Gloucester Place, London, W.1.

THE spring meeting of the British Society of Rheology on "The Flow of Powders and Granular Materials" will be held in Nottingham during April 7-8. Further information can be obtained from Mr. R. J. Cole, British Society of Rheology, Paint Research Station, Waldegrave Road, Teddington, Middlesex.

THE spring conference of the X-ray Analysis Group of the Institute of Physics and the Physical Society will be held in the University of Edinburgh during April 8-9. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "Microbiological Deterioration in the Tropics", arranged by the Microbiology Group of the Society of Chemical Industry, will be held in London during April 8-9. Further information can be obtained from the Honorary Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

A CONFERENCE on "Atomic Spectra and Radiation Processes", arranged by the Institute of Physics and the Physical Society, will be held in the Clarendon Laboratory, Oxford, during April 12-14. Further information can be obtained from the Administration Assistant, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A RESIDENTIAL conference on "The Educational Requirements for the Professional Inspection and Quality Engineer", arranged by the Institution of Engineering Inspection, will be held at the Chelsea College of Science and Technology during April 11-14. Further information can be obtained from the Secretary, Institution of Engineering Inspection, 616 Grand Buildings, Trafalgar Square, London, W.C.2.

## OVERSEAS DEVELOPMENT AND SERVICE BILL

THE Overseas Development and Service Bill had an unopposed second reading in the House of Commons on February 24. The Minister of Overseas Development, Mrs. B. Castle, claimed that the Bill reflected the spirit of the discussions of the United Nations Conference on Trade and Development, and in moving its second reading explained that Clause 1 would assist good planning of colonial development and welfare by providing a year's overlap. Under this Clause, a further £50 million was provided for schemes financed by grants, and a further £20 million for loans, which with the outstanding amounts gave totals of £95 million for grants and £40 million for loans for the five years up to 1970, for a much smaller number of dependent territories than was covered by the £68.5 million and £32 million, respectively, provided for the three years 1963-66 under the 1963 Act. About thirty Governments still came within the scope of the Colonial Development and Welfare Acts, but Mrs. Castle thought it unlikely that actual expenditure would reach the ceiling of £25 million for grants for the first three years or £10 million for loans. The Bill also lowered the proportion of loans to grants and provided 70 per cent of this aid in grants, and she hoped to see the aid given in a form in which it could be speedily used for development.

Clause 2 of the Bill extended the provisions of the Overseas Service Act, 1961, which it replaced, under which 41 Governments and administrations had entered into agreements, and during the present year more than 11,000 officers were receiving benefits at a cost to the British Government of more than £16 million. During 1964, nearly 1,800 officers had been recruited by the Ministry and requests were still increasing. So far, the scheme had been limited to officers in the central public services of overseas Governments who were expatriate members of the Overseas Civil Service or expatriate contract officers appointed in the same way. Under this

Clause, the Minister was authorized, with the agreement of the Treasury, to extend the scheme to bodies and organizations overseas other than Governments who were employing expatriate officers, and to public or social services such as education or local government. Mrs. Castle said that while no general offer of help would be made, she would consider any requests which might be received on their merits. At the third Commonwealth Education Conference at Ottawa in August 1964, financial assistance had been offered towards the salaries of British teachers in overseas universities, and Mrs. Castle said she proposed to honour this undertaking through the extension of the aid scheme if the Bill were passed. She also emphasized that under the present scheme Britain had been at pains not to disturb the relation between overseas Governments and the expatriate officers they employed or to encourage any division of loyalty: she believed that this principle was right. Finally, she stressed the importance of enlarging and improving Britain's technical assistance.

The Bill was generally welcomed in a well-informed debate, and in winding up for the Government, the Parliamentary Secretary to the Ministry of Overseas Development, Mr. A. E. Oram, said that whereas under the 1963 Act £109 million was provided over three years for a population of 18.75 million, under the present Bill £135 million over five years was made available for 5.5 million. In a written answer on the same day, Mrs. Castle stated that bilateral economic aid to developing countries disbursed by the Government in 1963 amounted to £69.2 million in grants and £69.7 million in loans; for 1964 the corresponding figures were £81.9 million and £92.5 million. In addition, £13.3 million in 1963 and £13 million in 1964 were contributed in multilateral aid to international organizations and £5.7 million and £3.0 million, respectively, in multilateral aid in other ways. In 1963 private investment contributed about £65 million.

## PROBLEMS IN SETTING UP NEW UNIVERSITIES

A PAPER, *The Creation of New Universities\**, has been prepared by Dr. C. I. C. Bosanquet and Mr. A. S. Hall, based on a conference held at the University of Keele in July 1964. The conference was attended by representatives of all the existing new universities, and its purpose was to assess experience in planning, their physical layout and their academic strategies. Moreover, it was hoped that it would indicate the distinctive features of the British contribution to thought and design, and the common features of the technique used in establishing new universities and of the public pressures they illustrate. By and large, the authors conclude that disappointingly little use has been made of the opportunity for innovation. While the new universities are being created with remarkable speed and administrative skill, the range of subjects and types of teaching offered are unlikely to be altered significantly, but the kind of education hitherto confined to Oxford and Cambridge would be more widely distributed in the United Kingdom.

The sites so far chosen, with the exception of Warwick, lie on previously undeveloped land near the outskirts of towns of medium size, usually of historic interest, lacking major industry, but centres of local administration. The

older industrial areas, as a rule, have failed even to create a 'promotions committee'. The form of constitution evolved does not differ significantly from the constitution of twenty universities that have come into being in Britain since 1890. No daring experiments have as yet been tried—the main reason for this is regarded as being money: no university feels it can be independent of some kind of local support.

The four formal sessions of the conference covered the shaping of university development plans; curriculum and teaching; staff and student residential arrangements and social life; academic organization and university government.

While there was some sharp difference of opinion over internal organization, such as the extent to which it should centre on the college system or around schools of study, how far a university should impose its guardianship on its students, the degree of responsibility exercised by the University Grants Committee in regard to subjects of study and the relation of universities to Parliament, there were two main points of agreement. First, in development, strategy as well as tactics must be subject to continuous review, taking into account experience gained by the individual university, overall national needs, and the availability of reserves. Secondly, the greatest single difficulty has been inadequate reserves during the planning

\* *The Creation of New Universities*. (Based on a Conference at the University of Keele, July 14 to 18, 1964.) By C. I. C. Bosanquet and A. S. Hall. Pp. 14. (Keele: The University, 1964.)

period. The policy of the University Grants Committee to make no recurrent grants until a university receives its first students has postponed the appointment of key academic staff and necessitated the taking of decisions affecting the shape of a university for years to come, without the benefit of continuous academic advice. The very short period in advance for which capital grants have

been indicated has also delayed physical planning, and while it is essential that a new university should be assured of the sums of money that may be committed over each of the next five years, it would be still more helpful to indicate capital grants for ten years ahead. This financial assurance would also assist the start of the scholarly and scientific work of the university.

## REPRODUCTION OF THE AFRICAN ELEPHANT

CONTINUING his researches on the reproduction of the African elephant, Dr. J. S. Perry has examined eight foetal specimens collected during a two-year stay in Uganda\*. In one paper Prof. E. C. Amoroso and Dr. Perry have investigated the foetus *in utero* in a series ranging from the smallest, weighing only 2 g, to a near-term male of 120 kg. Adequate preservation in the field enabled the authors to describe not only the gross structure of the foetal membranes, but also the microscope anatomy of the placenta. The placenta of the elephant is zonary, but the allanto-chorionic villi are thinned or interrupted at intervals on the circumference of the chorionic sac. The large allantois is quadrilobular; its four sacs completely separate amnion and chorion, fusing with the former and meeting over the back of the foetus. A yolk sac was present in the youngest foetus of approximately two months gestation. The endodermal lining of the allantois bears a large number of mushroom-shaped "allantoic pustules" of unknown function.

The placenta is of the vaso-chorial type of Amoroso and Wislocki. The single-layered trophoblast is partly cellular and partly syncytial. Marginally maternal blood

exudes and is ingested by the cytotrophoblast. Centrally on the placental band active phagocytosis occurs. The elephant, hyrax and manatee "share a number of features in the arrangement of the foetal membranes and in the detailed structure of the placenta". Fourteen half-tone plates illustrate the work.

In the second paper, which is an extension of that published in 1953, Dr. Perry describes the reproductive organs of the female African elephant in foetal and young animals. The remarkable feature of the external genitalia is the similarity between male and female, in that the urogenital canal has been carried forward on the belly of the female to a point close behind the umbilicus, to a position similar to that of the male opening. The uterine horns undergo a ram's horn flexure, as in some ungulates, before uniting to form the body of the uterus, but the lumina remain separate for most of its length, and it is here that a one-sided conception occurs. The muscles of the external portion of the female genitalia are described. There are four plates of photographs of dissections and transverse sections of the reproductive tract, and seventeen text figures.

These papers give us for the first time a clear picture of the foetal membranes and placenta of the African elephant.

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\* *The Foetal Membranes and Placenta of the African Elephant (Loxodonta africana)*. By E. C. Amoroso and J. S. Perry. *The Structure and Development of the Reproductive Organs of the Female African Elephant*. By J. S. Perry. *Phil. Trans. Roy. Soc., B*, 248, 1, 35 (1964).

## CHEMISTRY AND PHYSIOLOGY OF 'DORMINS' IN SYCAMORE

### Identity of Sycamore 'Dormin' with Abscisin II

GROWTH-inhibitory compounds ('dormins') play a part in the regulation of bud-growth of some woody perennials<sup>1-6</sup>. In sycamore and in birch, a change from long-day to short-day conditions produces a marked increase in dormin concentration and the buds stop growing; dormin-containing extracts of leaves from birch plants grown under short-day conditions inhibit the growth and induce the formation of resting buds in actively growing birch seedlings maintained under long-day conditions. Owing to the considerable technical difficulties attending the determination of bud-inducing activity, concentration of dormin is usually estimated by inhibition of wheat coleoptile growth.

On the other hand, Addicott *et al.*<sup>6,7</sup> have isolated a plant hormone (abscisin II) from young cotton fruit by following its abscission-accelerating effect in cotton explants. Abscisin II was also found to inhibit oat coleoptile growth<sup>6,7</sup>. We have isolated the dormin from sycamore (*Acer pseudoplatanus*, L.) in crystalline form and have established its identity with abscisin II.

Chopped, dried sycamore leaves, collected in the autumn of 1963, were extracted at 4° with 4:1 (v/v) methanol:water. The methanol was removed under reduced pressure; the residual aqueous solution, decanted from the precipitated chlorophyll and acidified to pH 3.5, was extracted with ether. The dormin was extracted from the ethereal solution into aqueous sodium bicarbonate; continuous ether-extraction of the bicarbonate solution, adjusted to pH 8.0, removed most of the weakly

acidic inactive compounds; subsequent acidification to pH 3.5, ether-extraction, and evaporation of the ethereal solution yielded a rancid-smelling gum containing the dormin. This was dissolved in a slight excess of aqueous sodium hydroxide and treated with aqueous lead acetate which precipitated much inactive material, but left dormin in solution, from which it was extracted with ether after acidification. Further purification was by column chromatography (silicic acid, eluted with benzene-ether mixtures) followed by thin-layer chromatography ('Kiesel-gel-G'), developed with a propanol-butanol-ammonia-water (6:2:1:2 v/v) mixture. This gave a colourless partly crystalline gum, recrystallization of which from benzene yielded 260 µg of the dormin as minute platelets, m.p. ~ 160° (Kofler block), which sublimed at ~ 130° in a high vacuum. For abscisin II, Addicott *et al.*<sup>6,7</sup> give m.p. 160°–161°, subl. 120°.

Inhibitory activity was followed by germinating excised wheat embryos<sup>8</sup> for 48 h at 27° in the dark in 0.01 M sodium phosphate buffer (pH 7.4) containing the test material at five or more geometric dilutions. Probit percentage reductions in coleoptile length were plotted against log<sub>10</sub> concentration to give a straight line. The concentration reducing length by 50 per cent was used to compare the activities of the test material. We have detected only one compound showing high growth-inhibitory activity in sycamore leaves.

The molecular weight of the dormin, determined by mass-spectrometry, was 264, identical with that reported<sup>6,7</sup> for abscisin II. The infra-red spectrum (potassium



chloride pressed disk) was identical with the published spectrum<sup>6,7</sup> of abscisin II, except for the intensity of the peak at 1,155  $\text{cm}^{-1}$  (sycamore dormin, *vv*; abscisin, *m*); in the 2,000–1,500  $\text{cm}^{-1}$  region the dormin had  $\nu_{\text{max}}$  1,670 (*vs*, shoulder), 1,650 (*vs*) 1,630 (*s*, shoulder), 1,600 (*s*, sharp). In the ultra-violet, the dormin absorbed at 260 and 245 (shoulder)  $\text{m}\mu$  in neutral or acidified ethanol, and at 244  $\text{m}\mu$  (with a 20 per cent increase in the extinction coefficient) in ethanol made alkaline with sodium hydroxide. The sensitivity of the spectrum to pH, and a low end-absorption ( $\epsilon_{200} : \epsilon_{260} \approx 0.5$ ), are characteristic of the purified dormin. We were unable to measure the extinction coefficient, which was of the order  $\epsilon_{200} = 15,000$ . For abscisin II, Addicott *et al.*<sup>6,7</sup> report  $\lambda_{\text{max}}$  (methanol) 252  $\text{m}\mu$  ( $\epsilon$  25,200), which agrees with the  $\lambda_{\text{max}}$  of sycamore dormin in weakly alkaline ethanolic solution. Sodium borohydride in aqueous-ethanolic solution reduced the dormin slowly to a compound with  $\lambda_{\text{max}}$  268  $\text{m}\mu$  in acid solution and 252  $\text{m}\mu$  in alkaline solution. It has already been shown<sup>9</sup> that the biological activity of crude dormin extracts is slowly destroyed by treatment with sodium borohydride.

The dormin appears to be a carboxylic acid having in addition a hydroxyl and an unsaturated ketone function. Its methyl ester, prepared by treatment with ethereal diazomethane for 3 min at 0°, showed  $\nu_{\text{max}}$  3,245 (*s*), 1,710 (*vs*), 1,655 (*vs*), 1,630 (*m-s*, shoulder), 1,600 (*s*, sharp), 1,232 (*vs*), 1,157 (*vs*)  $\text{cm}^{-1}$ ; and  $\lambda_{\text{max}}$  (ethanol) 264 and 238 (shoulder)  $\text{m}\mu$ , unaffected by addition of acid or alkali.

From a comparison of the properties of the dormin and abscisin II, particularly the molecular weights, infra-red spectra and melting-points, we conclude that the two compounds are identical.

It has been pointed out by Osborne<sup>10</sup> that abscission is an unspecific phenomenon which can be induced by auxins, kinins and gibberellins. Growth inhibition is also rather unspecific. Both abscisin II<sup>6,7</sup> and sycamore dormin<sup>2,9</sup> have been shown to inhibit coleoptile extension, and the dormin has proved to have abscission activity<sup>11</sup>. The role of this type of hormone in the plant has still to be defined, but the presence of high levels<sup>9</sup> in leaves of sycamore during early September when resting buds have been formed, but long before the leaves fall, suggests that the term dormin may give a better description of function.

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## Action of the Sycamore 'Dormin' as a Gibberellin Antagonist

THE hypothesis that endogenous growth inhibitors may regulate dormancy was first put forward by Hemberg<sup>1</sup>, and recently additional evidence has been brought forward in support of the view that bud dormancy in sycamore (*Acer pseudoplatanus*) and birch (*Betula pubescens*) is regulated by endogenous inhibitors<sup>2,3</sup>. This evidence may be summarized as follows: (1) A consideration of the photoperiodic phenomena in seedlings of woody plants suggests that when the leaves are maintained under short-day conditions they exert an inhibitory effect on the shoot-apex<sup>4</sup>. (2) Investigations on the levels of the growth-inhibiting substances in seedlings of woody plants under different photoperiodic conditions have shown that greater amounts are present in the leaves and apical buds of sycamore and other species under short days than with long days<sup>3,5</sup>. (3) When inhibitor extracted from the leaves of birch is applied to seedlings of this species growing under long-day conditions, extension growth ceases and apparently normal resting-buds are formed<sup>3</sup>; similar results have recently been obtained with seedlings of sycamore and blackcurrant. Thus, the evidence that endogenous inhibitors may function as dormancy regulators is gradually accumulating, and hence they have been referred to as 'dormins'. The sycamore inhibitor preparations used in our tests do not appear to contain more than one inhibitory substance of high activity<sup>2</sup>.

The question arises as to the mode of action of dormins in the plant. Studies with partially purified preparations of the inhibitor from sycamore suggest that it does not interact competitively with indolyl-3-acetic acid (IAA) in the coleoptile test<sup>3</sup>, and this conclusion is consistent with the observation that application of IAA does not overcome the dormancy of buds. On the other hand, several pieces of evidence suggest that the inhibitor may be antagonizing the action of endogenous gibberellins in the plant. Thus, it is well known that gibberellic acid (GA) will overcome the dormancy of buds induced by short days in several woody species, including sycamore, birch and blackcurrant. Moreover, birch seedlings which have been induced to form resting buds by externally applied inhibitor will resume growth if GA is then applied, or if the GA is supplied simultaneously with the inhibitor the seedlings will continue in active growth<sup>3</sup>, so that the effect of the inhibitor is nullified. Experiments of this type do not easily lend themselves to precise quantitative treatment, however, and consequently a series of other tests has been carried out with the sycamore inhibitor, in an attempt to determine the nature of its interaction with GA; for example, whether it is: (1) acting competitively with gibberellins; (2) inhibiting gibberellin synthesis; (3) acting in some other way. A series of tests has been carried out with the *Avena* coleoptile and mesocotyl tests, using inhibitor extracted from sycamore leaves and partially purified by paper chromatography in isopropanol-ammonia water and butanol-ammonia-water by the method described previously. This method separates the inhibitor from phenolic material. Although some workers have reported a response of coleoptile sections to gibberellic acid, the response is usually slight or absent. However, if coleoptile sections are supplied with an optimal level of IAA (1.0 mg/l.), together with various concentrations of inhibitor, then the growth is markedly reduced by the inhibitor, but it can be restored to about the level of the IAA controls by simultaneously supplying GA (Fig. 1). Thus, although the coleoptiles do not respond to GA in the absence of inhibitor, in the presence of the latter they become GA-requiring. The shape of the curves suggests a rather specific interaction between the inhibitor and GA in this test.

Seedlings of dwarf mutants of maize and dwarf varieties of pea are frequently used for assay of gibberellin activity.

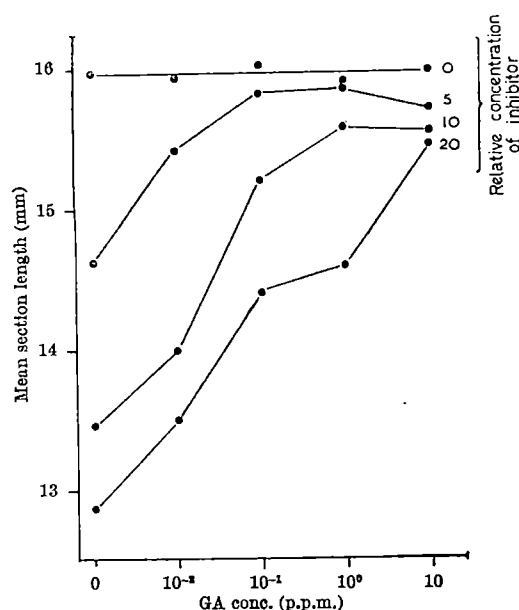


Fig. 1. Interaction between sycamore inhibitor and gibberellic acid in *Avena* coleoptile section test. All sections received indolyl-3-acetic acid at 1.0 p.p.m. The relative concentrations of the inhibitor are given

It was found that the inhibitor showed little activity when applied to dwarf mutants ( $d_1$ ,  $d_2$ ,  $d_3$  and  $d_5$ ) of maize, using the leaf section test, whether the inhibitor was applied alone or in conjunction with GA (except in the case of  $d_3$  mutants, where there was some inhibition of GA-stimulated growth). On the other hand, the inhibitor markedly reduced the growth of sections of tall (non-dwarf) maize, and this inhibition was almost completely overcome by GA (Fig. 2). By contrast, the sycamore inhibitor did not reduce the normal growth of dwarf ('Meteor') or tall ('Alderman') peas, but it markedly reduced GA-induced growth of both types of pea seedlings. The results of these tests are difficult to interpret, but in both species it would seem significant that the inhibitor is only active in reducing GA-induced growth.

In the foregoing tests, the sycamore inhibitor appeared to be interacting with GA in cell extension, and the following evidence suggests that it is also active in inhibiting cell division. Thus, liquid shake cultures of sycamore cambial tissue supplied by Dr. D. H. Northcote were established, using a modified Heller salt medium with added vitamins (but no coconut milk). When sycamore inhibitor was added to the culture medium cell division

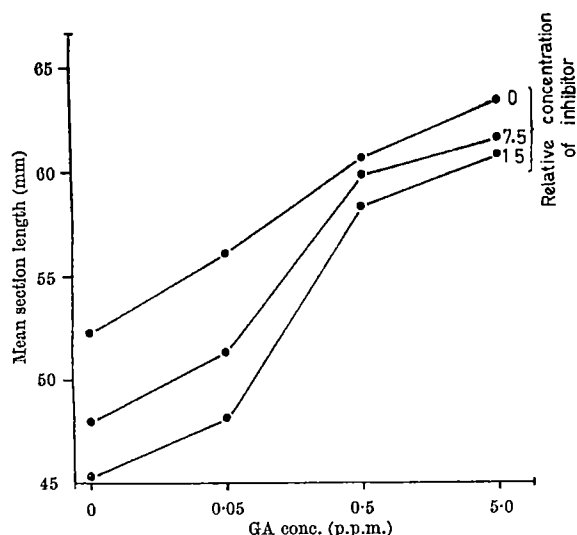


Fig. 2. Interaction between sycamore inhibitor and gibberellic acid in test with leaf sections of a tall variety of maize, using the method of Frankland and Wareing (ref. 13)

was completely inhibited, and it was impossible to overcome this effect by supplying GA, although GA alone stimulates cell division in liquid cultures of this tissue<sup>6</sup>. Addition of IAA or kinetin to the culture solution reduced the effect of the inhibitor, but the form of the response curves suggested a non-competitive interaction.

Although the results with the *Avena* coleoptile test suggested a direct competitive interaction between the sycamore inhibitor and GA, other evidence suggests that possibly the inhibitor may affect hormone biosynthesis. Thus, it has been observed by ourselves for seedlings of sycamore and other species that there is a reciprocal variation in the levels of growth-promoters (both auxins and gibberellins) and of inhibitors under different day-length conditions, there being high levels of promoters and low levels of inhibitors under long days and vice versa under short days<sup>7</sup>. Libbert<sup>8</sup> and Dorffling<sup>9</sup> have suggested that the 'β-inhibitor' found in many plant tissues regulates auxin biosynthesis. Direct evidence in support of this hypothesis has been obtained by ourselves with birch seedlings, by applying inhibitor from leaves of this species, either in lanolin or in solution, to the leaves of the seedlings, when it was found that the levels of auxins and gibberellins both in the leaves and in the shoot apical region were markedly reduced. On the other hand, addition of the sycamore inhibitor to cultures of the fungus *Gibberella fujikuroi* did not appreciably reduce the yields of GA by the fungus.

It is known that gibberellic acid will stimulate the synthesis of α-amylase by isolated barley endosperms<sup>10</sup>, and interaction between the sycamore inhibitor and gibberellic acid has been studied using this test. The technique used was that of Nicholls and Paley<sup>11</sup>, in which sections of barley endosperm were placed in tubes containing a range of GA and inhibitor concentrations in various combinations. After incubation for 30 h at 25° C the sugar released into the solution was determined by modification of the Somogyi method, using a colorimeter. It was found that the sycamore inhibitor suppressed the production of reducing sugars when used in combination with low concentrations of GA, but this inhibition was partly overcome at higher concentrations of GA (Fig. 3). Hemberg<sup>12</sup> has reported that the 'β-inhibitor' from potato inhibits the activity of α-amylase, but tests with sycamore inhibitor gave negative results with preparations of α-amylase. Thus, the sycamore inhibitor appears to inhibit the synthesis of α-amylase, but not the action of the enzyme itself.

It is clear that considerable further work will be needed to elucidate the nature of the interaction between gibberellin and sycamore inhibitor, but the observation that GA appears to counteract the effect of the inhibitor and vice versa, in several different tests, strongly suggests that the inhibitor may function as a gibberellin antagonist *in vivo*.

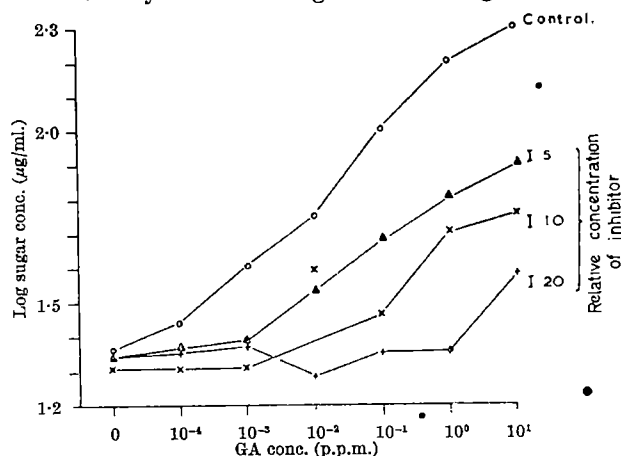


Fig. 3. Interaction between sycamore inhibitor and gibberellic acid in synthesis of α-amylase by barley endosperm. The concentration of reducing sugar produced is a measure of the amount of α-amylase formed

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## SYNCHRONOUS CULTURE OF STRAIN-L MOUSE CELLS

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**M**ETHODS for the preparation of synchronously dividing mammalian cells already include the use of temperature shock<sup>1</sup>, the mechanical or enzymatic dislodgement of cells in mitosis<sup>2</sup>, and the blockage of protein or DNA synthesis by antimetabolites or excess of thymidine<sup>3</sup>. A method recently developed by Mitchison and Vincent<sup>4</sup> for making synchronous cultures of yeast and bacteria by sedimentation may also be adapted to the mammalian cell system and overcomes the problem of providing sufficient material for the biochemical investigation of the events of the cell cycle, and the problem of avoiding the chemical derangement of cell metabolism. The method is simple and consists of centrifuging logarithmically growing cells through a sucrose gradient, then reculturing the small cell fraction. We present an analysis of the application of this method to strain-L mouse cells growing in suspension in a chemically defined medium.

The medium was a modified form of the Waymouth MB 752/1 formula<sup>5</sup>, substituting the amino-acid mixture (Medium C) devised by Mohberg and Johnson<sup>6</sup>. Strain L cells grow continuously in suspension in this medium with a maximum mean doubling time of 36 h (ref. 7). A culture of high viability to begin the experiment was obtained by taking suspension-grown cells through one generation in monolayer roller culture, removing dead cells by a complete medium change, then resuspending by treatment with trypsin, in partially used medium<sup>8</sup>. Addition of fresh medium was made at a dilution ratio of 0.4 day<sup>-1</sup> to maintain exponential growth.

Cells ( $2 \times 10^6$ ) from this culture were collected by centrifugation (100g for 10 min) at room temperature, resuspended in 1-ml. medium and layered on the surface of 14 ml. of a 5–15 per cent linear sucrose gradient in a heavy-walled test-tube. The gradient was prepared aseptically with a gradient machine using 5 per cent (w/v) sucrose in 0.07 M sodium chloride and 0.01 M phosphate buffer, and 15 per cent (w/v) sucrose in 0.01 M phosphate buffer. The choice of the gradient around the isotonic concentration of about 10 per cent sucrose was dictated by the osmolarity which these cells can tolerate<sup>9</sup>. In order to minimize the formation of streamers, the 1-ml. layer of cells was carefully stirred into the upper part of the gradient to occupy twice its original depth and immediately centrifuged at 80g for 6 min.

After centrifugation it was observed that a proportion of the cells had formed a pellet at the bottom of the tube, but the remainder were distributed in a broad band occupying two-thirds of the gradient. A syringe fitted with a long needle was lowered mechanically into the suspension to remove the upper 1-ml. fraction of this band. These cells, equal to 4 per cent of the initial number, were washed once with used medium and resuspended in a mixture of 18 ml. of used medium, 2 ml. of fresh medium

and 1 drop of crystalline trypsin solution (50 µg/ml.) in a 50 ml. round-bottom flask under an atmosphere of 5 per cent carbon dioxide in air. A control preparation of cells was made by gently mixing all the cells remaining in the gradient, and washing and resuspending a small portion in the same way. Samples of both cell populations were retained for sizing by photography. The cultures were stirred magnetically at 200 r.p.m. at 37°, and were afterwards sampled and fed every 4 h by removing 1 ml. of the cell suspension and replacing it with 1 ml. of fresh medium. The atmosphere was flushed with 5 per cent carbon dioxide in air at each sampling, and 1 drop of crystalline trypsin solution was added every 24 h. The sample was counted, fixed in 4 per cent (v/v) formaldehyde in 0.9 per cent sodium chloride, and stained by the Feulgen method to measure the mitotic index.

A comparison of the diameters of selected cells and the control group is shown in Fig. 1. It is clear that the majority of the cells selected for synchronous growth were in the lower range of cell diameters. The mean diameters were 14.1 µ and 15.4 µ, respectively, and these differ significantly at less than the 0.1 per cent level. Fig. 2 illustrates the calculated number of cells present in each culture obtained by counting at least 1,000 cells per sample in a haemocytometer and correcting the actual concentration for the semi-continuous sampling schedule which was maintained. The cell concentration was well below the steady-state concentration which would be expected for this dilution ratio and, in any event, steady-state conditions would not have been achieved during the

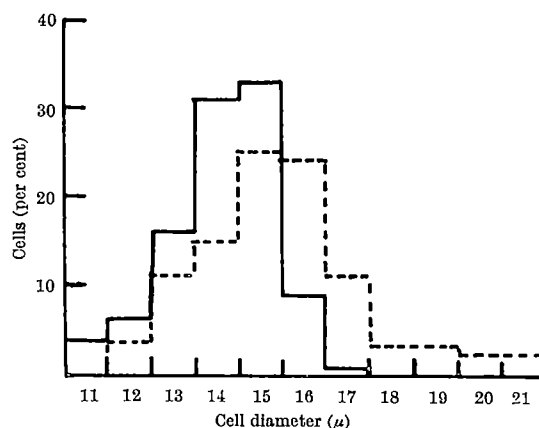


Fig. 1. Frequency histogram of cell diameters. Logarithmically growing cells ( $2 \times 10^6$ ) were centrifuged at 80g for 6 min on a 5–15 per cent sucrose gradient. One ml. was removed from the top of the resulting cell band, added to 8 ml. of used medium and the cells recovered by centrifugation at 100g for 10 min. A sample was photographed and the cell diameters of 200 cells determined (continuous line). A control from the remainder of the cells on the gradient was similarly treated (broken line). Cell diameters are expressed as a percentage of the total

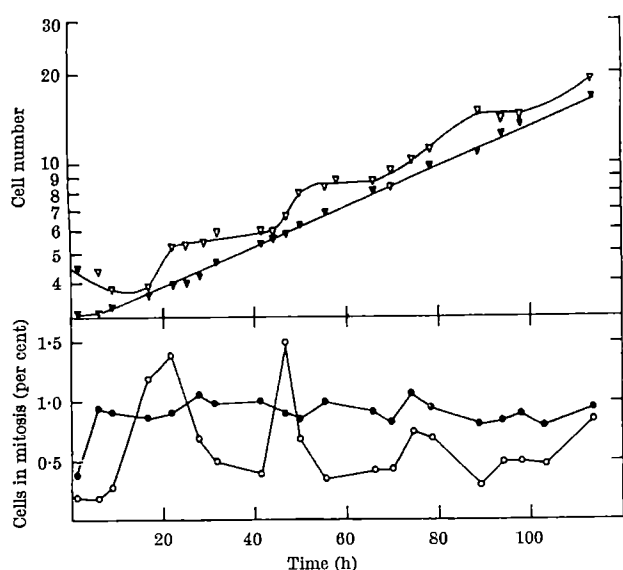


Fig. 2. Cell numbers and mitotic indices of synchronous and control cell cultures. Cells ( $8 \times 10^6$ ) separated on a sucrose gradient and freed from sucrose (Fig. 1) were suspended in 18 ml. used medium, 2 ml. fresh medium, 1 drop of trypsin solution (50  $\mu$ g/ml.) and gassed with 5 per cent carbon dioxide in air. Samples (1 ml.) were removed periodically, counted ( $\nabla$ , synchronous culture;  $\blacktriangledown$ , control culture) and fixed in 4 per cent formaldehyde for determination of the mitotic index by the Feulgen method ( $\circ$ , synchronous culture;  $\bullet$ , control culture). After sampling, 1 ml. fresh medium was added and each culture was regassed. The total cell number has been calculated by correcting the measured number of cells by the dilution factor.

period of the experiment<sup>7</sup>. The small loss of cells over the first 16 h in the synchronous culture probably represents dead cells which sedimented with the small cell fraction rather than cells damaged by the manipulations, since the control did not undergo a similar loss. Each

plateau on the synchronous curve represents the doubling of about 80 per cent of the cells present. The progressive loss of steepness in consecutive steps illustrates the expected decay in synchrony with time.

In estimating the mitotic index, late prophase, metaphase, and anaphase were scored for at least 2,000 cells per sample, and the results are shown in the lower curves in Fig. 2. After an initial delay in mitosis, the control index remained constant during the experiment, while the index in the test culture passed through a series of peaks corresponding to the steps in the cell count. Since the cultures were growing at the same overall rate, the total number of mitosis in each should be the same. This was not observed, because samples were removed every 4 h and mitosis of these cells probably occupies less than 2 h. An analysis of the degree of synchrony at each step (by Engelberg's method<sup>10</sup>) will be presented elsewhere.

While this method is not better than the best published methods of establishing synchrony in mammalian cells, its value lies in its simplicity, in providing at least two well-defined synchronous steps, and in the ease with which it can be scaled up to furnish sufficient material for biochemical analysis.

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## A NEW HAEMOGLOBIN, D IBADAN ( $\beta$ -87 THREONINE $\rightarrow$ LYSINE), PRODUCING NO SICKLE-CELL HAEMOGLOBIN D DISEASE WITH HAEMOGLOBIN S

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AT University College, Ibadan, Nigeria, all blood donors are screened for abnormal haemoglobins to ensure that no blood from patients with haemoglobinopathies is transfused. The haemoglobin of a 25-year-old member of the Yoruba tribe showed on paper electrophoresis at alkaline pH a single band which was indistinguishable from that of Hb S (Fig. 1). He was working as a carpenter and there was nothing to suggest sickle-cell anaemia in his previous history or in his present state. On examination there was no palpable spleen, the haemoglobin-level was 14.8 g/100 ml. blood, and the percentage of reticulocytes was <1. The red cells sickled on incubation with 2 per cent  $\text{Na}_2\text{S}_2\text{O}_8$ , but the morphology was normal and no sickled cells were seen in the stained smear of peripheral blood. The findings were those usually met with in sickle-cell trait, the heterozygous state for the genes controlling Hbs A and S respectively. No Hb A was found, and the non-sickling haemoglobin if present had to have a closely similar or identical electrophoretic

mobility to Hb S. Of the family, one sister and the mother could be examined. The sister had the sickle-cell trait (A+S), while the findings in the mother were identical with those of her son. Her red cells sickled, and although on electrophoresis of the haemoglobin only a single band in the Hb S position was found, there was no evidence of anaemia or disease. The propositus was kept under observation for three years; he remained in excellent health and was never absent from work because of illness. In order to demonstrate conclusively that the nature of the haemoglobin caused no disadvantage, the red cell survival was determined after labelling his cells with chromium-51. The half-life was found to be 28 days (normal 24-34 days).

To elucidate further the nature of the non-sickling component in the haemoglobin, the haemolysate was examined by various electrophoretic methods on paper and starch, at varying pH, and by chromatography at pH 6 with 'IRC' resin<sup>1</sup>. A single band was always obtained.

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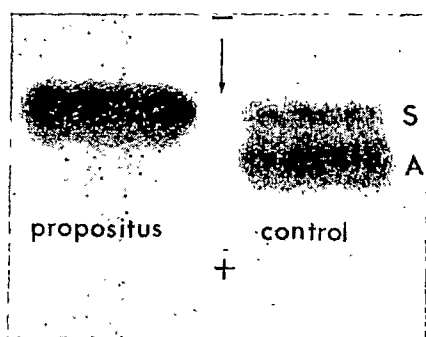


Fig. 1. Paper electrophoresis (tris buffer pH 8.9) of the propositus's haemoglobin, showing a single band in the position of Hb S

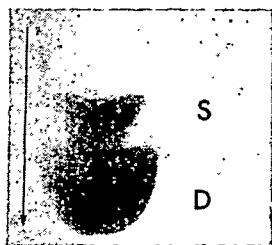


Fig. 2. Agar-gel electrophoresis (pH 6) of the propositus's haemoglobin, showing two bands, one in the position of Hb S and one in the position of Hb D

A haemoglobin which does not cause sickling, but is indistinguishable from Hb S by conventional methods of electrophoresis and by chromatography, is Hb D. This Hb separates, however, from Hb S on agar-gel electrophoresis at pH 6 (ref. 2). By this method the propositus's haemoglobin separated into two fractions, one in the position of Hb S and the other in the position of Hb A where among others Hb D would be expected to appear (Fig. 2). Contrary to the findings in sickle-cell Hb D disease neither Hb F was found, nor was the proportion of S greater than 50 per cent (on scanning it was found to be 20 per cent). The solubility according to Itano's test<sup>3</sup> was greater than that obtained in sickle-cell Hb D disease. It was in the upper range of that found in sickle-cell trait of the A + S type, namely, 180 mg/l. 2.24 M phosphate buffer.

Up to now we had considered the new haemoglobin to be D because it did not separate from Hb S on electrophoresis and on agar-gel electrophoresis was found to move like Hb D. It was still possible that its mobility was different from that of Hb S, but not sufficiently different to allow electrophoretic separation. To separate the non-sickling Hb from Hb S, 1 g of the propositus's haemoglobin dissolved in 40 ml. water was added to 160 ml. of 2.8 M phosphate buffer containing 2 g  $\text{Na}_2\text{S}_2\text{O}_4$ , mixed and incubated at 25° for 1 h. Insoluble haemoglobin was removed by centrifuging, and the supernatant haemoglobin solution was concentrated by dialysis *in vacuo*. The absence of Hb S could be demonstrated in a repeated Itano test when no precipitate was formed. A mixture of the isolated new haemoglobin with Hb A was compared with one of Hbs A + S by electrophoresis. The new haemoglobin had exactly the same mobility as Hb S (Fig. 3). Chromatography of the two mixtures on 'IRC 50' resin at pH 6 showed that Hb S and our new haemoglobin moved identically. Thus the non-sickling haemoglobin of our propositus was a true Hb D.

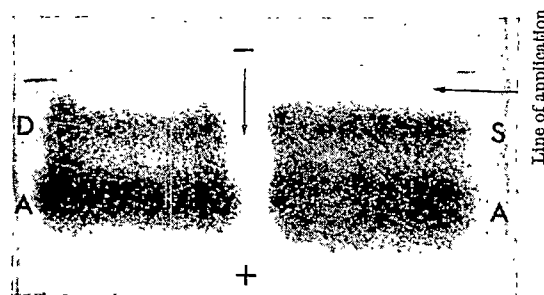


Fig. 3. Paper electrophoresis (tris buffer pH 8.9) of a mixture containing Hb A and the propositus's non-sickling Hb, and of Hbs A + S. The propositus's haemoglobin moves like Hb S, and thus is a Hb D

On electrophoresis at alkaline pH only Hbs S and D had been found in the propositus and in his mother. If the Hb D were an  $\alpha$ -chain abnormal variant, Hb A and a doubly abnormal hybrid moving more slowly than S + D would also have been expected. Thus our Hb D seemed to be a  $\beta$ -chain variant. This was confirmed by 'hybridization' of the isolated Hb D fraction with canine haemoglobin<sup>4,5</sup>. Mixtures of canine haemoglobin and Hbs A and D respectively were dissociated into their sub-units and these were then recombined. The electrophoretic mobility on starch at pH 8.2 of the  $\alpha_2$ human  $\beta_2$ canine hybrids was the same in both mixtures. The combination of the  $\alpha_2$ canine sub-unit with the  $\beta_2$  sub-unit of the HbD, however, moved more slowly than the  $\alpha_2$ canine  $\beta_2^A$  hybrid, indicating that the difference between the electrophoretic mobility of Hbs A and D was due to an increase in positive charge of the  $\beta$ -chains of the Hb D, that is, that the Hb D was a Hb D  $\beta$ .

To obtain more information on the chemical nature of this difference between Hb A and our Hb D  $\beta$ , 'fingerprints' of the purified Hb D were prepared. The haem was removed with acid/acetone<sup>6</sup>, and the globin digested with trypsin in 0.1 M  $\text{NH}_4\text{HCO}_3$  (pH 8.2) for 2 h at 38°. The pH was then adjusted to 6.4 with 0.5 M acetic acid and the insoluble 'core' was removed by centrifuging. After lyophilization of the supernatant, fingerprints were prepared by electrophoresis in a Michl type tank at pH 6.4 (ref. 7) using a potential gradient of 55 V/cm, followed by chromatography in iso-amyl alcohol/pyridine/water<sup>8</sup>. The peptides were rendered visible with 0.2 per cent ninhydrin in acetone, and submitted to the usual colour tests for specific amino-acids<sup>9-13</sup>. None of the peptides seen in fingerprints of Hb A was missing from the Hb D fingerprints. An additional peptide, however, was found just below  $\alpha\text{TpIII}$  (Fig. 4). The electrophoretic mobility



Fig. 4. Fingerprint of soluble tryptic peptides of Hb D Ibadan; 1, neutral peptides; 2, lysine ( $\alpha\text{TpVIII}$ ); 3,  $\alpha\text{TpIII}$ ; 4, new peptide

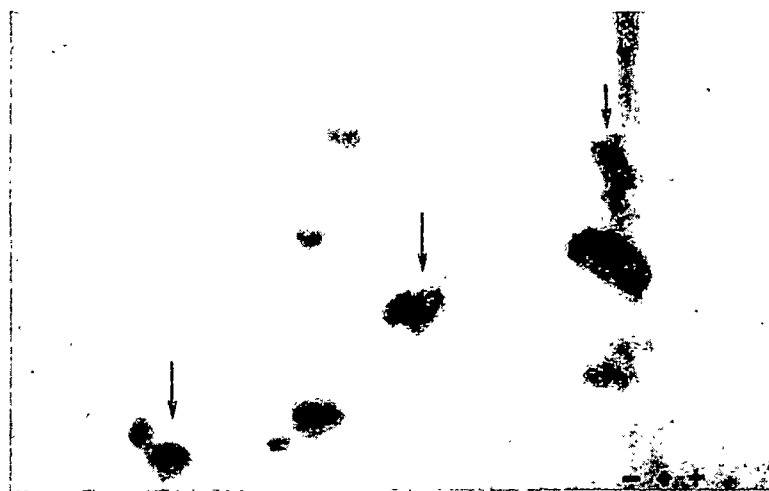


Fig. 5. Fingerprint of chymotryptic peptides of the Hb A core. The arrows indicate three peptides staining transiently yellow with ninhydrin. The right arrow also indicates the area of neutral peptides



Fig. 6. Fingerprint of chymotryptic peptides of the Hb D core. The arrows indicate two peptides staining transiently yellow with ninhydrin

at pH 6.4 was +0.49 (lysine = +1.0) and the  $R_F$  was 0.12 (25°). With ninhydrin it showed a transient yellow colour suggesting N-terminal glycyl. The specific colour tests for tyrosine<sup>9</sup>, tryptophan<sup>10</sup> and methionine<sup>11</sup> were negative, as were those for histidine<sup>12</sup> and arginine<sup>9,13</sup>.

As the peptide was a tryptic peptide its positive charge was presumably due to C-terminal lysyl, and its electrophoretic mobility suggested a sequence of four to five amino-acid residues. All the soluble tryptic peptides of Hb A were present, thus the additional peptide had to come from the usually insoluble core of the  $\beta$ -chain (residues  $\beta$ -83- $\beta$ -120).

The new peptide was isolated by paper electrophoresis at pH 6.4 and chromatography (24 h)<sup>8</sup>. The suspected presence of N-terminal glycyl was confirmed by a modified DNP method<sup>14</sup> and amino-acid analysis<sup>15</sup> demonstrated in equal amounts Thr, Gly, Phe and Lys (Table 1). The partial sequence could now be written as Gly-(Thr, Phe, Ala)-Lys. No glycine residue in the  $\beta$ -chain<sup>16,17</sup> has in its immediate sequence any combination of Thr, Phe, Ala except the Gly in position  $\beta$ -83, the N-terminal glycyl of the core peptide  $\beta$ TpX.

To determine the amino-acid sequence in the new peptide it was treated with chymotrypsin which hydrolyses phenylalanyl bonds. After digestion in 0.1 M  $\text{NH}_4\text{HCO}_3$  (pH 8.2) at 38° for 3 h, electrophoresis at pH 6.4 demonstrated two peptides. Their mobilities were +0.80 and 0 respectively (Table 2). The first was suggestive of the

dipeptide Ala-Lys for which at pH 6.5 a mobility of +0.77 had been reported<sup>14</sup>. Alanine was demonstrated to be its N-terminal residue, and hydrolysis of the peptide followed by high-voltage paper electrophoresis at pH 2.0 (ref. 18) showed only alanine and lysine to be present.

The neutral peptide showed a transient yellow colour with ninhydrin, indicating N-terminal glycyl, presumably the N-terminal residue of the original tryptic peptide. As in the original peptide the phenylalanyl bond was the only one easily susceptible to chymotryptic hydrolysis, Phe would be expected to be the C-terminal residue. This was confirmed by treatment of the peptide with carboxy peptidase-A which acts by splitting off the C-terminal residue. On high-voltage electrophoresis at pH 2 the amino-acid released was found to be phenylalanine.

The two chymotryptic peptides, therefore, had the sequences Gly-Thr-Phe and Ala-Lys, and the original peptide had to be Gly-Thr-Phe-Ala-Lys. From the known amino-acid sequences of Hb A (refs. 16 and 17) this peptide could correspond to the beginning of the core peptide  $\beta$ TpX Gly-Thr-Phe-Ala-Thr- ( $\beta$ -83- $\beta$ -87) if the threonine residue at position 87 of the  $\beta$ -chain of Hb A had been replaced by a lysine residue in Hb D  $\beta$ . As the residues 83-87 are the first five of the  $\beta$ -chain core a mutation of 87 Thr  $\rightarrow$  Lys would cause an additional soluble tryptic peptide to be found in the fingerprint of our Hb D  $\beta$ .

To confirm this origin of the additional D  $\beta$  peptide, fingerprints were prepared of the cores of Hb A and D  $\beta$ . The precipitates left after tryptic digestion were washed three times with Michl buffer pH 6.4 and then three times with water. They were then dissolved in 0.1 N hydrochloric acid precipitated with acetone and washed well with acetone by centrifuging. Chymotryptic digestion was carried out as described before, after which the pH was adjusted to 6.4 with acetic acid. A faint precipitate was centrifuged off and the supernatant was fingerprinted as described for the tryptic digest. The core fingerprint of Hb A (Fig. 5) showed three transiently yellow peptides when developed with ninhydrin, but that of the Hb D  $\beta$  core (Fig. 6) showed two. The additional 'yellow' peptide in the fingerprint of the Hb A core was in the neutral area. The neutral peptides of both cores were submitted to electrophoresis at pH 2. In each case several peptides separated, but one—transiently staining yellow with ninhydrin—was found only in the material from the Hb A core. Hydrolysis, followed by high-voltage electrophoresis at pH 2, showed the peptide to consist of glycine, threonine and phenylalanine. The peptide—missing in the core of Hb D—could only represent the first

Table 1. MOLAR RATIO OF AMINO-ACIDS AFTER ACID HYDROLYSIS OF TWO DIFFERENT PREPARATIONS OF THE ADDITIONAL PEPTIDE FROM Hb D

Threonine	0.9	1.0
Glycine (N-terminal)	1.0	1.0
Alanine	1.1	1.1
Phenylalanine	0.9	1.1
Lysine (C-terminal)	1.2	1.1

Table 2. AMINO-ACID ANALYSIS OF TWO CHYMOTRYPTIC PEPTIDES

	1	2
Mobility (Lys = +1.0)	+0.80	0
Threonine	+	+
Glycine (N-terminal)	—	+
Alanine (N-terminal)	+	—
Phenylalanine (C-terminal)	—	—
Lysine (C-terminal)	+	—

three residues of the  $\beta$ -chain core (83-85) of Hb A, and as this sequence of Hb D  $\beta$  was found in the soluble tryptic digest, it could not be expected to be present in the core of that Hb variant.

The formula of the Hb D  $\beta$  described here is therefore  $\alpha_2\beta_2^{87}\text{Thr} \rightarrow \text{Lys}$ , a new variant of Hb A: -D  $\beta$  Ibadan. It is the first example of a fully investigated mutation in the core, although another core mutation in Hb O Indonesia has been preliminarily reported as 116 Glu  $\rightarrow$  Lys (ref. 19). This is also the first mutation in which an additional polar group is introduced near the haem-linked histidine of human haemoglobin. No interference with the function of the haem was noted. Presumably the explanation is that this polar group—five residues away along the helical sequence—does not point in the direction of the ferrous atom.

It is of special interest that this haemoglobin is formed in preference to Hb S in the D + S heterozygote. D Ibadan seems to be as normal as Hb A, and its combination with Hb S does not result in sickle-cell Hb D disease. This preferential formation of a non-A haemoglobin in a sickle-cell heterozygote has only been reported once before—for a person with Hb J  $\beta$  Baltimore<sup>20</sup>. As more 'abnormal' haemoglobins are being examined, it is becoming quite certain that some of them are unusual rather than abnormal<sup>21</sup>.

Finally, Hb D Ibadan is the first example of a threonine  $\rightarrow$  lysine mutation in human haemoglobin. Recent investigations<sup>22</sup> of the triplet sequences of bases in RNA which determine the incorporation of amino-acids by a genetic code accept as the triplet code for lysine AAA

(A = adenine). The code suggested for threonine is AAC or ACA (C = cytosine), both of which would be compatible with a single mutation causing the step from threonine to lysine. Another proposed code for threonine, which lists in indeterminate order (ACC), would, however, not be compatible.

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## ACTIVITY AND MECHANISM OF ACTION OF 6-METHYLTHIOPURINE RIBONUCLEOSIDE IN CANCER CELLS RESISTANT TO 6-MERCAPTOPURINE

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FOR a number of years basic biochemical and biological research has been directed toward gaining new knowledge regarding the mechanism of action of purine antagonists, particularly 6-mercaptopurine (MP) because of its useful temporary anti-leukaemic activity in man<sup>1</sup>. One of the goals of such efforts has been to gain information which could serve as a rationale for design and synthesis of purine antagonists that might circumvent the most prevalent biochemical mechanisms associated with cellular resistance to MP.

A common biochemical alteration (observed in bacteria, experimental cancer cells including leukaemic cells, and human cancer cells in culture) associated with the development of cellular resistance to MP is the loss of activity of a specific nucleotide pyrophosphorylase (IMP-GMP pyrophosphorylase) responsible for catalysing the conversion of MP to its cytotoxic form, MP-ribonucleotide<sup>2</sup>. It

has not been possible to circumvent this loss of pyrophosphorylase activity by exposure of MP-resistant cells to MP-ribonucleotide, because this nucleotide either fails to enter the cell, or is degraded in the process of entry. MP-ribonucleotide is also ineffective against MP-resistant cells; its inactivity may be due either to its rapid degradation to MP (which has been observed in Ehrlich ascites cells<sup>3</sup>), to the absence of the necessary nucleoside phosphokinase activity (presumably 'inosine kinase', an enzyme which has not been reported<sup>4</sup>), or to both these factors. Efforts to overcome resistance to MP by employment of bis(thioinosine)-5',5'''-phosphate have shown some promise<sup>5</sup>, but MP-resistant cells in culture show some degree of cross-resistance to this compound (about 20- to 40-fold as compared with >1,000-fold resistance to MP). Such cross-resistance is too great to offer encouragement for *in vivo* activity against MP-resistant neoplasms, because of the relatively low therapeutic index of all known purine antagonists.

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Table 1. RELATIVE EFFECTIVENESS OF 6-METHYLTHIOPURINE RIBONUCLEOSIDE AND RELATED COMPOUNDS AGAINST *H. Ep. No. 2* CELLS IN CULTURE AND A 6-MERCAPTOPYRINE-RESISTANT VARIANT

Compound	Average $ED_{50}$ ( $\mu\text{g/ml.}$ )*	
	<i>H. Ep. No. 2/S</i>	<i>H. Ep. No. 2/MP</i>
6-Mercaptopurine	0.21	400
6-Mercaptopurine ribonucleoside	0.36	>100
6-Mercaptopurine ribonucleotide	0.61	>100
6-Methylthiopurine	73	111
6-Methylthiopurine ribonucleoside†	0.10	0.07
6-Methylthiopurine ribonucleotide†	0.17	0.04
6-Ethylthiopurine ribonucleoside	0.29	0.29
6-Benzylthiopurine ribonucleoside	3.7	2.8
6-Cyclopentylthiopurine ribonucleoside	>100	>100
6-Thioguanine	0.04	52
6-Thioguanosine	0.38	>100
6-Thioguanic acid	0.28	>100
6-Methylthioguanine	44	32
6-Methylthioguanosine	>100	>100

\*  $ED_{50}$  is that concentration of drug inhibiting cell growth by 50 per cent. Cells were grown on glass and growth was measured by determination of protein content (method of Oyama and Eagle (ref. 30)) after 4 days growth in the presence of drug.

† Average of 9 independent and internally consistent experiments.

‡ Barium salt monohydrate.

The present article is concerned with the biological activity (including the *in vivo* anti-leukaemic activity), some intermediary metabolic effects, and the metabolism of 6-methylthiopurine ribonucleoside (MeMP-ribonucleoside), a simple derivative of MP with high activity in culture against human cancer cells resistant to MP.

**Inhibition studies with *H. Ep. No. 2* cells in culture.** The isolation of a line of human epidermoid cancer cells (*H. Ep. No. 2*) resistant to MP has already been described<sup>6</sup>; this line is completely devoid of IMP-GMP pyrophosphorylase, which converts MP to MP-ribonucleotide<sup>7</sup>. A second line of *H. Ep. No. 2* cells resistant to MP was also used in studies of metabolic effects described later; this line shows the same loss of IMP-GMP pyrophosphorylase activity as the first MP-resistant line, but since it represents an independently isolated cell line and since it has been carried in the presence of 6-thioguanine rather than MP, it is given a separate designation (*H. Ep. No. 2/MP/TG*) to distinguish it from the first resistant line (*H. Ep. No. 2/MP*) and the parent line (*H. Ep. No. 2/S*). These cell culture lines were propagated in stationary culture in Eagle's basal medium supplemented with 10 per cent calf serum. The drug response was quantitatively assessed by a standardized procedure<sup>8</sup>. The results, presented in Table 1, show the inability of MP, MP-ribonucleoside and MP-ribonucleotide to inhibit the resistant line, and also show the cross-resistance of this line to 6-thioguanine,

6-thioguanosine, and 6-thioguanic acid. In contrast, MeMP-ribonucleoside was equally effective against *H. Ep. No. 2/S* and *H. Ep. No. 2/MP* cells, as were MeMP-ribonucleotide and the *S*-ethyl and *S*-benzyl (but not the *S*-cyclopentyl) derivatives of MP-ribonucleoside. The free base, 6-methylthiopurine (MeMP), was relatively non-toxic to both the sensitive and resistant lines, the  $ED_{50}$  concentrations being 700- to 1,000-fold higher than those of MeMP-ribonucleoside. It is also of interest that a similar relationship did not hold in the thioguanine series: both 6-methylthioguanine and 6-methylthioguanosine were relatively without inhibitory activity against both MP-sensitive and MP-resistant cell lines.

**Evaluation of the response in vivo of L1210 leukaemia and an MP-resistant variant thereof to MeMP-ribonucleoside.** Using standardized procedures<sup>9</sup>, MeMP-ribonucleoside and MP have been compared with regard to capacity to increase life-span of *BDF<sub>1</sub>* mice bearing L1210 or L1210/MP leukaemias. The design and results of these experiments are shown in Table 2. MeMP-ribonucleoside was about as effective against the MP-resistant leukaemia as was MP against the parent sensitive leukaemia, and MeMP-ribonucleoside produced about the same increase in life-span in mice with sensitive and with MP-resistant leukaemic cells. These results have been confirmed repeatedly.

**Pseudo-feedback inhibition of purine biosynthesis.** Many purine analogues, including MP, inhibit an early step of purine biosynthesis<sup>10-13</sup> presumably the first step of the pathway<sup>14</sup>, as a result of the capacity of their nucleotides to mimic the action of natural purine nucleotides as feedback inhibitors. Earlier investigations with MP and other purine analogues in resistant cells have shown that pseudo-feedback inhibition of purine biosynthesis occurs only in cells that have the capacity to form the nucleotides<sup>13,15</sup>. Since the activity of MeMP-ribonucleoside against MP-resistant cells suggests that MeMP-ribonucleoside is being converted to a nucleotide in cells where such conversion of MP or MP-ribonucleoside does not occur, it was of interest to determine the effectiveness of this compound as a pseudo-feedback type of inhibitor.

Azaserine specifically blocks an early step in purine biosynthesis, and the feedback or pseudo-feedback inhibition can be assayed conveniently by determining the effectiveness of a given agent in decreasing the amount of <sup>14</sup>C-labelled formylglycinamide ribonucleotide (FGAR)

Table 2. A COMPARISON OF THE *in vivo* RESPONSE OF 6-MERCAPTOPYRINE-RESISTANT L1210 LEUKAEMIA TO 6-METHYLTHIOPURINE RIBONUCLEOSIDE AND 6-MERCAPTOPYRINE\*

No. of L1210/MP cells inoculated	Treatment	Dosage (qd 1-15 days)			Median host life-span (days)		% Increase in host life-span†
		mg/kg	mg/m <sup>2</sup>	Relative to the $LD_{10}$	Untreated controls	Treated	
10 <sup>6</sup>	6-Mercaptopurine	40	120	1.4	10	9.0	-10
		27	81	0.96		9.5	-5
		18	54	0.64		9.0	-10
		12	36	0.43		10.0	0
		8	24	0.29		10.0	0
		5	15	0.18		10.0	0
10 <sup>6</sup>	6-Methylthiopurine ribonucleoside	36	108	ca. 2.4	10	12.5	25
		24	72	1.6		17.0	70
		16	48	1.1		15.0	50
		11	33	0.73		14.0	40
		7	21	0.47		12.0	20
		5	15	0.33		13.0	30
10 <sup>4</sup>	6-Mercaptopurine	40	120	1.4	12	12.0	20
		27	81	0.96		11.5	-4
		18	54	0.64		12.5	-4
		12	36	0.43		13.0	8
		8	24	0.29		12.0	0
		5	15	0.18		12.0	0
10 <sup>4</sup>	6-Methylthiopurine ribonucleoside	36	108	ca. 2.4	12	8.5	-29
		24	72	1.6		15.5	29
		16	48	1.1		19.0	58
		11	33	0.73		18.0	50
		7	21	0.47		18.0	50
		5	15	0.33		18.0	50

\* The above are results from concurrent experiments in which randomly distributed leukaemic mice were used. Leukaemic cells were inoculated intraperitoneally and all therapy was by the intraperitoneal route. The MP-resistant leukaemia used was a line (L1210/MP (III)) isolated by Hutchison (ref. 31). The maximum percentage increase in host life-span of animals bearing L1210/MP leukaemia provided by MeMP-ribonucleoside is approximately the same as that observed in animals bearing the parent L1210 leukaemia on treatment with the optimal chronic dosage of MP (ref. 32). In experiments carried out so far, the activity of MeMP-ribonucleoside and MP against the parent line of L1210 leukaemia appear to be about the same.

† Groups of 20 control and 10 treated animals were used. There were no 30-day survivors in either control or treated groups.



that accumulates in cells provided  $^{14}\text{C}$ -formate and treated with azaserine. The procedures used, which have been described in detail elsewhere<sup>13,15,16</sup>, are modifications of those used by LePage, Henderson *et al.*<sup>11,12</sup>. The details of the experiments are contained in Table 3, which presents the results obtained with the two separate MP-resistant *H. Ep.* No. 2 lines and the parent sensitive line in culture and with L1210/MP cells *in vivo*. Each of these resistant cell lines previously has been shown to be cross-resistant to MP-ribonucleoside and to be devoid of IMP-GMP pyrophosphorylase activity, the enzyme which converts MP and 6-thioguanine to nucleotides<sup>7,13</sup>.

Table 3. EFFECTS OF 6-METHYLTHIOPURINE RIBONUCLEOSIDE AND RELATED COMPOUNDS ON THE AZASERINE-INDUCED ACCUMULATION OF FORMYL-GLYCINAMIDE RIBONUCLEOTIDE (FGAR)

Cell line	Inhibitor and concentration	$^{14}\text{C}$ in FGAR (% of azaserine control)
Exp. 1* (stationary culture)	$\mu\text{g/ml.}$	
<i>H. Ep.</i> No. 2/S	6-Methylthiopurine ribonucleoside 5.0	1
	0.5	3
	0.1	15
<i>H. Ep.</i> No. 2/MP	6-Mercaptopurine ribonucleoside 5.0	1
	0.5	13
	0.1	57
<i>H. Ep.</i> No. 2/MP	6-Methylthiopurine ribonucleoside 5.0	1
	0.5	13
	0.1	57
<i>H. Ep.</i> No. 2/MP	6-Mercaptopurine ribonucleoside 50	92
	5.0	92
	0.5	92
Exp. 2* (suspension culture)		
<i>H. Ep.</i> No. 2/S	6-Methylthiopurine ribonucleoside 9.82	3
	0.98	3
	0.098	3
<i>H. Ep.</i> No. 2/MP	6-Methylthiopurine ribonucleoside 98.2	4
	9.82	5
	0.98	4
<i>H. Ep.</i> No. 2/MP	6-Mercaptopurine 50	95
	6-Methylthiopurine 48.8	78
	6-Mercaptopurine ribonucleoside 83.5	60
<i>H. Ep.</i> No. 2/MP	6-Thioguanine 50.0	91
	5.0	91
	0.5	91
Exp. 3 ( <i>in vivo</i> )	$\text{mg/kg}$	
L1210/MP	6-Methylthiopurine ribonucleoside 98.2	9
	49.1	7
	24.5	10
L1210/MP	6-Mercaptopurine ribonucleoside 83.5	94
	8.35	94
	0.835	94

\* Cells were treated with azaserine (10  $\mu\text{g/ml.}$ ), and sodium formate- $^{14}\text{C}$  (25  $\mu\text{C.}$  per flask or bottle) was added 1 h later. Purine analogues were added 30 min after the azaserine. Controls (azaserine control) received only azaserine and formate- $^{14}\text{C}$ . Cells were collected 2 h after addition of the radioactive formate.

† Azaserine (0.5 mg/kg) was administered intraperitoneally to groups of 3 mice each, bearing 7-day-old implants of leukaemia L1210/MP (ref. 31), followed 30 min thereafter by the purine analogue. Sodium formate- $^{14}\text{C}$  (50  $\mu\text{C.}$ /mouse) was given 1 h after the azaserine, and the cells were collected 2 h after administration of the tracer. The soluble fraction of the cells was prepared and assayed by the same methods used for *H. Ep.* No. 2 cells.

MeMP-ribonucleoside at concentrations of 0.5  $\mu\text{g/ml.}$  or less blocked the formation of FGAR almost completely in both the sensitive and resistant cell culture lines. MP-ribonucleoside was effective in blocking FGAR synthesis in *H. Ep.* No. 2/S cells, but not in *H. Ep.* No. 2/MP or *H. Ep.* No. 2/MP/TG cells. MP was also without effect on FGAR synthesis in the *H. Ep.* No. 2/MP/TG line; its high effectiveness in the parent line and its ineffectiveness in the *H. Ep.* No. 2/MP line have been reported earlier<sup>13</sup>. MeMP-ribonucleoside, at therapeutic doses, was also highly effective in blocking synthesis of FGAR in L1210/MP leukaemic cells *in vivo*, whereas MP-ribonucleoside was without effect (Table 3). The effectiveness of MeMP-ribonucleoside as a pseudo-feedback inhibitor has also been noted by Henderson<sup>12</sup> in studies with Ehrlich ascites cells *in vitro*.

**Metabolism of MeMP-ribonucleoside- $^{35}\text{S}$ .** MP-ribonucleoside- $^{35}\text{S}$  was prepared by exchange between sulphur-35 dissolved in pyridine and unlabelled MP-ribonucleoside as described by Moravsek and Nejedly<sup>17</sup>. Methylation of MP-ribonucleoside- $^{35}\text{S}$  with methyl iodide gave MeMP-ribonucleoside- $^{35}\text{S}$ , obtained in about 80 per cent overall yield from sulphur-35. Two batches of labelled compound with specific activities of 2.6  $\mu\text{C.}/\text{mg}$  and 16.5  $\mu\text{C.}/\text{mg}$  were used in metabolism studies. *H. Ep.* No.

2/S and *H. Ep.* No. 2/MP/TG cells in suspension culture ( $4-6 \times 10^7$  cells in 200 ml. medium) were exposed to MeMP-ribonucleoside- $^{35}\text{S}$  (5  $\mu\text{g/ml.}$ ) for 4 h. At this time, the cells were collected by centrifugation, washed, and extracted with hot 80 per cent ethanol as described elsewhere<sup>16</sup>. Three to four per cent of sulphur-35 added was present in the ethanol extract. The alcohol extract was analysed by two-dimensional paper chromatography-autoradiography (phenol-water followed by *n*-butanol-propionic acid-water) and by one-dimensional paper chromatography (butanol-propionic acid-water), as described previously<sup>16</sup>. Eighty to eighty-five per cent of the total sulphur-35 on the chromatograms was located in a spot falling in the nucleotide area of the chromatograms ( $R_F$  0.30 in butanol-propionic acid); 5-11 per cent of the total sulphur-35 was in a spot of  $R_F$  0.71, and the remainder in several small spots falling below the principal spot. Similar uptake and distribution were observed in both cell lines, and 24 h exposure to the labelled drug did not change either the extent of uptake or the distribution of radioactivity among the spots on the chromatogram. By co-chromatography in three solvents the spot with  $R_F$  value 0.71 was identified as unchanged MeMP-ribonucleoside. The principal spot ( $R_F$  0.30) was identified as the 5'-mono-phosphate of MeMP-ribonucleoside by (a) co-chromatography with an authentic synthetic sample of 6-methylthioinosine-5'-phosphate (prepared by the methylation of thioinosinic acid (J. A. Montgomery and H. J. Thomas, unpublished observations)), in three solvents (butanol-propionic acid-water; dicyclohexylamine, butanone,  $\text{H}_2\text{O}$  (2:10:5, v/v); *t*-butyl alcohol, butanone,  $\text{H}_2\text{O}$ , diethylamine (4:4:2:4, v/v)), in all of which there was complete coincidence of ultra-violet absorption and radioactivity; and (b) by treatment with calf intestinal phosphatase (Pentex, Inc., Kanakee, Ill.) for 1.5 h, 37° C, pH 9-10, which converted the principal spot completely to a compound indistinguishable from MeMP-ribonucleoside by co-chromatography. The identities of the spots with  $R_F$  values less than that of the nucleoside monophosphate have not been determined. MeMP has been observed to undergo some demethylation *in vivo* and *in vitro*<sup>18-20</sup>; MP-ribonucleotide was therefore a probable metabolite of MeMP-ribonucleotide. However, in the butanol-propionic acid solvent, MP-ribonucleotide ( $R_F$  0.09-0.13) and MeMP-ribonucleotide can be distinguished easily; very little radioactivity at the  $R_F$  of MP-ribonucleotide was noted on chromatography-autoradiography of extracts of cells exposed to MeMP-ribonucleoside- $^{35}\text{S}$ ; and when the material falling at this  $R_F$  value was eluted and treated with alkaline phosphatase, no radioactivity was detected at the  $R_F$  of MP-ribonucleoside. There was, therefore, little or no conversion of MeMP-ribonucleoside to MP-ribonucleotide.

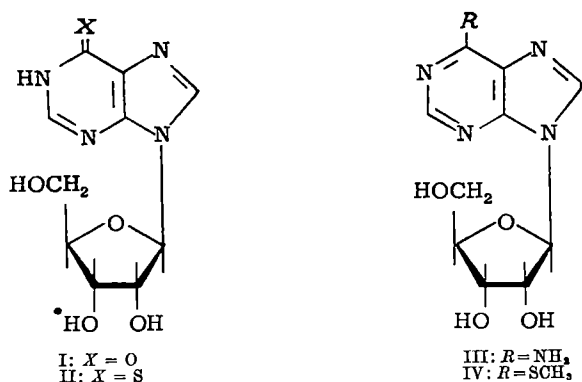
The possible incorporation of MeMP-ribonucleoside into polynucleotides is a question yet unanswered. When *H. Ep.* No. 2/S cells were grown in the presence of MeMP-ribonucleoside- $^{35}\text{S}$ , very small amounts of radioactivity were isolated with the nucleic acids. Determination of whether this radioactivity represents only contamination or a true incorporation is made difficult by the very small amount of sulphur-35 isolated with the polynucleotides and by the instability of MeMP-ribonucleotide—an instability which we observed in the present work and which has recently been noted also by Pfeleiderer *et al.*<sup>21</sup>. Additional evidence against incorporation into polynucleotides is the fact that on chromatograms made from the 80 per cent alcohol extracts of cells grown in the presence of MeMP-ribonucleoside- $^{35}\text{S}$  there was little radioactivity in the area to which the di- and tri-phosphates of MeMP-ribonucleoside would be expected to migrate.

It is also worth noting that no MeMP was detected on the chromatograms made from the 80 per cent alcohol extracts—results indicating that MeMP-ribonucleoside is not degraded by nucleoside phosphorylases in intact cells.

This finding is in accord with the recent report of Paterson and Sutherland<sup>3</sup> that MeMP-ribonucleoside is not converted to MeMP by cell-free extracts of Ehrlich ascites cells.

In separate experiments, cell-free extracts of *H. Ep.* No. 2/S cells were incubated for varying lengths of time with MeMP-ribonucleoside-<sup>35</sup>S (in *tris* buffer, 0.05 M, pH 7.4, containing ATP at a final concentration of 3.1  $\mu$ moles/ml.), after which the incubation mixtures were analysed by the usual chromatography-autoradiography procedures. The autoradiographic patterns thus obtained were similar to those obtained in intact cells; MeMP-ribonucleoside was the principal spot, and the maximum amount of nucleotide was produced during the first 20 min of incubation.

The various types of data presented here show MeMP-ribonucleoside to be an interesting compound on several counts. From the practical point of view, it is a simple derivative of MP that overcomes a mechanism of resistance to MP that is found in many species. From the biochemical point of view, it is of interest that *S*-methylation of MP-ribonucleoside apparently completely changes its substrate specificity for some of the enzymes of purine metabolism. Thus, in intact cells MeMP-ribonucleoside would appear not to be a substrate for nucleoside phosphorylases, and, *in vitro*, MeMP-ribonucleoside remains intact under conditions that produce extensive cleavage of MP-ribonucleoside to the free base<sup>8</sup>. The extensive conversion of MeMP-ribonucleoside to the nucleotide in cells which fail to convert MP-ribonucleoside to the nucleotide shows MeMP-ribonucleoside to be a substrate for a nucleoside kinase that does not act on MP-ribonucleoside. Kinases for inosine and guanosine have not been reported<sup>4</sup>, whereas adenosine kinase is known to occur in mammalian cells<sup>22</sup>; other ribonucleoside kinases known are uridine kinase, riboflavin kinase, and ribosyl-nicotinamide kinase<sup>22</sup>. These facts, and the fact that MeMP-ribonucleoside acts as a purine analogue in inhibiting purine synthesis by a pseudo-feedback action, make it likely that it is adenosine kinase that converts MeMP-ribonucleoside to the nucleotide. With regard to the possibility that MeMP-ribonucleoside is an analogue of adenosine, it is worth noting that, whereas inosine (I) and MP-ribonucleoside (II) both bear a proton at N-1 (since they exist largely in the lactam and thiolactam forms<sup>23</sup>), adenosine (III)<sup>24,25</sup> and MeMP-ribonucleoside (IV) do not. The absence of a proton at N-1 of the pyrimidine ring might, therefore, be more critical for activity as a substrate for the kinase than the nature of the group at the 6-position of the ring. In this connexion,



the inhibitory activity of other *S*-substituted derivatives of MP-ribonucleoside should be noted (Table 1). Another interesting point relating to substrate specificity is the fact that MeMP was not a substrate for any of the purine nucleotide pyrophosphorylases when assays were carried out with cell-free extracts of *H. Ep.* No. 2/S cells by procedures that have been described elsewhere<sup>7,26</sup>. This finding would suggest that the activity of MeMP *in vivo*<sup>27,28</sup>

is the result of demethylation to MP and its consequent conversion to the nucleotide by sensitive cells.

In so far as its metabolism in cultured cells is concerned, MeMP-ribonucleoside (and possibly its congeners) appears, then, to be a unique inhibitor in that it is not a substrate for enzymes degrading other purine nucleosides but is an excellent substrate for a kinase that converts it to the nucleotide, apparently the active inhibitory form. Since MeMP-ribonucleoside is converted to MeMP-ribonucleotide and not to 6-MP-ribonucleotide, it is an interesting question whether these two nucleotides inhibit cell division by means of the same mechanism. All facets of the action of MP are not yet understood; however, with regard to the mechanisms of action of MP and MeMP-ribonucleoside and to the possibility that MeMP-ribonucleoside acts as an adenosine analogue, it is noteworthy that both MP and MeMP-ribonucleoside (after intracellular conversion to the nucleotide) are potent pseudo-feedback inhibitors of purine biosynthesis in intact mammalian cells, and that this is an action that they have in common with several cytotoxic analogues of adenosine and adenosine, that is, 2-fluoroadenosine, 2-fluoroadenosine, 7-deazaadenosine (tubercidin), and others<sup>29</sup>.

The *in vivo* activity of MeMP-ribonucleoside against leukaemia L1210/MP suggests that this agent is worth considering for clinical trial against MP-resistant acute lymphatic and chronic myelocytic leukaemias.

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## INHIBITORY EFFECT OF *p*-BENZOQUINONE ON THE AGGREGATION BEHAVIOUR OF EMBRYO-CHICK FIBROBLAST CELLS

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IT has long been recognized that the adhesion and de-adhesion of cells in association with their movements from one place to another in an organism is a biological phenomenon of considerable importance. It lies at the root of such problems as embryogenesis, wound healing, and certain abnormal growth conditions. There is now abundant evidence that tumour cells, at least *in vitro*, are less adhesive than their normal counterparts<sup>1</sup>.

I have previously advanced the hypothesis that cellular adhesiveness is an expression of the degree of integration of the molecular complex (by cross-linkage) at the cell surface<sup>2</sup>. It was partly derived from the observation that quinone-tanned membranes, which have a relatively high surface viscosity due to bonding and contraction, are less adhesive than untanned ones<sup>2</sup>. Investigations on the reactions between a collagen monolayer and a tanning agent have shown that they are characterized by a considerable increase in surface viscosity together with increases in surface pressure and surface potential. It is considered that these particular changes are the result of cross-linking produced by tanning agents<sup>3</sup>. It is also likely that contraction of a cell surface after treatment with a tanning agent would be accompanied by an increase in the surface density charge.

A high surface viscosity, surface potential and surface density charge are regarded as being unfavourable to cellular adhesiveness<sup>4</sup>. It is significant that these properties are acquired by tumour cells, which seem to have relatively rigid surfaces and a high overall electronegative charge<sup>5</sup>. It has been suggested<sup>6</sup> that some form of discontinuous tanning of a cell surface, such as occurs when erythrocyte membranes are treated with tannate ions<sup>6</sup>, could increase surface rigidity without hindering pseudopod formation or pinocytosis.

It is legitimate to assume, therefore, that if the surfaces of cells were treated with a tanning agent, their adhesiveness would be reduced. Whether this could occur naturally is another question. But among the agents likely to bring this about are vitamin K<sub>1</sub> (2-methyl-3-phytyl-1,4-naphthoquinone), the ubiquinones, and their derivatives. It is interesting that the sensitivity of cells to radiation damage is relatively lower after treatment with menadione or synthetic vitamin K (2-methyl-1,4-naphthoquinone). It has been suggested that this compound tanned the surfaces of the cells to provide a charge distribution required for repairing the small radiation-induced lesions<sup>7</sup>. This effect recalls how the electrophoretic mobility of the cells of *Aerobacter aerogenes* is increased after treatment with phenols<sup>8</sup>. According to the investigators, the phenol caused a contraction of the cell surfaces, thereby raising the surface density charge.

It therefore seemed that the adhesiveness of cells would be reduced after treatment with a tanning agent, and this suggested an examination of the effect of *p*-benzoquinone on the aggregating ability of embryo-chick fibroblast cells.

The cells were derived from the muscle tissues of 10-day-old chick embryos. These were removed from the eggs under aseptic conditions, and the muscle tissues were washed thoroughly in warm Hanks's solution before being dissociated into free cells in a 1 per cent trypsin (Difco 1:250) solution for 10–15 min at 37° C. The cells were washed and re-suspended in Hanks's solution.

When the cells were treated with *p*-benzoquinone dissolved in Hanks's solution, it was found that the cells could tolerate exposure to concentrations not exceeding 10<sup>-6</sup> M for 10 min (ref. 9). Cell viability was little impaired after treatment with the quinone compound at concentrations within the range of 10<sup>-6</sup> M–5 × 10<sup>-6</sup> M. When the treated cells were re-suspended in a culture medium consisting of 10 per cent calf serum in Hanks's solution, only about 10 per cent of the cells showed an affinity for eosin stain after they had been in the medium for 5 h. Treated cells also proliferated in culture, although the length of the initial lag phase in cell growth was proportional to the concentration of *p*-benzoquinone to which the cells had previously been exposed. When the cells had been treated with *p*-benzoquinone at 10<sup>-6</sup> M or 2 × 10<sup>-6</sup> M they proliferated in culture as readily as untreated cells. Accordingly, it was decided to test the effect of *p*-benzoquinone at concentrations ranging from 10<sup>-6</sup> to 5 × 10<sup>-6</sup> M, which were non-lethal, on the adhesiveness of the cells.

Experiments designed to test the effect of chemical and physical factors on the aggregating capacity of cells in suspension have recently been initiated<sup>10</sup>. They have been based on the principle of bringing cells into contact with each other by gently swirling at about 70 r.p.m. in solutions contained in flasks inserted in a gyratory shaker. In the present investigation, 2-ml. samples of cell suspensions in siliconized test-tubes were placed in absorptiometers (E.E.L. 'Quantitrator' with 'Univalvo 20' attached), and stirred with a magnetically rotated rod covered with siliconized glass at 37° C. Optical density was continuously recorded at a wave-length of 600 mμ. A decrease in optical density occurs when the cells aggregate and an increase if cell aggregates disperse. This turbidimetric method is the same as that which has been successfully used in studies on platelet clumping<sup>11</sup>.

The fibroblast cells, after trypsinization, were re-suspended in Hanks's solution. The cell density was adjusted to 10 million cells/ml. by using a haemocytometer, and the cell preparation was divided into four aliquots. The cells of three of them were re-suspended for 10 min in Hanks's solution containing concentrations of *p*-benzoquinone at 10<sup>-6</sup> M, 2 × 10<sup>-6</sup> M, and 5 × 10<sup>-6</sup> M respectively. The cells of each of these samples were washed in Hanks's solution and re-suspended in a medium composed of 10 per cent calf serum in Hanks's solution. The untreated cells of the remaining sample were also re-suspended in this medium. Each of the four cell preparations was placed in an absorptiometer, the tube being inserted in a water-heated 'Perspex' holder fixed to the test platform. In each experiment changes in optical density in the three treated and one untreated cell preparations were recorded over a period of about 5 h, or until the process of cell aggregation had come to an end.

Fig. 1 shows that in both the control and treated cell preparations there was a significant increase in optical density in the first half-hour. Thereafter, it will be seen from the slopes of the curves that in both the treated and untreated preparations the optical density gradually decreased. The overall decrease in optical density is inversely proportional to the concentration of *p*-benzoquinone. The length of the first downward inflexion in

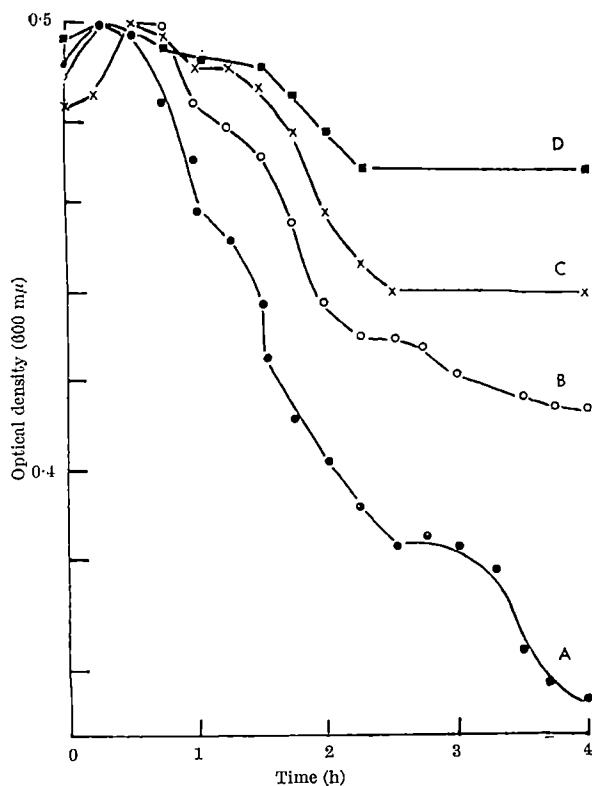


Fig. 1. Effect of pretreating embryo-chick fibroblast cells with *p*-benzoquinone on the optical density of a suspension of  $12 \times 10^5$  cells/ml. in a rotating medium. The curves are related to the following concentrations of *p*-benzoquinone: A, nil (control); B,  $10^{-6}$  M; C,  $2 \times 10^{-6}$  M; D,  $5 \times 10^{-6}$  M.

the curves is also related to the concentration of the quinone. This inflexion in the curves, however, is interrupted by a less steep slope in the 2nd hour. In the 3rd hour each curve flattens out. In the case of the preparations in which the cells were previously treated or tanned with *p*-benzoquinone at a concentration of  $5 \times 10^{-6}$  M (curve D), the rate of decrease in optical density was relatively slow, and there was no further decrease after 2 h. When the cells had been previously treated with *p*-benzoquinone at a concentration of  $2 \times 10^{-6}$  M (curve C), the pattern was similar except that the decrease in optical density was relatively more rapid before it ended after 2.5 h, as signified by the plateau. The plateau in curve B is a small one, reflecting a short delay in the decrease in optical density. The plateau in the control curve A was equally small, but it was followed by a steep inflexion in the 4th hour. In control preparations in particular, the process of cell aggregation was liable to continue for up to about 5 h. The pattern of these results (Fig. 1) was qualitatively reproducible. It is also of interest that the same pattern of results was obtained in Hanks's solution<sup>9</sup>.

When samples of the cell preparations were examined from time to time during an experiment, it became apparent that cell aggregates were formed within the first hour. These initial cell aggregates increased in size in the next 1.5 h. In the control preparations in particular, it was evident that in the last 1- to 2-h period of the experiment, the aggregates already formed tended to adhere to each other to produce larger ones. Relatively few fibroblast cells remained in the control preparations after cell aggregation had come to an end (Fig. 2A). In the treated cell preparations more cells remained free, particularly in the preparation in which they had been treated with *p*-benzoquinone at the highest concentration ( $5 \times 10^{-6}$  M, Fig. 2B). Eosin staining of the remaining free cells revealed that 85-90 per cent of them were viable.

The failure of these viable cells to take part in the formation of aggregates in the treated cell preparations was responsible for the relatively slighter overall decrease in optical density in these preparations. Treatment of the cells with *p*-benzoquinone, therefore, had antagonized their capacity to aggregate without impairing their viability. The adhesiveness of the cells had presumably been reduced by the tanning action of the *p*-benzoquinone molecules adsorbed at the surfaces of the cells. This is probable, because when exogenous quinones succeed in entering cells the effect is lethal, owing to the formation of hydrogen peroxide during antioxygenation of hydroquinones within the cell<sup>12</sup>.

How quinone molecules adsorbed at the surfaces of cells could inhibit cell aggregation is less easy to explain, because we have no clear understanding of the nature of the adhesive mechanisms at the surfaces of cells, or what alterations to the surface properties render cells less 'sticky'. The present findings suggest that a tanning reaction between *p*-benzoquinone and components (probably protein) at the surfaces of the cells is responsible for reducing cellular adhesiveness by increasing the surface viscosity. The same tanning reaction could also cause contraction at the cell surface and an accompanying increase in the surface density charge, an effect which is thought to occur when cells of *A. aerogenes* are treated with phenols<sup>3</sup>. In addition, quinones at the surfaces of cells would probably be reduced to quinols bearing negative groups, which would increase the overall electro-negative surface charge. All these changes, which can be brought about by the reaction of *p*-benzoquinone with

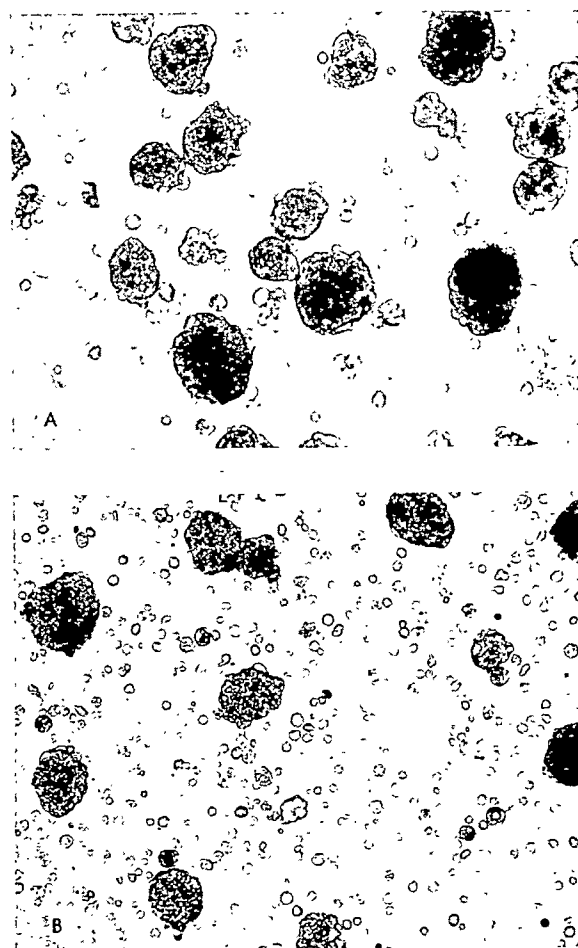


Fig. 2. Effect of pretreating embryo-chick fibroblast cells with *p*-benzoquinone on their aggregation in a rotating medium. A, Aggregates of untreated cells; B, aggregates of cells pretreated with *p*-benzoquinone at  $2 \times 10^{-6}$  M (note the relatively large number of free cells which had failed to take part in the formation of aggregates).



the surfaces of cells, are recognized as being unfavourable to cell adhesion<sup>4</sup>, and they provide an explanation for the inhibitory effect of *p*-benzoquinone on the aggregating ability of the embryo-chick fibroblast cells in the present experiments. The findings are also in line with the view, which formed a basis for this work, that cellular adhesiveness partly depends on the degree of integration (by cross-linking) of macromolecules at the cell surface<sup>5</sup>.

I thank Miss I. Cunningham for her help particularly in standardizing the methods adopted. I also thank Mrs. Howse and Mr. Hirst for their help in this work, which was aided by grants provided by the British Empire Cancer Campaign for Research and the Department of Scientific and Industrial Research.

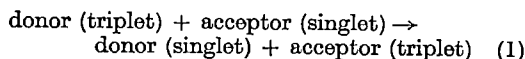
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## SELECTIVE AND MUTUAL SENSITIZATION OF DELAYED FLUORESCENCE

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THE process of triplet energy transfer to a singlet molecule in the ground state, as represented by the equation:



has been investigated in solution by flash excited phosphorescence measurement<sup>1-3</sup>, by flash absorption spectroscopy<sup>4</sup>, and by quenching or sensitization of photochemical reactions<sup>1,5</sup>. For a limited number of systems it has been found that, if the triplet level of the acceptor lies well below that of the donor, the transfer rate is diffusion-controlled, but if it lies well above that of the donor, energy transfer is negligible. If the values of the triplet energies are nearly equal, Bäckström and Sandros<sup>3</sup> concluded that process (1) is reversible. The ratio of the rates of the forward and back reactions should then be equal to  $\exp(\Delta E/RT)$  (where  $\Delta E$  is the difference of the two triplet energies) and the apparent transfer efficiency should be less than that for a diffusion-controlled reaction, and should be temperature-dependent.

Another method for the investigation of process (1) is suggested by the discovery that triplet energy can be transferred to a second triplet molecule. This leads to the formation of an excited singlet molecule and is responsible for the production of sensitized *P*-type delayed fluorescence<sup>6-8</sup>. By using as donor a molecule the excited singlet level of which is lower than that of the acceptor, the triplet level of the latter can be populated by light absorbed only by the donor, and the resulting sensitized anti-Stokes delayed fluorescence<sup>9</sup> can in principle provide a sensitive indication of the occurrence of process (1). We present here some preliminary results obtained with this technique, using three dyestuffs as donors and various aromatic hydrocarbons as acceptors.

### Selective Sensitization

The ratio of the delayed fluorescence efficiencies,  $\theta_A$ , to the prompt fluorescence efficiencies,  $\phi_A$ , of the acceptors at a concentration of  $10^{-6}$  M in ethanol at 22°C is shown in Table 1. At this temperature the lifetimes of the donor triplets, as indicated by the lifetimes of their *E*-type delayed fluorescence<sup>9</sup>, were of the order 5 msec for proflavine and acridine orange hydrochlorides and 2 msec for eosin di-sodium salt. Thus, if quenching is diffusion-controlled ( $k_T = 0.57 \times 10^{10}$  l. mole<sup>-1</sup> sec<sup>-1</sup> at 22°C), the donor triplets should be more than 90 per cent quenched at acceptor concentrations of  $10^{-6}$  M. At this concentration, therefore, the delayed fluorescence efficiency of the

acceptors should be high, low or zero according to whether their triplet levels lie well below, near, or well above those of the donors. The values of  $\theta_A/\phi_A$  in Table 1 are generally in agreement with this prediction, although the results for perylene with proflavine and acridine orange hydrochlorides are lower than would be expected.

Table 1. SELECTIVE SENSITIZATION OF DELAYED FLUORESCENCE AT 22°C

Compound	Triplet level $\mu\text{m}^{-1}$	$10^3 \theta_A/\phi_A$ with sensitization by:		
		Proflavine hydrochloride	Acridine O hydrochloride	Eosin di-sodium salt
Naphthalene	2.12 <sup>2</sup>	<0.01	<0.01	<0.01
Proflavine (donor)	1.71*	—	—	—
Pyrene	1.69 <sup>3</sup>	0.03	<0.01	<0.01
1:2-benzanthracene	1.65*	0.5	<0.01	<0.01
Acridine O (donor)	1.60*	—	—	—
Anthracene	1.48*	0.7	1	0.01
3:4-benzpyrene	1.47 <sup>10</sup>	2	1	0.06
Eosin (donor)	1.42*	—	—	—
Perylene	1.26 <sup>11</sup>	0.1	0.2	0.7

Rates of light absorption by donors were approximately  $6 \times 10^{-4}$ ,  $6 \times 10^{-4}$  and  $1 \times 10^{-5}$  einstein l.<sup>-1</sup> sec<sup>-1</sup> at 436, 436 and 546 nm for proflavine, acridine O and eosin. Acceptor concentrations were all  $10^{-6}$  M.

\* These values calculated from the maxima of the *T*→*S* emission spectra at -75°C.

At -75°C the diffusion-controlled rate constant ( $k_T$ ) in ethanol is 20 times smaller, while the lifetimes of the triplets of proflavine and acridine orange hydrochlorides are some 20 times greater than at 22°C. Thus, with an acceptor concentration of  $10^{-6}$  M, the delayed fluorescence efficiencies should show a variation qualitatively similar to that at 22°C, at least for those systems where the triplet levels of donor and acceptor are well separated. The results in Table 2 (with the exception of those for perylene) bear this out. Perylene is sparingly soluble in ethanol at room temperature and it is possible that at low temperature most of the perylene had crystallized from solution.

It is of particular interest to compare the efficiencies for those systems where the donor and acceptor triplets lie close together. Examples of two types are illustrated in Tables 1 and 2.

*Type 1. Acceptor triplet just below that of donor.* The efficiency of the proflavine-sensitized delayed fluorescence of pyrene at 22°C is much less than that of benzantracene (Table 1, column 3), as would be expected if reaction (1) were reversible. At -75°C the reverse rate of reaction (1) should be reduced and, as indicated in column 3 of Table 2,  $\theta_A/\phi_A$  is indeed greater in comparison with the value for benzantracene (see Fig. 1). It is increased still further by the presence of a greater concentration of pyrene (column 4 of Table 2).

*Type 2. Acceptor triplet just above that of donor.* At a concentration of  $10^{-6}$  M neither pyrene nor benzantracene

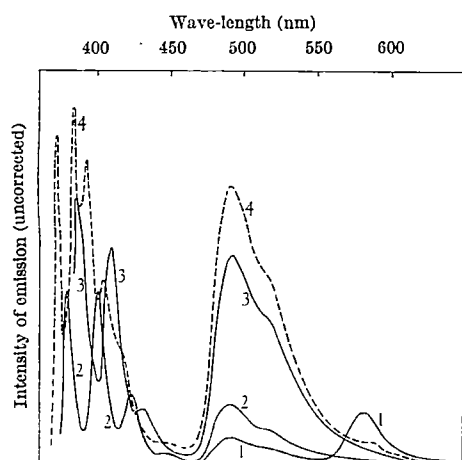


Fig. 1. Mutual sensitization of delayed fluorescence with proflavine hydrochloride as donor. Delayed emission spectra of  $8 \times 10^{-3}$  M proflavine hydrochloride in ethanol at  $-75^\circ\text{C}$  with (1) no addition; (2)  $10^{-4}$  M anthracene; (3)  $10^{-6}$  M 1:2-benzanthracene; (4)  $10^{-4}$  M pyrene. Rate of light absorption was  $6 \times 10^{-6}$  einstein  $\text{l}^{-1} \text{sec}^{-1}$  at 436 nm. Spectral sensitivity of apparatus was that indicated in ref. 8

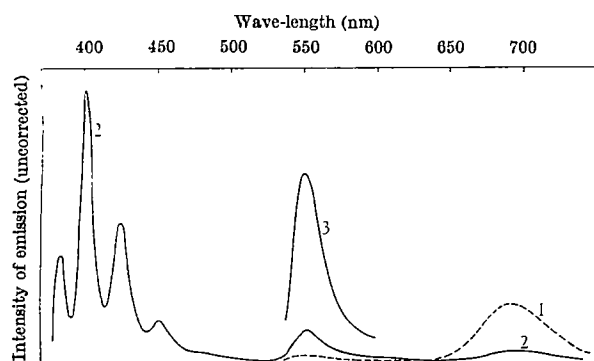


Fig. 2. Mutual sensitization of delayed fluorescence with eosin as donor. Delayed emission spectra of  $5 \times 10^{-4}$  M eosin di-sodium salt in ethanol at  $-75^\circ\text{C}$  with (1) no addition, and (2)  $10^{-4}$  M anthracene. (3) Prompt fluorescence emission spectrum of (1) and (2) at 3,000 times lower sensitivity. Rate of light absorption was  $1 \times 10^{-6}$  einstein  $\text{l}^{-1} \text{sec}^{-1}$ . Spectral sensitivity of apparatus was that indicated in ref. 8

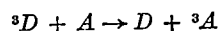
gives any detectable delayed fluorescence with acridine orange hydrochloride as donor. By increasing the pyrene concentration to  $10^{-3}$  M or the benzanthracene concentration to  $10^{-4}$  M, a quite large intensity of delayed fluorescence is observed (column 6 of Table 2). Similarly,  $10^{-6}$  M anthracene, with eosin as donor, shows very weak sensitization at  $22^\circ\text{C}$  (column 5 of Table 1) and no detectable sensitization at  $-75^\circ\text{C}$  (column 7 of Table 2). By increasing the concentration of anthracene to  $10^{-4}$  M, sensitization is observed even at low temperature (column 8 of Table 2 and Fig. 2). Incidentally, the system eosin-anthracene is notable for the considerable anti-Stokes displacement. Thus, when excited with light of wave-length 546 nm, the solution emitted some delayed fluorescence at wave-lengths shorter than 380 nm.

The accuracy of the data shown in Tables 1 and 2 requires some comment. The intensity of sensitized delayed fluorescence varied somewhat with the batch of ethanol used, and some solutions underwent photoreaction during irradiation. The values of  $\theta_A/\phi_A$  are therefore only approximate. The triplet energies of the donors were determined from the positions of the maxima of their  $T \rightarrow S$  emission spectra at  $-75^\circ\text{C}$ . The spectra consisted of single broad bands and it was not possible to locate precisely the O—O transitions. The triplet energies might therefore be situated at wave-numbers up to  $0.05 \mu\text{m}^{-1}$  greater than those recorded. In spite of these difficulties, the results provide a qualitative indication of the relationship between triplet energy-levels and triplet energy transfer efficiencies (process 1) in systems other than those previously investigated. Derivation of values for the efficiency of process (1) from measurements of sensitized delayed fluorescence cannot be attempted until certain anomalous effects (see later) have been satisfactorily explained.

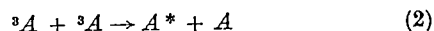
The fact that the delayed fluorescence of acceptor compounds can be selectively sensitized by the choice of a suitable donor suggests a method for the direct analysis of mixtures of fluorescent compounds. Some simple applications of this principle have been discussed elsewhere<sup>12</sup>.

### Mutual Sensitization

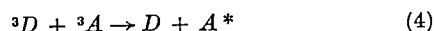
Two alternative mechanisms have previously been proposed for the production of sensitized *P*-type delayed fluorescence<sup>7</sup>. Both mechanisms require process (1) as an initial stage, namely:



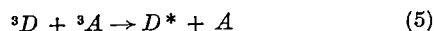
The first mechanism then proceeds by energy transfer between two acceptor triplets (with or without the formation of an excited dimer as an intermediate):



The second mechanism proceeds in an analogous manner by mixed triplet interaction:



The mixed triplet interaction could, in general, give rise to some single-excited donor molecules:



The addition of low concentrations of acceptor would then produce sensitized delayed fluorescence of the donor.

The results obtained with proflavine hydrochloride as donor and three aromatic hydrocarbons as acceptors are shown in Fig. 1. In the absence of acceptor the delayed emission spectrum (uncorrected for spectral sensitivity of the apparatus) shows weak bands due to the  $T \rightarrow S$  phosphorescence (582 nm) and the *P*-type delayed fluorescence (490 nm) of the donor. With acceptor

Table 2. SELECTIVE SENSITIZATION OF DELAYED FLUORESCENCE AT  $-75^\circ\text{C}$

Compound	Triplet level $\mu\text{m}^{-1}$	$10^3 \theta_A/\phi_A$ with sensitization by:					
		Proflavine hydrochloride		Acridine O hydrochloride		Eosin di-sodium salt	
		$[A] = 10^{-6}$ M	$[A] = 10^{-4}$ M	$[A] = 10^{-6}$ M	$[A] = 10^{-4}$ M	$[A] = 10^{-6}$ M	$[A] = 10^{-4}$ M
Naphthalene	2.12	< 0.01	< 0.01*	< 0.01	—	< 0.01	—
Proflavine (donor)	1.71	—	—	—	—	—	—
Pyrene	1.69	0.9	7	< 0.01	0.4*	< 0.01	< 0.01*
1:2-benzanthracene	1.65	3	6	< 0.01	0.5	< 0.01	< 0.01
Acridine O (donor)	1.60	—	—	—	—	—	—
Anthracene	1.48	1	2	0.7	0.6*	< 0.01	1
3:4-benzpyrene	1.47	5	2	—	0.7	0.1	0.4
Eosin (donor)	1.42	—	—	—	—	—	—
Perylene	1.26	0.03	—	0.03	—	0.01	—

Rates of light absorption by the donors were approximately  $6 \times 10^{-6}$ ,  $6 \times 10^{-6}$  and  $1 \times 10^{-5}$  einstein  $\text{l}^{-1} \text{sec}^{-1}$  at 436, 436 and 546 nm for proflavine, acridine O and eosin. \* Refers to acceptor concentrations of  $10^{-3}$  M.

concentrations of  $10^{-6}$  M, the  $T \rightarrow S$  phosphorescence is almost completely quenched, but the delayed fluorescence of the donor increases, and of course, in addition, the sensitized anti-Stokes delayed fluorescence of the acceptors appears at shorter wave-lengths. With benzantracene and pyrene (curves 3 and 4 in Fig. 1), and with benzpyrene (not shown), the sensitization of the donor delayed fluorescence at 490 nm is considerable. Similar results were obtained with acridine orange as donor. With eosin as donor and anthracene as acceptor (Fig. 2) sensitization of the delayed fluorescence of the donor was again observed, although process (1) was relatively inefficient since an anthracene concentration of  $10^{-4}$  M was required to reduce the  $T \rightarrow S$  emission of the eosin (690 nm uncorrected) to 20 per cent of its original value.

### Anomalous Effects

Although these results suggest that mixed triplet quenching can indeed populate the excited singlet state of the donor (by process 5) as well as that of the acceptor (by process 4), there are two features of the results which have not yet been explained. The first concerns the sensitized delayed fluorescence of the donor. At high acceptor concentrations the donor triplet is strongly quenched and process (5) should be almost completely suppressed. Nevertheless, with, for example, benzanthra-

cene at a concentration of  $10^{-4}$  M, the delayed fluorescence of the donor was still observed at an intensity comparable with that shown in Fig. 1. Complex formation between donor and acceptor in the ground state does not seem to be the explanation since the absorption spectrum of the mixture showed no new bands. The second puzzling feature concerns the shape of the sensitized delayed fluorescence spectra of pyrene and benzpyrene. With low acceptor concentrations, both spectra show distortions indicating the presence of another emitting species having a structured spectrum overlapping that of the monomer. Further investigation of both effects is in progress.

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## MECHANISM OF CELL DAMAGE DURING FREEZING AND THAWING AND ITS PREVENTION

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GLYCEROL, dimethyl sulphoxide (DMSO) and several other hydrophilic non-electrolytes, protect cells of many types from damage during freezing and thawing, and allow them to be stored for long periods at low temperatures<sup>1</sup>.

Theories put forward to explain the protective actions of these compounds include prevention of damaging ice crystal formation<sup>2</sup>, and reduction of the concentration of electrolytes at any temperature during freezing<sup>3</sup>. My recent results support the latter. Two new methods have been developed to prevent any rise in electrolyte concentration during cooling to low temperatures. As a result the functional recovery of an organized tissue after re-warming has been greatly improved. The theoretical basis of these methods is as follows.

Fig. 1A shows a solid three-dimensional phase diagram of the three-component system DMSO-NaCl-water. The letter *a* in Fig. 1B denotes a solution containing 0.154 M NaCl in a mixture of DMSO and water (1 : 9 v/v). Its freezing-point is at *b*. It is clear that during further cooling (*bcd*) ice only will separate out until very low temperatures are reached. As ice crystallizes out, the concentrations of both the NaCl and the DMSO in the liquid phase increase. For comparison, *efgh* represents the cooling of the two-component system of NaCl (0.154 M) in water, and *ijkl* indicates the cooling of the two-component system of DMSO (10 per cent v/v) in water.

The curves in Fig. 2 show the effects of varying the initial DMSO concentration on the NaCl concentration at different temperatures during freezing. The initial concentration of NaCl is 0.154 M. The inclusion of as little as 5 per cent v/v DMSO greatly reduces the concentration of NaCl at any temperature as ice separates. Increasing the concentration of DMSO not only lowers the freezing-point progressively, but also markedly reduces the build-up of salt concentration as the temperature is lowered.

With this quantitative information, either of two methods can be used in order to prevent the electrolyte concentration from rising during cooling.

**Method 1.** After incubating the tissue or organ with 5 or 10 per cent v/v DMSO until equilibration is reached at  $+37^{\circ}\text{C}$  (ref. 4), the sample is cooled to the freezing-point and seeded with an ice crystal to prevent supercooling. Afterwards the temperature is lowered very slowly. As ice separates out the volume of the residual liquid phase is kept constant by the addition of a small amount of a solution of DMSO in water at the same temperature. The concentration of DMSO added is the same as that in the liquid phase at that temperature. The addition of the DMSO-water solution can be carried out either in steps or continuously. The most important factor to be considered is that throughout the cooling process sufficient time must elapse to allow the electrolytes throughout the whole system, including the cells, to equilibrate by diffusion. Provided that the liquid volume remains constant the concentrations of all solutes, except DMSO, cannot increase despite the separation of ice. The same concentration-temperature curve can again be followed during the equally slow thawing process. As the ice melts, solutions containing more and more electrolytes but less and less DMSO can be added to keep the electrolyte concentrations constant. This method depends on the diffusion of the electrolytes.

**Method 2.** After the usual incubation in 5 or 10 per cent DMSO at  $+37^{\circ}\text{C}$ , to allow the DMSO to penetrate into the intracellular space, the sample is cooled to within a few degrees of the freezing-point but not allowed to freeze. The concentration of DMSO within the cells is then increased by transferring the tissue to another solution at the same temperature containing the same concentration of electrolytes but an increased concentration of DMSO. After sufficient time has elapsed for

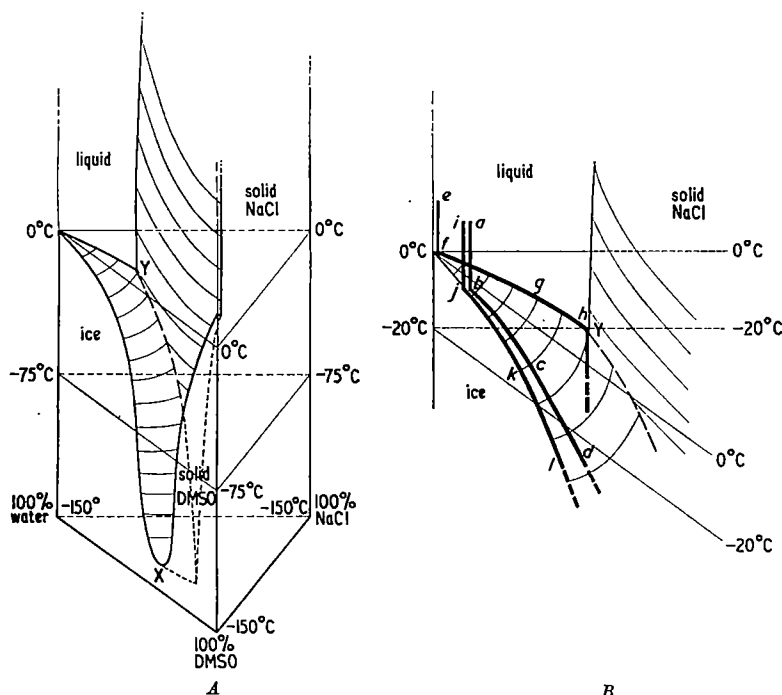


Fig. 1. *A*, Three-dimensional phase diagram of the three-component system DMSO-NaCl-water. The eutectic temperature of the two-component system DMSO-water is  $-136^{\circ}\text{C}$  (*X*), and of the two-component system NaCl-water  $-21^{\circ}\text{C}$  (*Y*). *B*, Magnified view of the ice-liquid interface; *a* is a three-component system of 0.154 M NaCl in a mixture of DMSO and water (1:9 v/v) which freezes at *b* ( $-3.6^{\circ}\text{C}$ ); *e* and *i* represent the two-component systems of NaCl (0.154 M) in water, and DMSO (10 per cent v/v) in water with freezing-points at *f* ( $-0.56^{\circ}\text{C}$ ) and *j* ( $-3.6^{\circ}\text{C}$ ) respectively. When *a* is cooled below its freezing-point ice only separates out (along the curve *bad*), both the DMSO and the NaCl remaining in solution but becoming more concentrated as the temperature is lowered.

diffusion to mix the DMSO completely, outside and inside the cells, the temperature can again be lowered to within a few degrees of the new freezing-point. As in Method 1, the change in DMSO concentration and the reduction in temperature can be carried out either in steps or continuously. Finally, the sample can be stored at  $-79^{\circ}\text{C}$  in the liquid state in a suitable concentration of DMSO (55 per cent v/v). The procedure can be carried out in reverse during slow re-warming. This method depends on the diffusion of DMSO.

Both these methods were theoretically derived using the assumption that NaCl was the only electrolyte present. However, they are still applicable even when the isotonic medium to which the DMSO is added contains a complex mixture of different electrolytes in solution. This is because the basic DMSO-water freezing-point curve is not altered appreciably by the small concentration of electrolytes present in physiological solutions, such as Krebs's solution. The main aim of both these methods is never to allow the solute concentration to exceed the normal levels. The effects of lowering the temperature of a multi-component mixture of electrolytes are complex, but the concentrations of the individual solutes cannot increase above the normal using methods 1 or 2; they can only decrease as each comes out of solution. As yet there is no evidence that cells are permanently damaged by the removal of one or more electrolytes from solution during cooling.

These two new methods of cooling tissues to  $-79^{\circ}\text{C}$  were compared experimentally with the usual cooling method<sup>1,5</sup> using smooth muscle preparations from the guinea-pig. Contractile function in these preparations depends not only on a high percentage survival of cells but also on complete maintenance of the co-ordination between the cells.

Uteri and taenia coli were taken from immature (200–350 g) albino guinea-pigs. The modified Krebs's solution used had the following composition (mM): NaCl, 118; KCl, 4.5;  $\text{CaCl}_2$ , 1.4;  $\text{MgCl}_2$ , 1.16;  $\text{NaH}_2\text{PO}_4$ , 1.16;

$\text{NaHCO}_3$ , 25; dextrose, 11.1. All solutions containing muscles above  $0^{\circ}\text{C}$  were bubbled with  $\text{O}_2$  (95 per cent) +  $\text{CO}_2$  (5 per cent). The functions of the same samples of smooth muscle were tested *in vitro* in an organ bath at  $+37^{\circ}\text{C}$  before and after cooling to  $-79^{\circ}\text{C}$ . Isometric contractile responses were recorded using an M.R.C. strain gauge and a 1-mV potentiometric recorder. Sub-maximal responses were induced by a regularly repeated standard dose of histamine ( $2-3\text{ }\mu\text{g}$  in 20 ml.). After being tested for function, all muscles were incubated for 20 min at  $+37^{\circ}\text{C}$  in 10 per cent v/v DMSO in Krebs's solution. The three methods of cooling and re-warming are described here. Storage time at  $-79^{\circ}\text{C}$  varied between 1 and 24 h.

**Method 1.** The sample was cooled to  $0^{\circ}\text{C}$  in the Krebs's solution containing 10 per cent v/v DMSO. Addition of the different solutions during freezing and re-warming was carried out in steps. The oscillations of the electrolyte concentrations were small and always below the normal value, in order to prevent any risk of a damaging rise in concentration.

**Method 2.** In this method the sample was cooled to  $0^{\circ}\text{C}$  in the Krebs's solution containing DMSO 10 per cent v/v as before. A thread had previously been tied to the muscle, and at each step of the cooling and warming process the muscle was transferred from one solu-

tion to the next. Freezing did not occur.

**Old method.** After being cooled to  $0^{\circ}\text{C}$  in the Krebs's solution with 10 per cent v/v DMSO the sample was cooled at  $2^{\circ}-3^{\circ}\text{C}$  per min to about  $-30^{\circ}\text{C}$  and then at  $4^{\circ}-5^{\circ}\text{C}$  per min to  $-79^{\circ}\text{C}$ . Thawing was accomplished by plunging the tube containing the muscle at  $-79^{\circ}\text{C}$  into a beaker of water ( $+40^{\circ}\text{C}$ ). All ice crystals had vanished within 2 min.

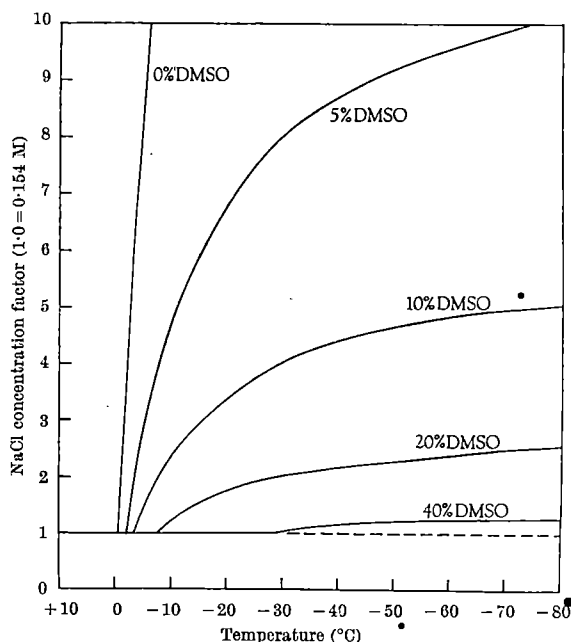


Fig. 2. The effects of varying the initial DMSO concentration on the NaCl concentrations of DMSO-NaCl-water solutions at different temperatures during freezing. In each instance the initial concentration of NaCl is 0.154 M. Increasing the concentration of DMSO reduces the build-up of salt concentration as ice separates during freezing.



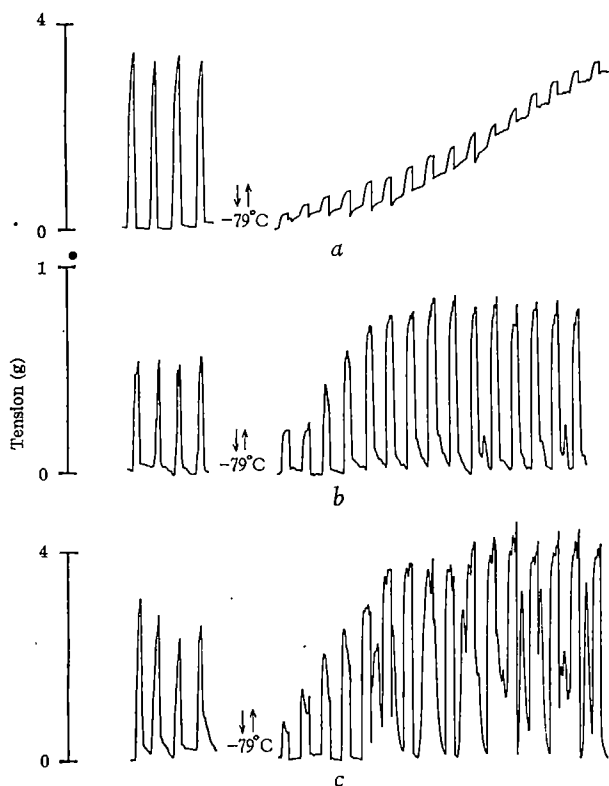


Fig. 3. Isometric contractile responses of uteri to a standard dose of histamine at  $+37^{\circ}\text{C}$  before and after freezing to  $-79^{\circ}\text{C}$ . (a) Cooled by the old method; thawed rapidly. (b) Cooled by method 1; thawed rapidly. (c) Cooled and thawed slowly by method 1. Standard doses of histamine ( $2\text{ }\mu\text{g}$  in a and b;  $3\text{ }\mu\text{g}$  in c) were given at 2-min intervals; contact time 30 sec. Volume of organ bath 20 ml.

Other experiments were performed in which muscles were cooled using methods 1 or 2 but were then thawed rapidly (within 2 min).

After re-warming, all muscles were transferred to a Krebs's solution without DMSO, set up in the organ bath, and tested for contractile function as before.

As previously reported<sup>5,6</sup>, all uteri frozen by the old method contracted spontaneously after thawing and remained contracted (Fig. 3a). Only the addition of EDTA or  $\text{Mg}^{++}$  induced relaxation<sup>6</sup>. In contrast, uteri thawed either slowly or rapidly from  $-79^{\circ}\text{C}$  using method 1 (Fig. 3b and c) or method 2 did not give this spontaneous long-lasting contraction. When either of the new methods was used the mean contractile responses of thawed uteri to the standard dose of histamine were either equal to or greater than the control responses before cooling (Fig. 3b and c). Uteri cooled to  $-79^{\circ}\text{C}$  by all three methods showed spontaneous rhythm after thawing; this effect was much reduced in muscles cooled by method 1 but rapidly thawed (Fig. 3b).

Taenia coli frozen to  $-79^{\circ}\text{C}$  by the old method never contracted after thawing either in response to histamine or spontaneously. By contrast, taenia cooled by methods 1 and 2 contracted in response to histamine after re-warming; unlike the thawed uteri, they showed no spontaneous contraction.

These results leave no doubt that the functional activity of smooth muscle was better preserved by these new techniques of cooling to low temperatures. The basic principle of these two new methods of cooling in the presence of a non-electrolyte is to prevent the concentrations of solutes in the liquid phase from rising above normal values. This was done by adding solutions during cooling and by depending on diffusion to restore equilibrium inside and outside the cells. The fact that cooling either by method 1 (with ice formation) or by method 2 (without ice formation) protects smooth muscle from damage strongly indicates that, for this mammalian tissue

at least, appreciable damage is not caused by ice. This is further evidence that the partial protective effects given by many non-electrolytes, including glycerol, DMSO, and methyl acetamide<sup>7</sup>, are due to their action in reducing the build-up of solute concentrations as the temperature is lowered. Lovelock first suggested this explanation for the protection of erythrocytes by glycerol during freezing. He found that haemolysis of erythrocytes occurred when the concentration of the NaCl in the liquid phase reached mole fraction 0.014 (0.8 M in water) irrespective of the concentration of glycerol<sup>8</sup>. With other cells, partial damage probably occurs before the electrolyte concentration reaches this comparatively high level. Nash has described a range of protective compounds and the physical properties they need in order to interact with water<sup>7</sup>. This interaction enables them to lower the freezing-point of water and reduce the build-up of solute concentrations during freezing in the same way as do glycerol and DMSO. The one criterion is that protective compounds should be non-toxic in the high concentrations which are reached during cooling. If this condition is fulfilled, then these new methods can be used with protective substances other than DMSO provided that an accurate freezing-point curve is determined initially.

Huggins has delayed the formation of ice during the cooling of red blood cells by using a high concentration of DMSO (5.5 M, 39 per cent v/v) in the original medium<sup>9</sup>. These simple non-nucleated cells can tolerate this high concentration of DMSO at  $+37^{\circ}\text{C}$ . Other types of cells cannot. With method 2 the concentration of the protective substance is deliberately increased, but only as cooling progresses. It is important to remember that all biological samples cooled in a 10 per cent v/v DMSO solution by the old method to  $-79^{\circ}\text{C}$  are in contact with a solution of 50.5 per cent v/v DMSO during storage at that temperature. In fact, the damaging or denaturing properties of compounds like DMSO must be greatly reduced by lowering the temperature. Further experiments are necessary to determine the relationship of temperature to the denaturing ability of different concentrations of DMSO and other non-electrolytes.

One other advantage of these new methods is to reduce the problem of the rate of cooling. Hitherto, rates of cooling have been important because, using the usual method of cooling, an increase in the solute concentration still occurs. The amount of damage caused by an excess of electrolyte will depend on three factors: the actual concentration of electrolyte, the temperature, and the time that the excess concentration is left in contact with the living cell. Despite the fact that, with the old method, reducing the temperature will increase the concentration of electrolytes during freezing, the lower the temperature the lower will be the chemical potential of the electrolytes. The combination of these factors will therefore determine the optimal rates of cooling. By contrast, using method 1 or 2, smooth muscle cells, at least, can be kept for several hours at temperatures as high as  $-5^{\circ}\text{C}$  or  $-15^{\circ}\text{C}$  without electrolyte damage. Since changes of concentration need only occur very slowly, damage due to sharp osmotic imbalances on either side of cell membranes can also be avoided.

If very long-term storage is required, samples should be kept at the temperature of liquid nitrogen ( $-196^{\circ}\text{C}$ ) rather than that of solid carbon dioxide ( $-79^{\circ}\text{C}$ ). The increase in electrolyte concentration which must occur just before the whole system solidifies between  $-79^{\circ}\text{C}$  and  $-196^{\circ}\text{C}$  will not damage the cells at these very low temperatures, provided that normal concentrations have not been exceeded between  $0^{\circ}\text{C}$  and  $-79^{\circ}\text{C}$ .

Until now, recovery of viable cells and tissues from low temperatures has necessitated rapid thawing. The small muscle samples used in the present experiments were easy to thaw rapidly and tolerated the process well. Rapid thawing has itself caused problems with larger samples of other cells and tissues as well as with organs

because of the difficulty of rapid heat transfer. The slow thawing procedures used in both method 1 and method 2 will avoid these difficulties.

The most important advantage of method 2 over method 1 is that, because freezing is avoided, it may be possible to devise a similar method for cooling organs, by perfusing higher and higher concentrations of protective substances at lower and lower temperatures. Experiments to attempt this are planned with the heart.

In the past many cells have been partially protected from damage during freezing and thawing by the use of added non-electrolytes. This is a tribute to the capacity of these cells to withstand exposure to raised electrolyte concentrations, even if these concentrations are much lower than would be reached without the non-electrolytes. The complete prevention of any increase in the concentration of salts may lead to the successful recovery of more sensitive cells and to an improved recovery of the others.

Two final words of caution should be given. First, the most important practical difficulty of these two new methods is in deciding when the process of diffusion has equilibrated the concentrations of ions (method 1) or of DMSO (method 2) at each temperature. Until quantita-

tive values for the diffusion of ions and DMSO have been computed for different conditions of temperature and tissue type and size, estimation of this time factor must be approximate. Secondly, the longer time taken for cooling and re-warming increases the relative importance of possible harmful effects of high concentrations of DMSO or of any other protective agent at low temperatures.

Once these problems are overcome it may be that these new methods will form the basis for the successful freezing, storage and thawing of tissues which require 100 per cent cell survival in order to function.

I thank Drs. J. E. Lovelock, T. Nash and Audrey U. Smith for their advice, and Miss L. Staniford and the staff of the Engineering Division of the National Institute for Medical Research for their technical assistance.

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<sup>2</sup> Mazur, P., *Ann. N.Y. Acad. Sci.*, **85**, 610 (1960).

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<sup>6</sup> Farrant, J., *J. Physiol.*, **170**, 33P (1964).

<sup>7</sup> Nash, T., *J. Gen. Physiol.*, **46**, 167 (1962).

<sup>8</sup> Huggins, C. E., *Transfusion*, **3**, 483 (1963).

## DISTRIBUTION OF TRITIATED THYMIDINE IN VARIOUS TISSUES

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AFTER injection into animals tritiated thymidine is distributed by the bloodstream and finds its way into cells. It is then incorporated into DNA by those cells that are in a suitable metabolic state, and thymidine not utilized is broken down. Measurements have shown that the average number of grains per nucleus reaches its maximum 40-60 min after injection<sup>1,2</sup>. It seemed of interest to determine directly the availability of tritiated thymidine to the various types of cells in a number of organs.

In one experiment adult mice weighing 22-25 g were given intraperitoneal injections of 50  $\mu$ Ci tritiated thymidine and killed 15 min, 30 min and 1 h after injection. After dissection, organs were immediately frozen by immersion in isopentane cooled in liquid nitrogen. Autoradiographs were prepared by a technique which allows the retention of the soluble labelled compounds as described by Appleton<sup>3</sup>. Frozen sections were cut at 5-6  $\mu$  on a 'Pearse' cryostat kept in a darkroom and operated under a red safelight. Coverslips were covered with Kodak 'AR10' stripping film so that the photographic emulsion faced outwards, then dried and cooled to -5° C. Frozen sections were transferred by touching the cold coverslips against the sections and the preparations exposed at a temperature of -20° to -30° C. After exposure the sections were fixed in 5 per cent acetic alcohol for 1 min, washed in tap water, and then processed in Kodak 'D19b' developer and acid fixer. After being washed and dried the preparations were stained with haematoxylin and eosin. Autoradiographs of well-labelled nuclei were invariably above the nuclei, indicating that the film and tissue were firmly bonded together, and that no slipping occurred during processing or exposure. Autoradiographs of fixed and embedded material were also prepared.

Most cells in all tissues investigated show nuclear and cytoplasmic labelling 15 min after injection, while after 30 min the autoradiographs are considerably weaker. 1 h after injection the grain numbers are lower still, though definitely above background, and after 24 h the counts are nearly equal to background except over cells which have incorporated tritiated thymidine into DNA.

Such cells are found in varying proportions at all times in epithelial cells and smooth muscle surrounding the colon and seminal vesicle, and in brain, as well as in the dividing organs (intestine, oesophagus).

The number of photographic grains per unit area was counted in various tissues by using a square eye-piece aperture. Heavily labelled nuclei were disregarded. Fig. 1 shows that after intraperitoneal injection the concentration of grains is highest in smooth muscle, somewhat lower in epithelial tissues, and lowest in brain, probably owing to the blood-brain barrier.

In another experiment adult mice were given an intraperitoneal or intravenous injection of 50  $\mu$ Ci tritiated thymidine and killed 2 min after injection; water-soluble autoradiographs were prepared as described. In the smooth muscle from the colon of a mouse which had been given an intravenous injection (Fig. 2) the label is evenly distributed throughout the muscle. On the other hand, in smooth muscle (colon) from a mouse which had been given an intraperitoneal injection (Fig. 3) thymidine can be seen diffusing in from the peritoneal fluid surrounding the intestine, irrespective of the direction of the muscle fibres or nuclear membranes. The speed of diffusion is approximately 2  $\mu$ /sec.

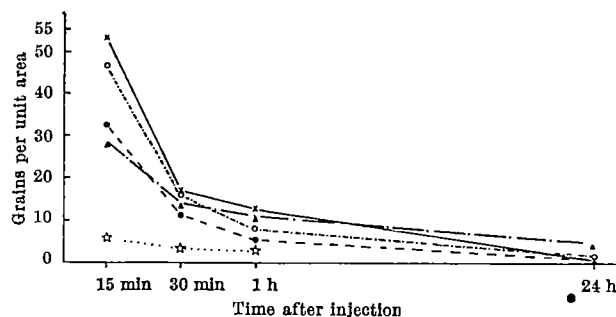


Fig. 1. Distribution of tritiated thymidine in various tissues of the mouse. The unit area for grain counting (72  $\mu^2$ ) was determined by an eye-piece aperture. Nuclei showing high labelling were assumed to have incorporated tritiated thymidine and were omitted. Crosses, smooth muscle (colon); triangles, colon; stars, brain; open circles, smooth muscle (seminal vesicle); closed circles, seminal vesicle.

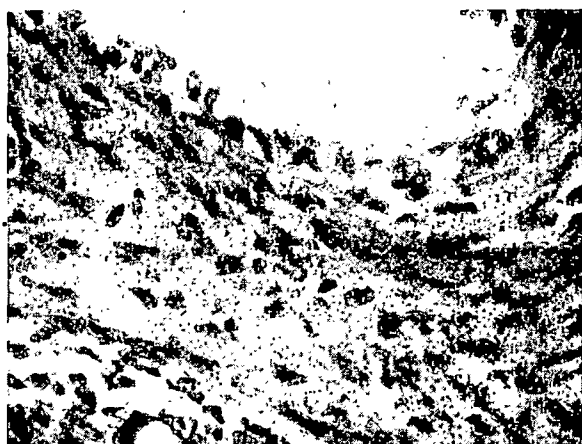


Fig. 2. Water-soluble autoradiograph of the distribution of tritiated thymidine in smooth muscle of the colon 2 min after intravenous injection. Note the even labelling throughout the muscle. (Haematoxylin and eosin,  $\times 400$ )

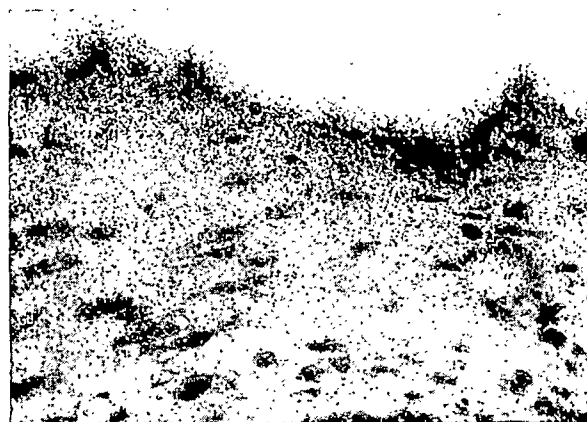


Fig. 3. Water-soluble autoradiograph of the distribution of tritiated thymidine in smooth muscle 2 min after intraperitoneal injection. The thymidine can be seen diffusing in from the peritoneal fluid bathing the outside of the gut; the diffusion appears to be unaffected by nuclei or muscle fibres. (Haematoxylin and eosin,  $\times 400$ )

Grain counts (Table 1) show that after intravenous injection the concentration in mouse intestine is approximately equal in the epithelium and in the smooth muscle.

Using an eye-piece aperture of apparent diameter equal to that of cell nuclei, the grain density over nuclei and cytoplasmic areas in brain was counted (Figs. 4, 5 and 6).

15 min after injection, the cytoplasm shows a reasonably random distribution of grain densities while some of the nuclei contain considerably more than others. After 30 min a few nuclei show more grains than the equivalent areas of cytoplasm, while after 1 h the distribution over nuclei and cytoplasm is almost equal. Similar differences between labelling of nuclei and cytoplasm were observed in other tissues, though no detailed counts were undertaken. In brain the average count per unit area over nuclei is 3.8 grains and 1.1 grains over cytoplasmic areas. Measurements have shown that approximately 10 per cent of the area of a section is nuclear, hence 27.8 per cent of the labelled material is in the nuclei and 72.2 per cent in the cytoplasm. After 30 min the concentrations are 0.9 grains per unit area over nuclei and 0.7 over cytoplasm, and at 60 min 1.2 grains for nuclei and 1.6 for

Table 1. TRITIATED THYMIDINE IN MOUSE INTESTINE AFTER INTRAVENOUS INJECTION

Time (min)	Grains/unit area	
	Epithelium	Smooth muscle
2	12.9	15.6
5	12.7	12.9
10	10.7	10.3
20	2.5	2.9

Time: time from injection to death of animals. Grains per unit area: see note to Fig. 1. Corrected for background.

cytoplasm. These figures have been corrected for background. After 30 min and 60 min the concentrations are approximately equal and therefore 10 per cent is in the nuclei and the remainder in the cytoplasm. Nevertheless a number of nuclei still show more grains than would be expected from a random distribution (Fig. 6). In previous work<sup>4</sup> tritiated thymidine was found to be incorporated into DNA in only 0.24 per cent of the cells of the central nervous system; the labelled nuclei counted here in considerable numbers are therefore unlikely to be permanently labelled.

Grain counts over well-labelled nuclei in water-soluble autoradiographs and stripping film autoradiographs of fixed and embedded organs showed no significant difference. Feinendegen and Bond<sup>5</sup> found higher concentrations of tritiated thymidine in bone marrow in cell types capable of division than in the non-dividing ones. Unless the discrepancy between their findings and ours is due to differences in technique it has to be assumed that bone marrow cells differ from those in other organs in permeability to thymidine.

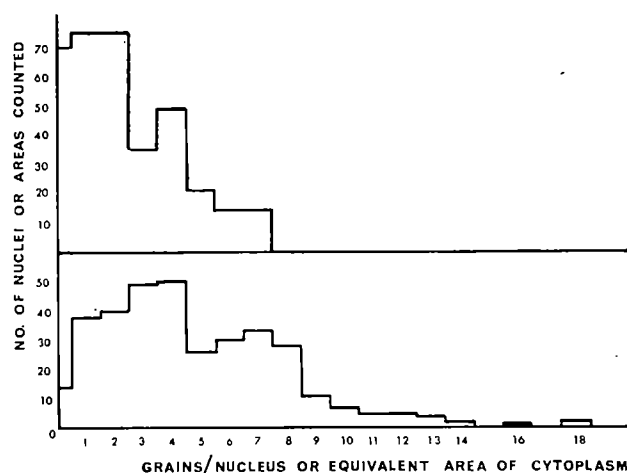


Fig. 4. Grain distribution in brain, 15 min after injection of tritiated thymidine. An eye-piece aperture of the apparent size of the nuclei was used to count grains over 300 nuclei and 300 cytoplasmic areas. Above, cytoplasm; below, nuclei

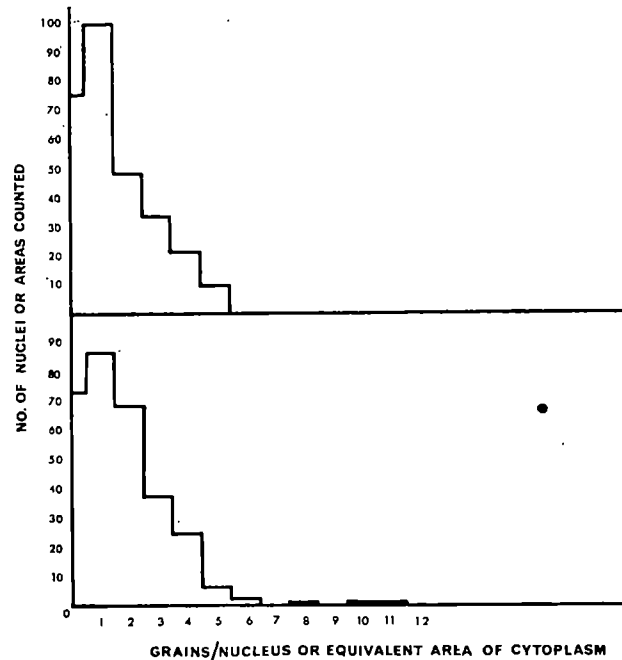


Fig. 5. Grain distribution in brain, 30 min after injection. See remarks to Fig. 4. Above, cytoplasm; below, nuclei

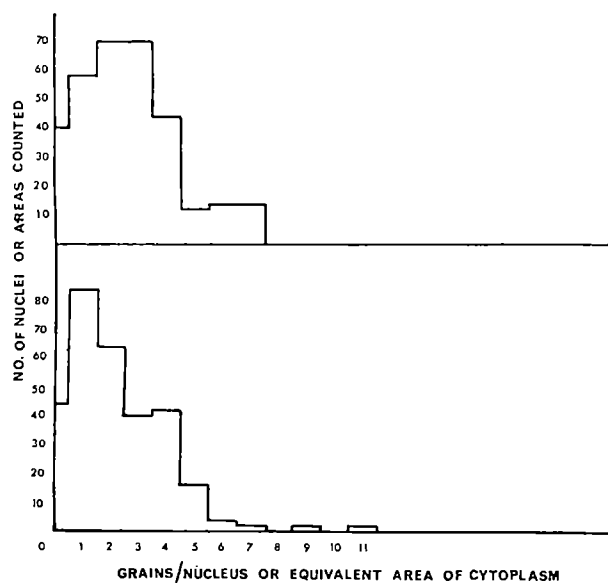


Fig. 6. Grain distribution in brain 1 h after injection. See remarks to Fig. 4. Above, cytoplasm; below, nuclei

It can be concluded that: (a) tritiated thymidine is available to the cytoplasm and nuclei of nearly all cells in the organs investigated; (b) since only a proportion of nuclei are found to be permanently labelled, availability of thymidine in itself is not sufficient to initiate incorporation of thymidine into DNA, nor does it to any extent stimulate it; (c) the amount of tritiated thymidine which diffuses into all cells in an organ is sufficient to explain the degree of uptake in well-labelled nuclei if it is assumed that such cells retain all the thymidine entering the cell and that the contents of soluble material are 'turned over' once every 3-5 min; (d) the incorporation of tritiated thymidine in the more heavily labelled nuclei of the central nervous system in excess of that expected from random labelling may be due to a different permeability of some nuclear membranes or the formation of an intermediate compound; (e) in view of the short time of availability of tritiated thymidine, the site of injection can seriously affect the total amount of tritiated thymidine reaching the cells, and therefore comparison of grain numbers over labelled nuclei in different organs may be subject to substantial errors.

<sup>1</sup> Koburg, E., and Maurer, W., *Biochim. Biophys. Acta*, **61**, 229 (1962).

<sup>2</sup> Quastler, H., and Sherman, F. G., *Exp. Cell Res.*, **17**, 420 (1959).

<sup>3</sup> Appleton, T. C., *J. Roy. Micro. Soc.*, **83**, 277 (1964).

<sup>4</sup> Pele, S. R., and Appleton, T. C. (in preparation).

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## SECRETION OF THYROCALCITONIN

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DURING recent months a good deal of controversy has centred round the hypocalcaemic factors secreted by the parathyroid and thyroid. Copp *et al.*<sup>1</sup> have demonstrated that hypercalcaemic perfusion of a dog's thyroid along with its parathyroid glands resulted in a more rapid fall in the systemic plasma calcium concentration than could be accounted for in terms of mere inhibition of parathormone secretion. Moreover, they showed that cross-transfusion of thyro-parathyroid venous plasma from such a stimulated gland preparation into an intact dog caused a sharp decrease in the plasma calcium concentration. No significant change in calcium concentration was associated with hypercalcaemic perfusion of the thyroid from which all parathyroid tissue had been removed. Using sheep, in which species two superior parathyroids are found near the apices of the thymus and only one in each lobe of the thyroid, Copp and Henze<sup>2</sup> demonstrated a hypocalcaemic response to hypercalcaemic perfusion of the superior parathyroid but no response when one lobe of the thyroid with its small parathyroid were similarly perfused. From these and other results, Copp *et al.*<sup>1</sup> conclude that a hormone having a hypocalcaemic action is secreted by the parathyroid in response to hypercalcaemia. This parathyroid hormone they called 'calcitonin'. On the other hand, using goats, in which the anatomical arrangement of parathyroids and thyroids is similar to that found in sheep, Foster *et al.*<sup>3</sup> failed to demonstrate the secretion of calcitonin in response to hypercalcaemic perfusion of the superior parathyroid.

In contrast to the foregoing work carried out on anaesthetized animals, a conscious sheep preparation<sup>4</sup> was devised, and with it Care and Keynes<sup>5</sup> clearly demonstrated that the parathyroids are a source of a hypocalcaemic hormone, similar in its time course of action to calcitonin.

Hirsch *et al.*<sup>6</sup> postulated the release of a hypocalcaemic factor from parathyroid-free thyroid which they termed thyrocalcitonin, and Hirsch and Munson<sup>7</sup> and Baghdiantz *et al.*<sup>8</sup> have all prepared purified extracts of thyroid tissue

which exhibit potent hypocalcaemic effects in both intact and parathyroidectomized animals. Both groups of workers suggest that calcitonin and thyrocalcitonin are the same substance and that this is secreted by the thyroid. However, no unequivocal evidence for the release of thyrocalcitonin in response to hypercalcaemic perfusion of a thyroid free from parathyroid and undamaged by surgical interference has so far been provided. Such evidence is presented here.

**Operative procedures.** Pig thyroid has been reported to contain no parathyroid tissue<sup>9</sup> and this was confirmed by the examination of serial sections by light microscopy. Two castrated Large White male pigs were used of live weight 35-40 kg. Anaesthesia was induced by the intravenous administration of a mixture of 30 per cent methohexital sodium (25 mg/ml.) and thiopentone (50 mg/ml.); it was maintained under halothane and oxygen. Food was withheld for the 24 h preceding the experiment.

The neck was incised longitudinally and the thyroid exposed. Since, in the pig, the manubrium of the sternum often extends over the thyroid vein, 1-2 cm of this was removed and the attached muscles were ligated and cut. The stump of the manubrium was plugged with bone wax. Careful dissection revealed the thyroid vessels. The right thyroid artery is a branch of the inferior cervical artery in the typical case, although it has been observed as a branch of the common carotid artery. The left and right thyroid veins soon fuse to form a single vessel which joins the junction of the left and right innominate veins (Fig. 1).

The right inferior cervical artery was catheterized with siliconed polyvinyl chloride tubing (ext. diam. 2.0 mm) cranial to the point of emergence of the thyroid artery. The left common carotid artery was catheterized with siliconed polyvinyl chloride tubing (ext. diam. 3.0 mm). A loose ligature was placed round the thyroid vein close to its junction with the innominate veins. The left external jugular vein was catheterized with polythene tubing (ext. diam. 1.2 mm).



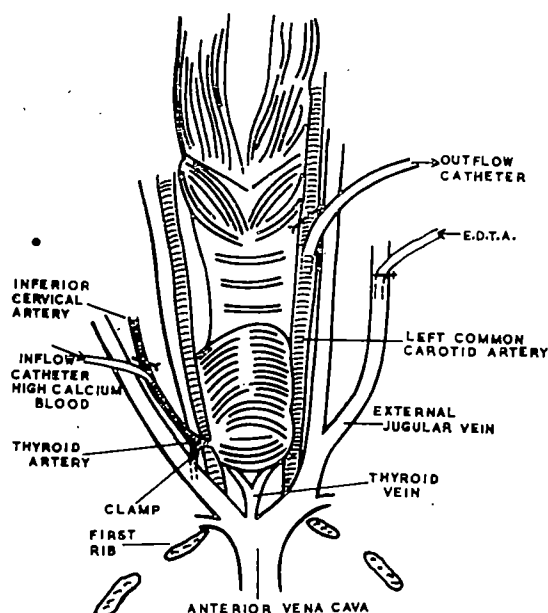


Fig. 1. The location and principal blood supply of the thyroid gland in the pig, showing the method of hypercalcaemic perfusion of the gland. A portion of the manubrium has been removed to show the whole of the thyroid vein. The parathyroid glands are in the thymus and have been left intact.

After surgery had been completed, each pig was heparinized initially with 7,500 i.u. heparin and a further 5,000 i.u. administered every 1-1.5 h. The outflow catheter was connected via siliconed polyvinyl chloride tubing to the inflow catheter in such a way that the rate of flow could be controlled by means of a roller pump so that the perfusion pressure was maintained at least 20 mm in excess of the systolic blood pressure. This flow rate was usually 5-8 ml./min. By means of a constant infusion pump Krebs-Henseleit Ringer solution, supplemented with calcium chloride, was added to the blood at the rate of either 0.1 or 0.2 ml./min. Inflow blood samples could be obtained from a tap on the inflow catheter and systemic blood samples from a tap on the outflow catheter. When the circuit was established and the rate of calcium supplementation of the perfusing blood adjusted to give a suitable resultant plasma calcium concentration (7 m.equiv./l.), a small bulldog clip was placed on the right inferior cervical artery caudal to its junction with the thyroid artery.

In order to maintain a constant content of ionized calcium within the pig, a suitable concentration of the di-sodium salt of ethylenediamine tetraacetic acid (EDTA) dissolved in 0.9 per cent saline was infused at 0.5 ml./min via the catheter in the left external jugular vein. The EDTA concentration was adjusted to compensate for the additional calcium used to perfuse the thyroid.

Approximately 2 h after termination of surgery, hypercalcaemic perfusion of the thyroid commenced along with systemic infusion of compensatory EDTA. 2 h later the EDTA infusion was stopped, the thyroid vein quickly catheterized, using siliconed polyvinyl chloride tubing (ext. diam. 2.0 mm) of approximate length 25 cm, and thyroid venous blood collected for about an hour in an ice-cooled vessel held about 20 cm below the level of the thyroid. Systemic plasma samples were obtained at regular intervals and analysed for calcium, magnesium, inorganic phosphate and total solids.

The thyroid venous blood was quickly centrifuged and the plasma stored at 1° C. After 20 h at 1° C, 48 ml. of the plasma was injected intravenously into a similar pig and the effect on systemic plasma calcium concentration noted. In another experiment, 22 ml. thyroid venous plasma was injected intravenously into a 1½-year-old

Greyface female sheep (live weight 46 kg) and the calcium concentration in serial venous plasma samples measured.

**Analysis.** Plasma was usually separated within 15 min of sampling and calcium concentration was determined immediately afterwards by titration against EDTA with murexide indicator, using a photoelectric cell to locate the end-point. Plasma total solids were also quickly estimated by use of a T.S. meter (American Optical Co., Buffalo, N.Y.). Plasma magnesium concentration was measured by atomic absorption spectroscopy<sup>10</sup>. In the expression of the results, plasma calcium and magnesium concentrations have been corrected for changes in plasma total solids. The coefficient of variation associated with the plasma calcium concentration is  $\pm 0.7$  per cent and that with the plasma magnesium concentration  $\pm 1.3$  per cent. The estimation of the plasma inorganic phosphate according to the method of Hall<sup>11</sup> was also started immediately after the plasma had been separated. The coefficient of variation of this method is  $\pm 1.5$  per cent.

**Results.** Fig. 2 shows the pronounced fall (30 per cent) in plasma calcium concentration associated with hypercalcaemic perfusion of the thyroid for 2-2.5 h. There was no concomitant fall in plasma magnesium or inorganic phosphate concentrations. Drainage of thyroid venous blood resulted in a slow rise in not only the plasma calcium but also the plasma magnesium concentrations.

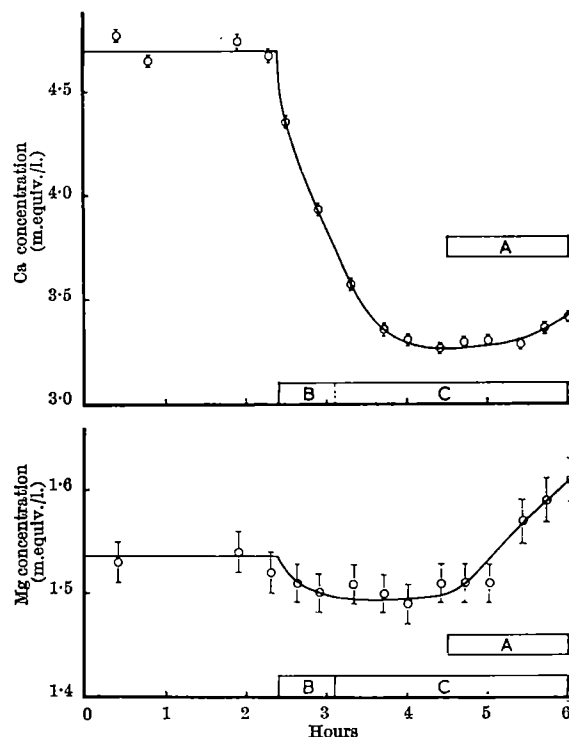


Fig. 2. The changes in systemic plasma calcium and magnesium concentration associated with hypercalcaemic perfusion of the pig thyroid *in situ* under halothane, oxygen anaesthesia. B, Perfusion calcium concentration 10 m.equiv./l. C, Perfusion calcium concentration 7 m.equiv./l. A, Drainage of thyroid venous blood. For each plasma sample the standard deviation associated with the analytical technique used is shown by a vertical line.

The intravenous injection of the perfused thyroid venous plasma into another pig resulted in a small but significant decrease in the plasma calcium concentration (Fig. 3). In another experiment a 12 per cent fall in plasma calcium concentration was observed in the donor pig, but no effect was obtained following the intravenous injection of the perfused thyroid venous plasma into a sheep. No significant change in magnesium concentration of the plasma of either sheep or pig was noted after cross-transfusion of perfused thyroid venous plasma.

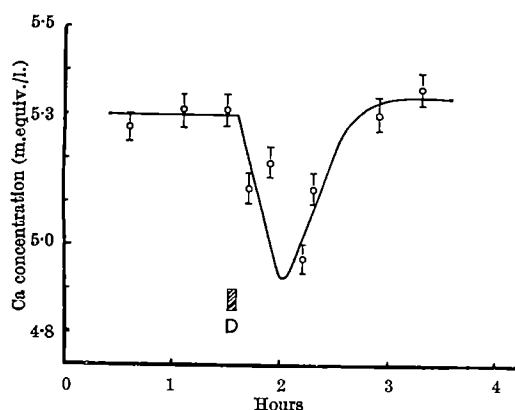


Fig. 3. The effect on the plasma calcium concentration of a conscious pig transfused intravenously with 48 ml. thyroid venous plasma (D) obtained by drainage of thyroid venous blood during hypercalcaemic perfusion of the thyroid

**Discussion.** The very degree of hypocalcaemia associated with hypercalcaemic perfusion of the thyroid is suggestive of the secretion of a specific calcium-lowering hormone rather than of some inhibition of parathormone secretion associated with the experimental technique. This conclusion is confirmed by the hypocalcaemic response to the cross-transfusion to another pig of venous plasma from such a stimulated thyroid. In view of the relatively large response noted in the donor pig, one might have expected an effect in the recipient pig larger than was in fact observed. Thyrocalcitonin may be unstable when stored in plasma at 1° C, which may account for the lack of effect after cross-transfusion to a sheep, since pig thyroid extracts, rich in thyrocalcitonin, have been shown to exert a hypocalcaemic action in the sheep (D. H. Copp, personal communication).

The slow rise in plasma calcium concentration which accompanied the drainage of thyroid venous blood was presumably the result of parathormone action, since the parathyroid glands were left intact. The similar rise in plasma magnesium concentration may have had a similar cause but may also have been affected by the drainage of thyroxine. In other experiments, the administration of either thyroxine or tri-iodothyroxine to sheep has been shown to result in a hypomagnesaemic response unaccompanied by hypocalcaemia.

The rapid hypocalcaemic response to hypercalcaemic perfusion of the thyroid is not unlike that observed during similar perfusion of the parathyroid<sup>2,5</sup>, although a good deal greater in magnitude. However, the mass of tissue perfused is also greater in the case of the thyroid. A difference, however, lies in the lack of effect of thyrocalcitonin on plasma magnesium concentration relative to that shown by parathyroid calcitonin.

There would now seem to be little doubt that both the parathyroid and the thyroid secrete calcium-lowering hormones in response to hypercalcaemic perfusion; however, the relative importance of each in calcium homeostasis remains to be determined.

<sup>1</sup> Copp, D. H., Cameron, E. C., Cheney, B. A., Davidson, A. G. F., and Henze, K. G., *Endocrinology*, **70**, 638 (1962).

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## AGAR GEL-ELECTROPHORETIC ESTERASE PATTERNS IN HOUSEFLIES

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**I**NVESTIGATIONS on esterases and organophosphate resistance in the housefly (*Musca domestica*) revealed the occurrence of a large number of different esterases<sup>1</sup>. Detailed agar gel-electrophoretic investigations of one particular strain of flies (strain C) demonstrated the occurrence of ten different esterases capable of hydrolysing  $\alpha$ -naphthyl acetate. Single individuals were found to contain 3–8 out of these 10 esterases. Genetical investigations showed the occurrence of these esterases to be controlled by 9 different genes on 8 different chromosomal loci<sup>1,2</sup>.

In this article emphasis will be laid on the great diversity of agar gel-electrophoretic patterns obtained with different strains of houseflies. Homogenates in distilled water of either at least 30 flies or individual flies, at a concentration of 10 flies/ml., were centrifuged at 20,000g for 30 min and the supernatants used for agar gel-electrophoresis on microscope slides according to Wieme<sup>3</sup>. Esterases were demonstrated as brownish bands in the gel by the addition of  $\alpha$ -naphthyl acetate and diazo-blue<sup>2</sup>.

Fig. 1 shows the patterns obtained for mass-homogenates of 11 strains of flies. Electrophoretic experiments on single individuals showed that the patterns obtained with mass-homogenates are built up from dissimilar

individual contributions. In Fig. 2 examples are given for the strains F<sub>c</sub> and H. Adding these results to those previously obtained for strain C, it seems reasonable to state that strains of houseflies, originating from different parts of the world and some of them also having particular histories of laboratory breeding and selection, show highly strain-specific electrophoretic esterase patterns; moreover, that these patterns are the resultant of both intensity and frequency of the esterase bands contributed by the individual flies in each population. Investigations carried out at intervals of 8 months to 2 years showed the strain patterns to be constant over periods of time representing 15–50 generations. Only one of the esterases was present in all individuals of all strains, that is, that nearest the cathode. This is not the cholinesterase, since this has previously been proved to move slowly to the anode<sup>5</sup>. Under the conditions used in the experiments recorded here, the cholinesterase does not show up as a distinct band, probably because it is largely particulate.

In a previous investigation<sup>6</sup> it was shown that nearly all the fly-strains in our laboratory which are resistant to organophosphorus insecticides have considerably lower esterase activities to  $\alpha$ -naphthyl acetate than the susceptible strains. In a series of papers<sup>1,4,6–8</sup> evidence has been produced that this is due to the absence from

Table 1

	Organophosphate-susceptible strains									
	<i>S</i>		<i>ct</i>		<i>bac</i>		<i>L</i>		<i>U<sub>s</sub></i>	
	-D	+D	-D	+D	-D	+D	-D	+D	-D	+D
Protein content	0.55	0.77	0.38	0.56	0.51	0.74	0.52	0.73	0.34	0.57
Esterase activity	0.12	0.66	0.07	0.53	0.09	0.64	0.12	0.55	0.06	0.52
	Organophosphate-resistant strains									
	<i>H</i>		<i>K<sub>a</sub></i>		<i>C</i>		<i>F<sub>a</sub></i>		<i>G</i>	
	-D	+D	-D	+D	-D	+D	-D	+D	-D	+D
Protein content	0.51	0.72	0.41	0.56	0.61	0.87	0.42	0.57	0.48	0.64
Esterase activity	0.07	0.10	0.05	0.08	0.07	0.11	0.06	0.10	0.06	0.09

Protein contents and esterase activities in the supernatants obtained after centrifugation of whole homogenates without deoxycholate (-D) and with deoxycholate (+D). Protein contents were determined according to the Folin-Ciocalteu method (ref. 9) after 10 times dilution of the supernatant, which gives a concentration of 1 fly/ml., and are expressed in mg/ml. Esterase activities in the presence of  $10^{-7}$  M eserine were determined according to a method that has been previously described (ref. 6). One ml. of 500 times diluted supernatant ( $\sim 0.02$  fly/ml.) was incubated with 5 ml. of eserinated  $\alpha$ -naphthyl acetate solution (0.003 M) for 30 min. After that 1 ml. of a diazo blue-lauryl sulphate reagent was added and optical density measured at 600 m $\mu$  and 1 cm light path. The values thus obtained are given as 'esterase activity'. Strains: *ct* and *bac* (= *bub*; *acv*; *car*), carrying recessive marker genes, were obtained from Dr. T. Hiroyoshi, Osaka University, Japan. See also legend to Fig. 1.

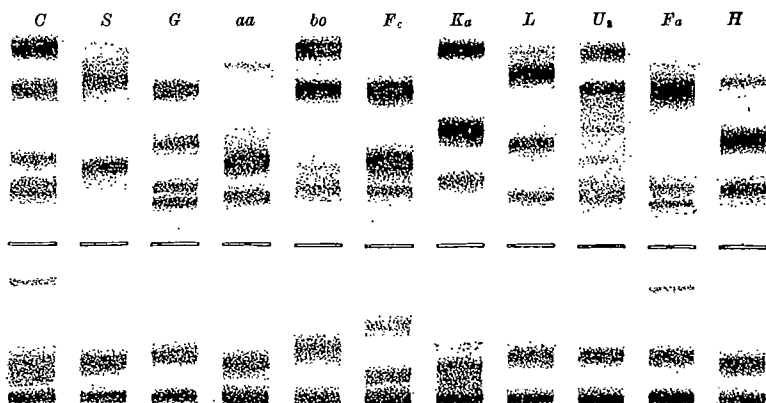


Fig. 1. Electrophoretic esterase patterns in 11 strains of houseflies. Top, anode; bottom, cathode. Data on the origin, further history and properties of the strains C, S, G, F<sub>c</sub>, K<sub>a</sub>, L, U<sub>a</sub>, F<sub>a</sub> and H are given elsewhere<sup>1,2</sup>. aa=strain *ar:ac* and bo=strain *bub:ocra*, each carrying two recessive marker genes, were obtained from Dr. R. Milani, Pavia, Italy

the organophosphate-resistant strains of a single ali-esterase, which in the susceptible strains is responsible for approximately 85 per cent of the total non-eserine-sensitive esterase activity. This ali-esterase has been named the ali-esterase *a*.

Ali-esterase *a* was found to be mainly particulate. High-speed centrifugation removes most of it, and consequently, if supernatant activities were compared, the difference between susceptible and resistant strains was much less than that observed if total homogenates were compared. It is, however, possible to solubilize the ali-esterase *a* by the addition of 0.005 M deoxycholate. This is clearly borne out by the results in Table 1, which gives the protein contents and ali-esterase activities of

the supernatants, obtained with and without deoxycholates of 5 organophosphate-susceptible and 5 organophosphate-resistant strains. Protein contents of all strains and ali-esterase activities in the resistant strains were only about 50 per cent higher with deoxycholate, whereas the ali-esterase activities in the supernatants of the organophosphate-susceptible strains were approximately six times higher after deoxycholate treatment. Electrophoretic esterase patterns of supernatants of deoxycholate-treated homogenates showed a very active anodically moving esterase to be present in the organophosphate-susceptible and not in the organophosphate-resistant strains. Its position is roughly similar to that of the fastest-moving esterase of strain S. Semi-quantitative determinations of the esterase activities in the agar gel by incubation

of gel-strips with  $\alpha$ -naphthyl acetate in test-tubes indicated that this esterase represents about 80 per cent of the total activity in the gel.

Unfortunately, nothing is known about the natural function and the natural substrate of the esterases examined, and the diversity of esterase patterns raises some questions. The many esterases may have some common function, which can be performed by different sets of iso-enzymes, that is, the difference in electrophoretic mobility could be caused by functionally non-essential differences in structure. However, flies which survived a rather low dose of the insecticide diazinon were found to be devoid of all esterase bands except the most cathodical one, but still were quite normal in behaviour. This could indicate that, with the possible exception of the most cathodically moving one, the esterases examined do not have a very essential function in normal adult life. Their evolutionary importance under natural conditions can only be guessed at, but little doubt now exists that the occurrence of some of the esterases is intimately connected with their ability to survive treatment with organophosphorus insecticides. The significance in this respect of the ali-esterase *a* and genetically related enzymes has been well established<sup>1,4,7</sup> and the results of further investigations will be published soon.

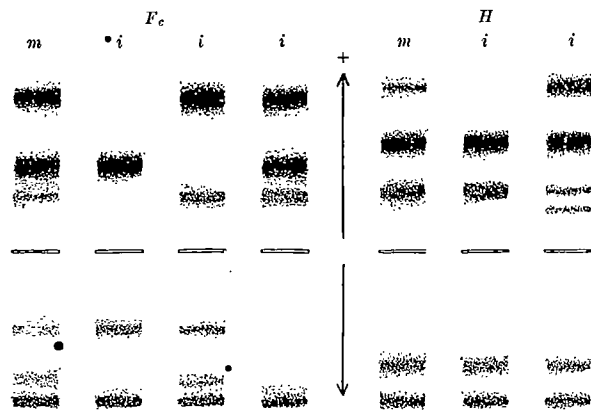


Fig. 2. Mass and individual electrophoretic esterase patterns of two strains. m=mass. i=individual

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## QUANTITATIVE EXAMINATION AND ANALYSIS OF THE PROZONE PHENOMENON IN THE HAEMAGGLUTINATION REACTION

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THE inhibition of agglutination associated with excess antibody has been attributed either to a saturation of the antigenic sites by excess antibody<sup>1</sup> or to a competitive inhibition by blocking antibodies<sup>2</sup>. Although these hypotheses are often presented as opposing views of the prozone phenomenon, Renton and Hancock<sup>3</sup> effectively showed their essential similarity. However, they also demonstrated the inadequacies of these hypotheses to explain fully the prozone phenomenon.

A recent investigation carried out in this laboratory was concerned with the analysis of the haemagglutination reaction<sup>4</sup> and emphasized the importance of factors which affect either the number or strength of antigen-antibody linkages formed between cells. Heterogeneity of both antigen and antibody combining sites with respect to their contribution to the stability of the antibody linkages formed between cells was assessed in this investigation. It was felt that a direct consideration of the influence of these factors on the agglutination occurring in excess antibody would provide a more comprehensive picture of the prozone phenomenon. Moreover, using a quantitative agglutination procedure the prozone phenomenon could be examined with greater accuracy and in greater detail than had been previously possible with conventional agglutination techniques.

The quantitative procedure used in this investigation depends on the fact that agglutination results in a reduction in free cell concentration. The Coulter electronic cell counter (Coulter Electronics, Hialeah, Florida) has provided a practical means of measuring free cell concentration and therefore a means of quantitating red cell agglutination<sup>5</sup>. In this procedure antiserum in graded dilutions is added to a series of silicone-treated 15-ml. cylindrical glass vials. Sufficient buffered saline is added to give a volume of 5 ml. Then 5.0 ml. of a washed red cell suspension containing  $4.0 \times 10^6$  cells per ml. is added to each vial. Cell standards consisting of only cells and buffered saline are routinely included. In this particular application of the quantitative agglutination procedure, all vials are allowed to stand undisturbed at 25° C for 1 h to permit sensitization. Because of the low cell concentration, little or no agglutination occurs during this period, but primary union of antigen and antibody does occur<sup>6</sup>. The vials are further incubated at 25° C for 2 h with mechanical mixing on a stirring device specially designed to provide uniform mixing. The free cell concentration is determined for the contents of each vial with the Coulter counter from which the percentage agglutination is calculated.

An example of the quantitative measurement of agglutination occurring over a wide range of antiserum concentrations is shown in Fig. 1. Agglutination is expressed as a percentage and plotted on an arithmetical scale. Antiserum concentration is plotted on an arithmetical scale in the inserted figure and on a log scale in the main figure. Expressing antiserum concentration as a logarithm has the advantage that proportional changes in antiserum concentration are graphically equivalent, allowing agglutination curves obtained with antisera of widely different titres to be directly compared. The quantitative agglutination curve shown in Fig. 1 exhibits

three general zones associated with the progressive increase in antiserum concentration. There is an initial zone of increasing agglutination, followed by a zone of maximum agglutination and a final zone (prozone) characterized by a progressive reduction in the percentage of agglutination.

Fig. 2 compares the quantitative agglutination curves of four anti-A and four anti-Rh<sub>0</sub> sera. Cells from a single donor were used to test the antisera of each group so that the curves within each group may be directly compared. Antiserum 15 shows the strongest agglutinating properties of the four anti-A sera. This serum gave the highest percentage agglutination and showed no detectable inhibition at the highest antiserum concentration tested. Antisera 19 and 28 gave somewhat lower agglutination maxima with inhibition of agglutination at moderate concentrations of antiserum. Antiserum 1891 appears to be the weakest, giving an agglutination maximum of only 47 per cent and inhibition at relatively low antiserum concentrations. Differences in the slopes of these curves in antibody excess indicate that these antisera contain different distributions of antibody molecules with respect to their agglutinating properties. The smaller secondary peak observed with antiserum 1891 suggests the presence of a minor secondary antibody population. The agglutination curves obtained with the anti-Rh sera show less variability than the curves obtained with the anti-A sera. In addition, the agglutination obtained with the anti-Rh<sub>0</sub> sera and papain-treated red cells<sup>7</sup> was inhibited by a relative low excess of antiserum as indicated by the comparative narrowness of the Rh curves.

In contrast to the previous experiment, agglutination curves obtained with different cell populations are compared in Fig. 3. Cells from four A Rh positive individuals were examined with a single anti-A serum and a single anti-Rh<sub>0</sub> serum. In their reaction with the anti-A serum cells 2 and 3 gave curves similar to each other, but different from the curves obtained with cells 1 and 4. These latter cells gave more agglutination at lower antiserum concentrations and their agglutination was also more readily inhibited by excess antibody. When these same cells were tested with the anti-Rh<sub>0</sub> serum a different pattern of curve was

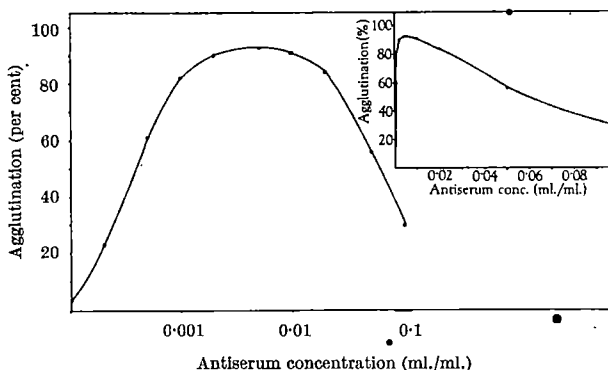


Fig. 1. Plot of percentage agglutination against log-antiserum concentration (anti-A serum and A<sub>1</sub> red cells). In the insert an arithmetic plot of antiserum concentration is shown for comparison.



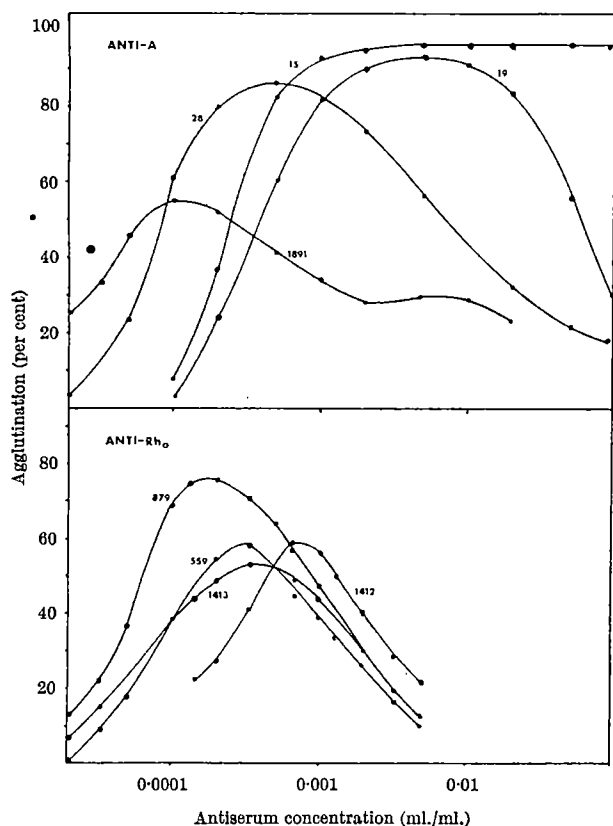


Fig. 2. Comparison of quantitative agglutination curves obtained with two different groups of antisera (anti-A and anti-Rh<sub>0</sub>). Each group of antisera was tested with cells obtained from a single donor. Differences within each group are, therefore, due to differences in the individual antibody populations

obtained. Cell 1 showed the least inhibition of agglutination by excess antibody and cell 4 gave the lowest maximum percentage agglutination. Cells 2 and 3 were intermediate in their behaviour. These results illustrate the variability of the antigenic characteristics found between cell populations and the effect this variability has on the quantitative agglutination curve.

In the foregoing experiment it was not possible to distinguish between the effect of number and binding strength of the antigenic sites. It was, therefore, of interest to design an experiment in which the number of antigenic sites/cell would be the sole experimental variable. This was accomplished by simply using different concentrations of a purified protein antigen (human 7S  $\gamma$ -globulin) coupled to aliquots of a tanned red cell suspension<sup>8</sup> and testing with an appropriate antiserum (rabbit anti-human  $\gamma$ -globulin). In Fig. 4 the effect of reducing the number of antigenic sites/cell on the quantitative agglutination curve is shown. A progressive weakness of the agglutination curve can be seen with reduction in the concentration of antigen used for coupling. In antigen excess a greater concentration of antiserum was required to obtain the same percentage agglutination at a reduced number of antigenic sites/cell with the maximum percentage agglutination correspondingly reduced. In antibody excess there was a progressive percentage increase in the inhibition of agglutination.

Differences in the agglutinability of A<sub>1</sub> and A<sub>2</sub> cells were previously concluded to be due to both a difference in the number and strength of antigenic sites/cell<sup>9</sup>, that is, A<sub>2</sub> cells have fewer and weaker antigenic sites than A<sub>1</sub> cells. For this reason it was of interest to examine the quantitative agglutination curves obtained with these cells. A comparison of agglutination curves obtained with a number of anti-A sera tested with A<sub>1</sub> and A<sub>2</sub> cells, rather

surprisingly, gave two extremes in behaviour. An example of each is shown in Fig. 5. Antiserum 23 gave an agglutination curve with A<sub>2</sub> cells shifted to the right and somewhat weaker than that obtained with the A<sub>1</sub> cells. Antiserum 1891 with A<sub>2</sub> cells also shows an agglutination curve shifted to the right; however, in contrast to what might be expected, the A<sub>2</sub> curve is higher and broader than the curve obtained with A<sub>1</sub> cells. The comparatively weak agglutination curve of antiserum 1891 with A<sub>1</sub> cells suggests that this antiserum contains a high proportion of weakly agglutinating antibody molecules. The stronger agglutination curve obtained with the A<sub>2</sub> cells further suggests that a considerable portion of these weakly agglutinating antibody molecules was unable to combine with the A<sub>1</sub> cells which provided an opportunity for only the more strongly agglutinating antibody molecules to combine with these cells.

The foregoing experiments provide a number of points pertinent to the prozone phenomenon. In order to clarify these findings, the prozone phenomenon with respect to the basic factors responsible for agglutination will be considered in greater detail.

Haemagglutination has been shown to be the result of the random collision of red cells<sup>6</sup> with the formation of cross-linkages between antigenic sites on the surface of the red cells by bivalent antibody molecules<sup>10,11</sup>. The formation of such antibody cross-linkages would require both the presence of free as well as bound antigenic sites. One could, therefore, assume that under idealized conditions, such as would be found in the interaction of homogeneous antibody and cell populations, 50 per cent satura-

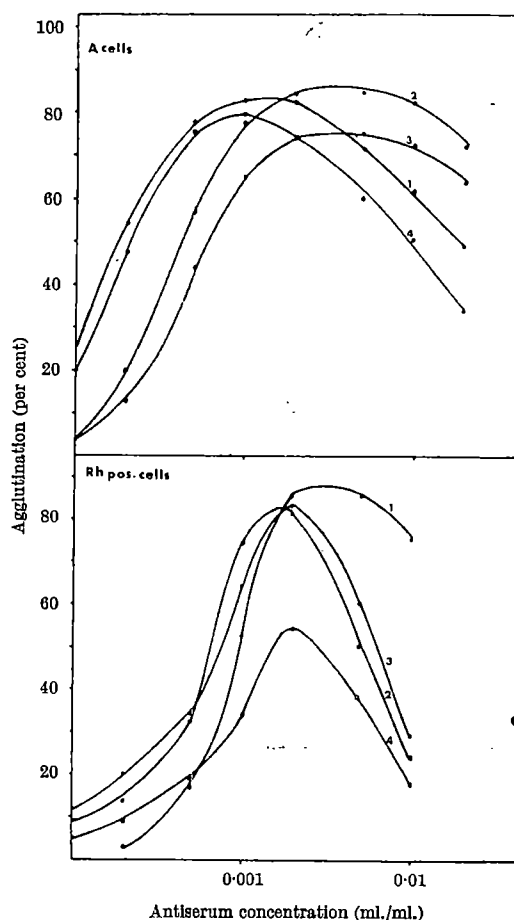


Fig. 3. Comparison of quantitative agglutination curves obtained with A Rh positive red cells obtained from four different individuals. Agglutination was obtained with a single anti-A serum (28) and a single anti-Rh<sub>0</sub> serum (1412). Papain-treated red cells were used only with the anti-Rh<sub>0</sub> sera

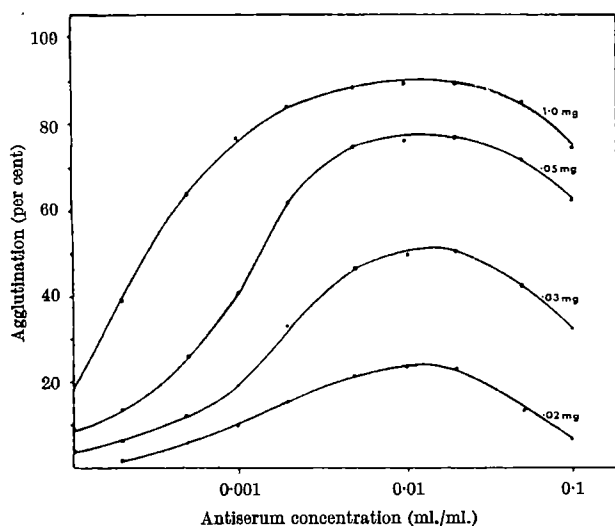


Fig. 4. Comparison of quantitative agglutination curves obtained with cells possessing different numbers of antigenic sites per cell. Aliquots of tanned human group O red cells were coupled with different concentrations of human 7S  $\gamma$ -globulins. Agglutination was obtained with a rabbit anti-human  $\gamma$ -globulin serum.

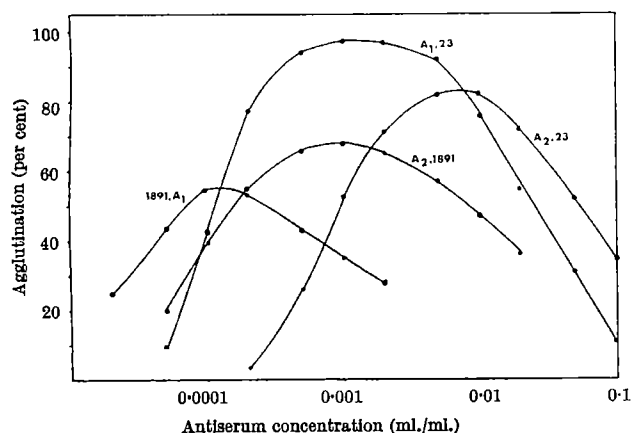


Fig. 5. Comparison of quantitative agglutination curves obtained with  $A_1$  and  $A_2$  human red cells. Two anti-A sera giving dissimilar results are shown.

tion of the antigenic sites would provide the maximum opportunity for antibody cross-linkages to be formed. It could also be assumed that above or below 50 per cent saturation there would be a proportional decrease in the opportunity for intercellular linkages and, as a consequence, a proportional decrease in the intercellular binding force. However, the magnitude of the intercellular binding force achieved between cells would be determined not only by the proportion of free and bound antigenic sites, but also by the stability and absolute number of the intercellular linkages formed. It is on the basis of the last-mentioned two parameters that antibody heterogeneity and variation in number and strength of the antigenic sites find expression in the prozone phenomenon.

The stability of the antibody linkage formed between cells is basically determined by the chemical and structural complementarity between the antigen and antibody combining sites<sup>12</sup>; the greater the degree of complementarity the more stable their union. Antibody molecules with the same antigenic specificity may vary widely in their binding strengths, even to the simplest of antigenic determinants<sup>13</sup>. As a consequence of this heterogeneity, antibody molecules of different binding strengths may be represented in given antisera in different proportions and concentrations<sup>9</sup>. In general, an antiserum composed of

more weakly binding antibody molecules would require a greater number of antigen-antibody linkages/cell to achieve a specific percentage agglutination than would be required by an antiserum composed of more strongly binding antibody molecules. Therefore, in terms of the quantitative agglutination curve, agglutination with a weakly binding antiserum would occur over a narrower range of antiserum concentrations than would be found with a more strongly binding antiserum. Correspondingly homogeneous antibody populations, with respect to their binding properties, could be expected to give symmetrical curves, whereas heterogeneous antibody populations could be expected to give agglutination curves with varying degrees of skewness and secondary peaks, dependent on the particular composition of the individual antibody population. These qualitative and quantitative differences would account for the dissimilarity in the quantitative agglutination curves shown in Fig. 2.

Blocking antibodies and their role in the prozone phenomenon<sup>14</sup> can be considered to represent one aspect of antibody heterogeneity. These antibodies can be broadly defined as antibody molecules capable of combining with the antigenic sites of a given cell population, but unable to form sufficiently stable intercellular linkages to result in agglutination. This would require one or both of the combining sites of a bivalent antibody molecule being deficient<sup>15</sup>. Such blocking antibody molecules may represent a major portion of an antibody population, as is found with the majority of human Rh antisera, or they may constitute only a small fraction of the total antibody population. Blocking antibodies, in a mixture of blocking and agglutinating antibodies, would effect agglutination by combining with available antigenic sites and prevent the combination of those antibody molecules capable of producing agglutination<sup>9</sup>. This would have a total practical effect of reducing the available number of antigenic sites/cell. Those antisera containing a higher proportion of blocking antibodies would have a more pronounced prozone, similar to the effect resulting from a direct reduction in the number of antigenic sites/cell (Fig. 4).

The expression of blocking activity, however, is not an independent characteristic, but rather highly dependent on the cell population used for testing. This was inferred in our definition in which blocking antibodies were defined in terms of their reaction with a 'given' cell population. This was necessary because antigenic properties and, therefore, agglutinating properties may differ from one cell population to another<sup>16</sup>. Such antigenic differences may be so extreme that an antiserum can be agglutinating with one cell population, blocking with another and unable to combine with a third<sup>17</sup>.

Antigenic differences between cell populations can be due to a difference in the number of antigenic sites/cell or, as well, to a difference in the binding properties of the antigenic sites. In terms of the agglutination reaction, the effect of these two factors would not be distinguishable. Within limits, it could be expected that an increase in the number of antigenic sites/cell would compensate for a deficiency in their binding strength and vice versa. The comparison of different cell population with a single antiserum (Fig. 3) showed the accumulative effect of antigenic differences resulting in characteristic agglutination curves, whereas in Fig. 4 the effect of purely quantitative differences in the number of antigenic sites/cell was illustrated. Finally, the combined effect of antibody heterogeneity and antigenic variation was evident in the comparison of the agglutination curves obtained with  $A_1$  and  $A_2$  cells shown in Fig. 5.

In summary, the ability to demonstrate a prozone with most of the antisera examined indicates that it is not a phenomenon of rare occurrence, but rather a basic phase of the agglutination reactions associated with antibody excess. It was concluded that the prozone phenomenon is, as it has been traditionally considered to be, primarily caused by antibody molecules occupying too great a

proportion of the available antigenic sites. This was considered to result in a mechanical deficiency in the number of antibody linkages formed between cells and, as a consequence, a reduction in agglutination. Contributing to this phenomenon are the factors which determine the stability and absolute number of intercellular linkages. Variation in the slopes of the quantitative agglutination curves in antibody excess was indicative of the heterogeneity found within and between antibody populations. Correspondingly, the differences in quantitative agglutination curves found when different cell populations were compared reflect the contribution of antigenic properties of the cell to the haemagglutination reaction in general and the prozone phenomenon in particular.

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## SMALL-ANGLE ELECTRON DIFFRACTION PATTERNS OF ASSEMBLIES OF SPHERES AND VIRUSES

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THE technique of small-angle electron diffraction introduced by Mahl and Weitsch<sup>1</sup> has since been used in investigations of the structure of polyethylene<sup>2</sup>, the scattering by ferromagnetic films<sup>3</sup>, and the structure of catalase<sup>4</sup> as outlined by the negative staining method<sup>5</sup>. It is the purpose here to show the value, in relation to biological specimens, of experiments similar in outline to those carried out by Mahl and Weitsch on specimens made up of particles of either evaporated metal or inorganic salts. Specimens which we have examined so far include polystyrene latex spheres (a useful organic test specimen) and the tobacco necrosis and turnip crinkle polyhedral viruses.

Specimens were prepared for electron microscopy by depositing droplets of the particle suspensions in distilled water on supporting grids covered with thin films of evaporated carbon. The final particle concentrations on the grids could readily be varied. In regions of high particle density, in favourable cases, two-dimensional paracrystalline lattices formed. In an ideal experiment, as the particle density was progressively reduced, the degree of ordering of the arrays would vary from the paracrystalline through a liquid-like state (in two dimensions) until the particles were randomly distributed in the specimen plane. (A random distribution would effectively occur when all the interparticle distances were much greater than the resolving power of the diffraction arrangement.) The specimens were examined in an Associated Electrical Industries E.M.6 electron microscope under similar small-angle electron diffraction conditions to those described by Ferrier<sup>6</sup>. The diffraction camera length (C.L.) used was about 60 m for electrons of 80-keV energy. The resolution in the diffraction pattern depends primarily on the size of the incident electron beam at the specimen (about 10  $\mu$  in this work) and the magnification chosen for the second projector lens. Under the conditions adopted, reflexions corresponding to Bragg diffraction angles  $\geq 3 \times 10^{-5}$  radians (or Bragg planes of spacing  $\leq 1350$  Å) could be recorded photographically. Exposure times were normally in the range from 5 to 30 sec. None of the specimens gave observable (above background) coherent diffraction corresponding to spacings less than about 60 Å.

In Figs. 1a-d are shown examples of small-angle patterns from paracrystalline arrangements of polystyrene spheres and tobacco necrosis virus, a liquid-like

distribution of turnip crinkle virus, and a random arrangement of polystyrene spheres. In all cases the coherent features of the patterns appear superimposed on an intense background photographic density due to the electrons elastically and inelastically scattered by the supporting film and the incoherent scattering by the particles themselves.

Normal transmission electron microscope observations of the specimen regions giving rise to the diffraction patterns (a) and (b) showed that the basic crystalline arrangements in the paracrystals were hexagonal but that many stacking faults were present together with other lattice distortions. If the paracrystalline reflexions are interpreted as the  $h\bar{h}k + k$  0 reflexions of an infinite hexagonal lattice and a geometrical calibration<sup>3</sup> of the diffraction camera length is used, then the following values for the mean interparticle separations are obtained: polystyrene latex spheres  $880 \pm 20$  Å; tobacco necrosis virus  $192 \pm 3$  Å. These values are in agreement with diameter measurements made by transmission microscopy using gold-palladium shadowed specimens.

The mechanism by which the reflexions arise from the paracrystals is intermediate between the mechanisms in X-ray and optical diffraction. In X-ray specimens each particle forms part of a three-dimensional assembly and each particle scatters X-rays in three dimensions. In the optical analogue to X-ray diffraction<sup>6</sup> the diffraction pattern is formed by combining the light transmitted through holes in a two-dimensional screen. In the electron diffraction patterns, electrons are scattered elastically (or effectively elastically) throughout the volume of each particle and are subject to similar phase relationships to those found in the X-ray case (that is, the Fourier transforms of the (dehydrated) particles are of the same form for electrons as for X-rays), but the lattices are two-dimensional. The limited number of reflexions from the paracrystals thus provide some information about the spherically averaged Fourier transforms of the polystyrene or virus particles since the paracrystalline patterns may be regarded as (rather poor) lattice samplings of these transforms.

The angular extents of the coherent patterns in Figs. 1a-c give an indication both of the linear extents of the regions of ordered packing of the particles and of damage to the specimen caused by the electron beam. In the specimens of turnip crinkle virus no regular arrangements of more

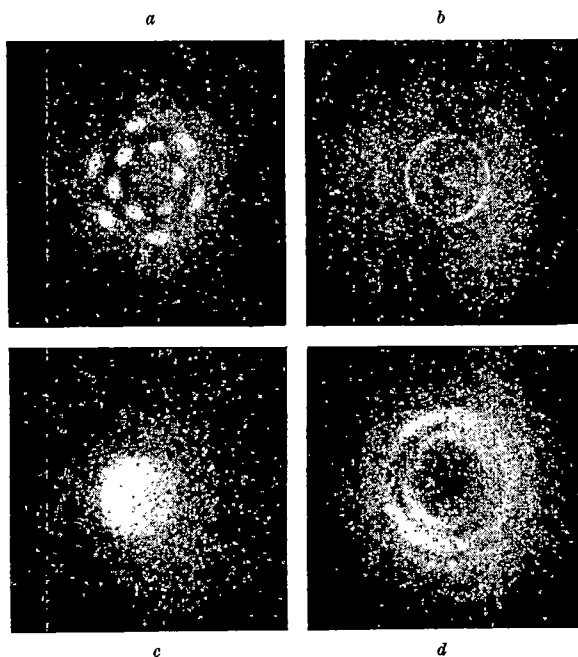


Fig. 1. Small-angle electron diffraction patterns (80 keV electrons) of: (a) Paracrystalline assembly of polystyrene latex spheres. Diffraction camera length as printed  $C.L.=92$  m. (b) Paracrystalline assembly of tobacco necrosis virus particles.  $C.L.=27$  m. (c) Liquid-like assembly of turnip crinkle virus particles.  $C.L.=92$  m. (d) Random assembly in two dimensions of polystyrene latex spheres.  $C.L.=71$  m.

than 1000 Å in linear extent were observed and consequently the turnip crinkle pattern (Fig. 1c) is similar to that expected from a statistical particle arrangement as found in a liquid<sup>7</sup>. The single ring at a spacing of  $308 \pm 10$  Å represents the interparticle distance which occurs with maximum probability, that is, the mean particle diameter. This value is again in reasonable agreement with the value found by transmission electron microscopy. It has not, so far, been found possible by changing the particle concentration in the specimens of turnip crinkle virus to obtain a paracrystalline pattern. However, a liquid-like diffraction pattern from tobacco necrosis virus has been observed.

Using low particle densities in specimens of polystyrene spheres we have directly photographed the continuous Fourier transforms arising in effect from single spheres. Such a pattern for spheres of mean diameter 880 Å is shown in Fig. 1d; similar patterns have been obtained for 1260 Å diameter spheres. The positions and relative intensities of the diffraction rings in Fig. 1d correspond closely to those expected from the expression for the transform of a sphere of the known radius<sup>8</sup>. Similar, but less extensive, information on Fourier transforms can also be found by the rather tedious and lengthy methods

of small-angle X-ray diffraction applied to extremely dilute particle suspensions<sup>9</sup>. The electron diffraction method, besides having advantages in greater angular range and speed of recording the patterns, has the further advantage that each pattern refers to a definable group of particles for which the polydispersity of size (and to some extent shape) can be measured from electron micrographs; its main disadvantage is that the continuous transform is superimposed on a high background intensity of incoherently scattered electrons. The last factor, even for specimens undistorted by the electron beam, sets a lower limit to the particle diameters for which 'random' patterns (with the implied large interparticle distances) are observable even with the thinnest specimen supporting film.

It may be noted that the angular extents (regardless of exposure time) of the coherent diffraction patterns of the type shown in Fig. 1d are greater than those characterized by Fig. 1a. This indicates that the blurring of the continuous particle Fourier transform due to the polydispersity of the particle diameters is less than the blurring of the discontinuous lattice transform due to the distribution of interparticle separations in the paracrystals. The Fourier transforms of isolated virus particles have not yet been satisfactorily observed; a difficulty of specimen preparation is that when the viruses are in a suitable concentration for observable diffraction they tend to clump on the specimen grids and the clumps give rise to very weak liquid-like diffraction patterns.

Emphasis has been given here mainly to the observational aspects of the small-angle electron diffraction patterns from particle assemblies in the absence of any deliberate attempts to crystallize the specimens on the supporting film. However, an examination of the intensities of the reflexions from viruses and other assemblies is of interest both because of the structural information that may be derived from it (especially from crystalline rather than paracrystalline specimens) and from the point of view of the theory of small-angle electron diffraction; both these aspects are being investigated.

We thank Dr. R. P. Ferrier for his advice. The virus specimens were supplied by Mr. H. L. Nixon and Dr. B. Kassanis. One of us (J. S.) thanks the Department of Scientific and Industrial Research for a maintenance award.

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## •✓ READING RATE, WORD INFORMATION AND AUDITORY MONITORING OF SPEECH

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DURING the past twelve years there has been a great deal of research on the part played by auditory feedback in the control of speech production. Lee<sup>1</sup> first showed that if subjects were allowed to hear their own voices only with a delay of about 0.2 sec, their speech was

remarkably disrupted. They stuttered, stammered, drawled or broke down completely. Since then many people have worked on more detailed aspects of the breakdown, such as the different effects on vowels and consonants<sup>2</sup>, on vocal rate and intensity<sup>3</sup>, and on articulation<sup>4</sup>.



and the effects of variation in the length of the delay<sup>5</sup>. At what level is the auditory feed-back monitored? Lee suggested that it primarily controls the 'voice loop' producing syllabic units. Chase<sup>6</sup> showed that delaying the feed-back can facilitate the repetition of a single sound or phoneme 'b', and Fairbanks and Guttman found that the majority of errors were one phoneme long. However, the problem is unlikely to have a single, simple solution. Fairbanks showed that the type of disturbance varied with the delay interval used, and concluded that the "units of speech control should not be identified with any of the conventional units such as the phoneme". Control may be at many levels from phonation and articulation to whole phrase or sentence.

Little of the work so far has investigated control at the highest level. This problem could be tackled by varying the information rate in the words of different passages, and using them in delayed feed-back reading tests. If any monitoring were carried out at the high level of word or phrase, delayed feed-back might be expected to produce variations in disturbance with passages of differing redundancy, while the control of the simpler motor elements of phonation and articulation would be unaffected, since while verbal information varies, phonetic information remains the same. One might be able to separate out the different components of the total reading time in normal conditions and with delayed feed-back by comparing reading rates under these conditions with those obtained in silent reading and in reading aloud with auditory feed-back completely masked by noise.

The passages used in the experiment reported here were a set of statistical approximations to English<sup>7</sup> prepared in the manner described by Miller and Selfridge<sup>8</sup>. The set included a 1st-, 2nd-, 4th-, 6th- and 8th-order passage and a passage of normal prose taken from Conrad's novel *Lord Jim*. Each was 100 words long and typewritten without punctuation on a separate sheet of paper. Estimates of the information content of these passages were made by taking  $\log_2 p$  of the probabilities estimated from the frequencies with which two samples of 10 missing words from each passage were correctly replaced by two groups of 100 subjects<sup>9</sup>. These missing words were deleted at regular intervals of one word in ten, chosen to be at the asymptote where predictability does not increase with increased context or decreased deletion rates. The values obtained were 4.8, 3.9, 3.6 and 3.3 bits for 2nd-, 4th-, 6th- and 8th-order approximations respectively, and 2.6 bits for a prose extract from Conrad's novel *Lord Jim*. The 1st-order passage was a random selection of words from a novel and the mean information per word was taken from the frequencies of the twenty missing words given in the Thorndike-Lorge word count<sup>10</sup>; the value was 9.2 bits.

There were five groups of subjects (all university students or graduates), each of whom read the passages under a different condition. Within each group the order in which the passages were given was randomized. Group A (12 subjects) read them aloud at a natural speed; Group B (12 subjects) read them silently at a natural speed; Group C (11 subjects) read them aloud while a loud masking noise was played over headphones to both ears; Group D (7 subjects) read them backwards as quickly as possible, starting with the last word and working back from right to left along the lines to the first word; Group E (21 subjects) read the passages aloud with delayed auditory feed-back. Their speech was recorded and played back about 0.25 sec later at a high intensity to both ears over headphones. This group was first given one passage of normal prose to read, to introduce them to the delayed feed-back. In Group E a clear division between two types of subjects emerged: one sub-group of 9 subjects was not affected at all by the delayed feed-back and their results did not differ from those of Group A; the other sub-group of 12 subjects showed the typical disturbances reported in the literature—slowing

up, drawing, stammering and so on. The results of these two groups were analysed separately. The criterion for assigning subjects to the latter group was that their mean time for reading a passage with delayed feed-back should be more than 40 sec. The mean for Group A was 35.4 with a range of 28.5–41.2 sec. The mean for subjects in Group E who showed no overt disturbance was 36.5 sec.

Each experiment was recorded and the time taken to read the passages was measured. Fig. 1 shows the mean time per word plotted against the average information per word in the different passages. Results of an experiment by Morton<sup>11</sup> are also included: his subjects (also university students) read a set of approximations aloud as quickly as possible. The regression for subjects in Group E, who were unaffected by the delayed feed-back, is not shown as there was no significant difference between this and the regression for Group A. The equation was  $T = 1.52 \text{ Inf.} + 29.6$ . All the regression co-efficients are significant except that for reading backwards which is quite insignificant, and the departures from linearity are not significant in each case. The only significant differences in slope of the regressions are between the delayed feed-back condition and the others, and the reading backwards and the others. The delayed feed-back regression is significantly steeper than the regression for normal reading aloud ( $P < 0.02$ ), and the regression for reading backwards is significantly less steep ( $P < 0.001$ ). Thus the difference between silent reading, rapid reading aloud (Morton's results) and normal reading aloud is a difference in the constant component of the reading time

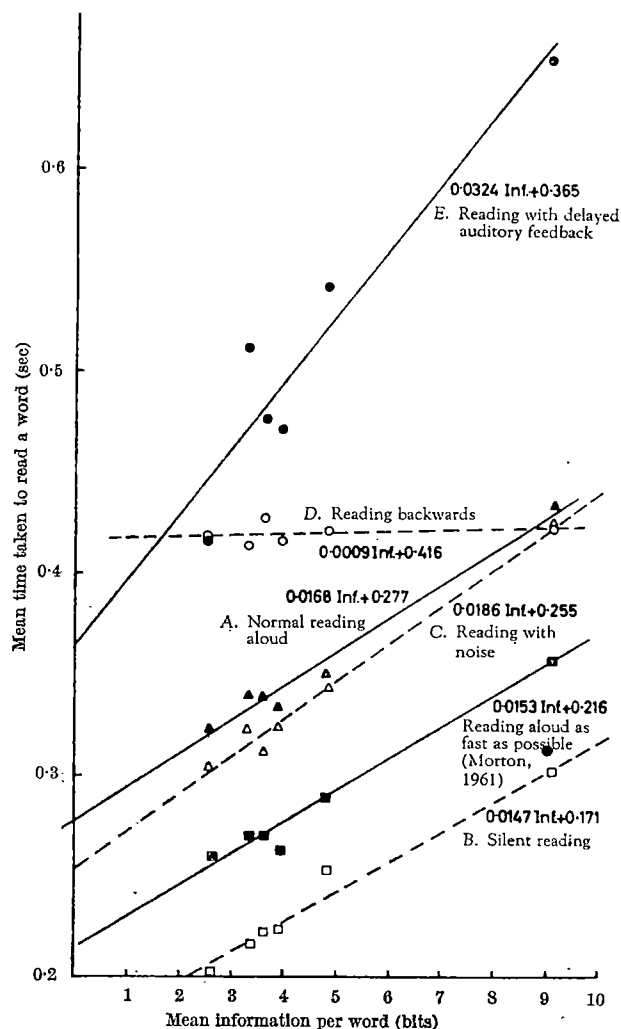


Fig. 1. Reading times and word information

rather than in the component which varies with verbal information content. The delayed feed-back, on the other hand (for those subjects who were affected), halved the rate of gain of information as well as increasing the intercept.

What do these results imply about the different factors contributing to total reading time? The fact that the time taken to read the passages backwards was constant shows that the variation in the other conditions must be due to the varying information content resulting from the sequential dependencies between words, and not to differences in word length or difficulty. It seems plausible to divide the total time into two main components, which may or may not overlap in practice: (I) the perception time taken to read the passage; (II) the time taken to organize and perhaps to monitor the response. Each of these may be composed of (a) a verbal component which varies with the information content of the words, and (b) a non-verbal component independent of word information. For (I), the visual input, one can distinguish (a) the information taken from the verbal context; (b) that taken directly from the print. The latter visual discrimination factor would be constant for all passages, while the former would vary with information content. For (II), the vocal output, (a) the variable component might be the selection and perhaps the monitoring of responses at the level of word or phrase, while (b) the constant component was the control of the mechanics of articulation.

The silent reading condition probably reveals the effect of the input component only. The constant of 17.1 sec could reflect the visual discrimination time, while the slope shows the information component and implies the high rate of gain of 68 bits of information per sec. Normal reading aloud makes no significant change in the information component, but it adds a constant of 10.6 sec to all passages, which, in terms of the account given here, would be the time taken for the control of articulation. The fact that the slope does not change shows that the information time hypothesized for response selection cannot be independent of the information time required to identify the input. It seems that, once the words or phrases have

been recognized, no further time is necessary at this level to select and organize the words as vocal responses. It also suggests that whatever monitoring of auditory feed-back is normally carried out does not require any extra information capacity. This is confirmed by the result from Group C who read aloud with noise: there is no significant difference in reading time between this condition and normal reading aloud, so that the absence of auditory feed-back has no effect on either component of the reading time. The instruction to read aloud as fast as possible again has no effect on the slope of the regression (if Morton's subjects and passages were comparable with the present ones). Subjects appear to be processing contextual information at the verbal level as fast as possible in normal reading; but they are able to reduce the constant component of the response time, either by shortening the time taken for articulation or by reducing the extent of monitoring at the non-verbal level. Fig. 2 illustrates these suggestions.

The account so far assumes that the informational and non-informational components of the total time are independent and do not overlap or mask each other, as did Crossman's choice and movement time in card-sorting when his subjects saw the cards in advance<sup>12</sup>. It seems *a priori* more likely that they should overlap in reading, since subjects can look ahead of the words they are speaking. The apparently very high rate of gain of information taken from the slope would then be an artefact. However, the fact that instructions (to read fast) and the presence or absence of vocalization affect the constant component without altering the information rate suggests that one is justified in treating the two components separately. If this way of measuring the information rate is valid, it gives an unusually high value—about 60–70 bits per sec. The usual finding in reaction time experiments is 5–7 bits per sec<sup>13</sup>; but it is interesting that in an auditory-verbal reaction task—repeating letters, numbers or nonsense syllables—Davis, Moray and Treisman<sup>14</sup> also obtained a rate of gain between 60 and 70 bits per sec, although they questioned whether this measure was meaningful. Both tasks involve a high degree

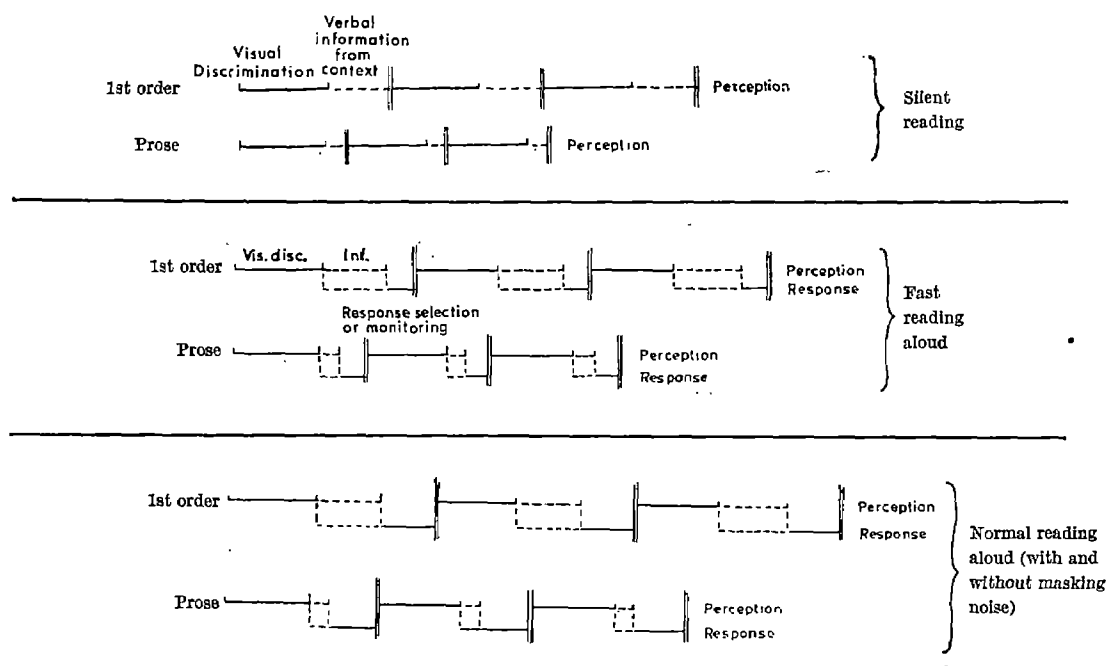


Fig. 2. Suggested components of reading time for passages of different information content. || Segments represent the time taken over a constant number of words; — components represent proportion of time for non-verbal information processing; - - - components represent proportion of time for verbal information processing. The order of the components between these || segments is arbitrarily chosen in the diagram. The lengths represent the proportion of time taken on each component, but the division between the two components may follow a different pattern.

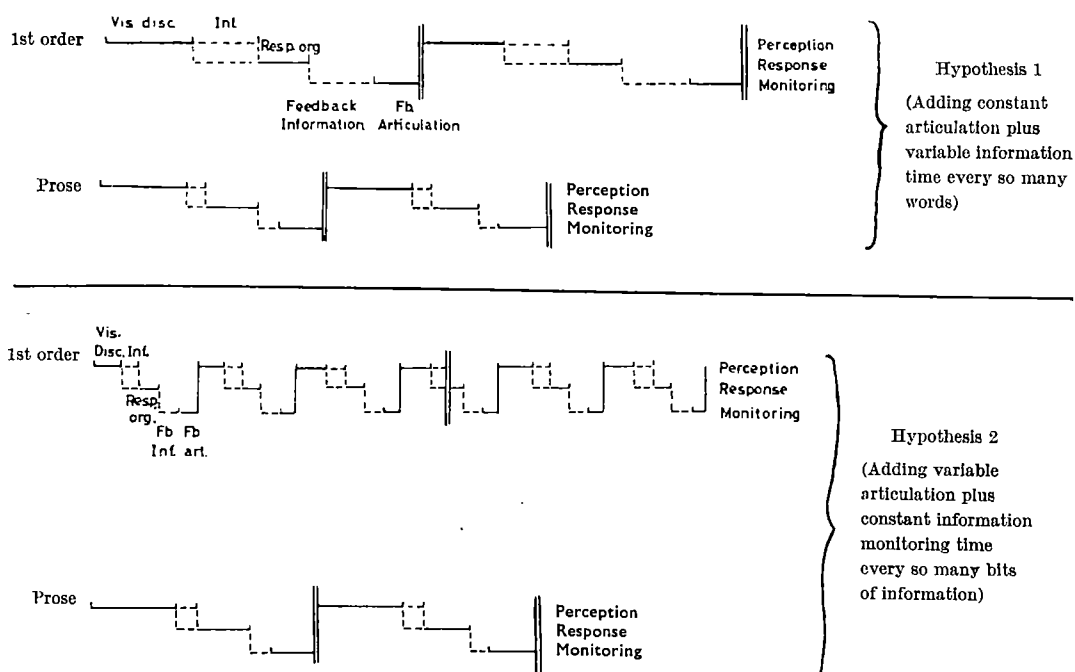


Fig. 3. Suggested components of reading time with auditory feed-back

of familiarity and 'stimulus-response compatibility', which might leave a large capacity available for transmitting information.

The introduction of delayed auditory feed-back is the only condition which affects the information rate as well as the constant component. The increase in the constant reflects the auditory monitoring at a non-verbal level. The most plausible explanation of the change in slope is that the feed-back is now monitored at the verbal as well as at the phonemic level. This might be because the delay in the feed-back causes a phonemic mismatch at the normal interval at which the vocal output and the feed-back are compared. The words in the feed-back thus become unpredictable and are treated as new information to be processed completely a second time; this increases reading time by doubling the slope and halving the rate of gain of information. The fact that the regression coefficient is approximately doubled agrees well with this hypothesis, but the factor of 2 is not crucial. It might be, for example, that at shorter or longer delays the slope would be less than twice as steep: this would be explained by the fact that the mismatch between vocal output and auditory feed-back was less disturbing and easier to ignore, so that only a proportion of the words were processed twice at the level of verbal information. If some other condition made the mismatch still more disturbing (for example, increasing the intensity of the feed-back), this might force subjects to process some words more than twice, so that the slope could be more than doubled. The crucial point for this hypothesis is that the slope should be steeper for delayed auditory feed-back than for other conditions.

Finally, one might distinguish two forms which the verbal monitoring could take: it might be that subjects monitor their speech every so many words—a fixed number for all passages—and that the monitoring adds a certain time which varies with the information content of the words; or alternatively the monitoring might be made every constant number of bits, so that it occurs more often for the incoherent than the coherent passages, but adds a constant component to the total reading time every time it is made. These suggestions are illustrated in Fig. 3. The results of four subjects were examined in

more detail to throw light on these alternatives. If monitoring were carried out at intervals which vary with the information content, one should expect to find the overt disturbances, such as lengthening and repetitions of sounds, also occurring at intervals which vary with information. The mean number of these per passage is given in Table 1. It reveals that, on the average, one correction or pause is made every four words for the 1st-order passage and every eight words for the prose. The interval does appear to vary with the coherence of the passage. This would be consistent with the idea that speech responses are organized or programmed in discrete segments which vary with the information content and which are monitored intermittently as wholes.

Passage	1st-order	2nd-order	4th-order	6th-order	8th-order	Prose
Mean number of repetitions, pauses or lengthened words per 100 word passage	25.3	22.5	19.0	19.3	18.8	12.3
Speech disturbance every how many words	4.0	4.4	5.3	5.2	5.3	8.1

Since this work was carried out, Fillenbaum has reported a similar experiment with different results (Fillenbaum, S., *J. Acoust. Soc. Amer.*, **33**, 1800, 1961). I am at present unable to explain the discrepancy.

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## LETTERS TO THE EDITOR

## RADIO ASTRONOMY

## Occultation of the Crab Nebula by the Solar Corona at Centimetre Wave-lengths in June 1964

DURING the occultation of Taurus in June 1961 and 1962, centimetre wave-length observations were undertaken by U.S. Air Force Cambridge Research Laboratories scientists to investigate small-scale irregularities, if any, in the solar corona which might produce wide-angle scattering at metre wave-lengths and also be observed at centimetre wave-lengths. Results for 1962 were interpreted by Basu and Castelli<sup>1</sup> to indicate a progressive broadening of the source and a decrease in source intensity as the angular separation between Taurus and the Sun became smaller. The reverse tendency was noted on the egress part of the cycle. The broadened maximum extent of the source was found to be about 20 min of arc at  $\lambda = 10$  cm.

In 1964, using the identical technique as in 1962, no apparent broadening or change in source intensity was detected.

Observational wave-lengths were 25 cm and 10 cm. Equipment consisted of conventional radiometers and a single 84-ft. parabolic antenna with a dual frequency linearly polarized feed. The pencil beam produced was less than 17.5 min of arc at  $\lambda = 10$  cm and about 36 min at  $\lambda = 25$  cm.

The observing technique used in 1962 and again in 1964 was to make 4 or 5 right ascension drifts of the source and/or solar contribution each day between June 10 and 20. Then, on control days during the final week of June, drifts were taken with the antenna directed at points in the sky corresponding to the angular separation between the Sun and the radio source, Taurus, during the actual occultation period.

During the occultation period, temperature calibrations were made before and after each drift. Sufficient time was allowed for each drift to establish a far-out steady sky level free from solar contribution. On the control days, equipment gains were adjusted to agree with those on corresponding data days. This was done using noise generator calibrations.

The solar flux was found for each day both locally and by referring to the Ottawa 2,800-Mc/s flux, and control day drifts were effectively normalized to comparison days in respect to solar contribution through the antenna side lobes.

Finally, the actual curves and control day curves were subtracted from each other. We were careful to reference curves at far-out zero points. For each day's equipment gain settings, linearity was determined and corrections were made where applicable.

The actual response curves due to Taurus alone after subtraction of the solar component were constructed by plotting signal levels taken at 6-sec intervals from the analogue records. At  $\lambda = 10$  cm, points for plotting were read with an accuracy of  $\pm 1$  sec. Half-power widths of the constructed response curves were read with an accuracy of better than  $\pm 1.5$  sec of time.

The time-width of each constructed drift due to Taurus alone at half-intensity level was compared with drifts made early or late in June when there was no question of solar contribution in the response drift.

It is also possible (and this has been done) to compute the angular extent of Taurus whether unbroadened or otherwise from the expression:

$$C^2 = A^2 + B^2$$

where  $C$  = the actual source response where minutes of arc = (min of time)  $15 \cos$  declination;  $A$  = the actual antenna half-power beam width;  $B$  = the Gaussian source half-intensity width.

Theoretical maximum intensity calibration errors at  $\lambda = 10$  cm of  $\pm 3^\circ$  K due to precision variable attenuator resettability and absolute calibration over the operating range in the 10-cm equipment scarcely apply. Practically, by peak calibration of many drifts of various sources with the same equipment, it is felt that in the present case the maximum probable relative intensity error is under  $\pm 1.5^\circ$  K.

The validity of the whole method is based on the extraordinary stability of the antenna side lobes and on how well we are able to calibrate out the solar contribution.

Results are presented in Table 1.

Table 1

Date	1964 = 10 cm Source extent at half- intensity—min		Source temp. at receiver	Signal percentage solar
	Time	Arc		
June 3-5, control	1:275'	4:6'	—	0
June 11	1:25	3:1	24.7° K	17
June 12	1:25	3:1	23:8	18.8
June 13	1:275	4:6	23:72	50
June 14	1:275	4:6	24:04	57
June 15	1:29	5:4	23:71	17.8
June 16	1:25	3:1	24:68	14.3
June 17	1:275	4:6	24:8	15.9
June 26, control	1:275	4:6	23:8	0

Uncertainty: time duration of drift half-intensity—3 sec. Angular extent of source possible error 2.8 min of arc. Intensity uncertainty limits  $24^\circ \pm 1.5^\circ$  K.

In 1963, other observers have detected no significant broadening at 6, 13, 18 and 21 cm. Air Force Cambridge Research Laboratories personnel did not repeat the 1962 measurements in the same form. Therefore, we can only assume that the 1963 evidence of others is correct for 1963. In view of the results of the present 1964 investigation, which seems to justify the method, an explanation is sought for our 1962 results. Such an explanation is not to be found in a comparison of the fluxes for the various days and years. However, it is significant that, in 1962, an active region was present at about  $10^\circ$  south of the solar equator. It is the southern corona which is probed by the measurement at the time of minimum angular separation of the Sun and Taurus. At the Union Radio Scientifique Internationale General Assembly in Tokyo, Japan, in 1963, Vitkevich commented on an unusual scattering effect observed on June 10, 1962, at metre wave-lengths and lasting for several hours.

It is anticipated that similar investigations will be continued in the coming year.

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## ASTRONOMY

## Lunar Luminescence

In a recent article, Kopal<sup>1</sup> has directed attention to a report by Sir William Herschel<sup>2</sup> of 'volcanoes' in the neighbourhood of the lunar crater Aristarchus seen in April of 1787. Both he and Middlehurst<sup>3</sup> note the similarity between Herschel's description and two recent reports by Greenacre<sup>4,5</sup> of reddish luminescence near Aristarchus in October and November 1963. In Table 1 are listed 16 additional transient luminescent events in the vicinity



of Aristarchus observed during the period 1783–1963. Included in the second column of Table 1 are the colour, the visual magnitude, and the dimensions of those luminescent spots for which these data were reported. Of the 19 observations listed, the first 16 were made 2–6 days after new Moon; that is, when Aristarchus was on the dark side of the lunar surface far from the terminator. The three most recent observations were made shortly after full Moon, with the crater in sunlight. In addition to these reports, the literature contains references to luminescent events in the vicinity of several other lunar features including Pitatus<sup>23</sup>, Heraclides Promontory<sup>24</sup>, Alpine Valley<sup>25</sup>, Carlini<sup>26</sup>, Alphonsus<sup>27</sup>, and Kepler<sup>28</sup>. All these events, except those involving Alphonsus and Kepler, were on the dark side of the Moon. The latter two events as well as the luminescence of Aristarchus observed by Kozyrev<sup>22</sup> in 1961 were recorded photographically. Several of the remaining events were observed visually through different telescopes by two or more independent observers.

Table 1. OBSERVATIONS OF LUMINESCENCE IN THE VICINITY OF ARISTARCHUS

Date of observation	Description	Observer and ref.
May 4, 1783	Red; fourth mag.; < 3 in. arc	Herschel <sup>6</sup>
April 19–20, 1787	Red; < fourth mag.; 3 in. arc	Herschel <sup>12</sup>
March–May, 1789		Bode <sup>7</sup>
Feb. 4–7, 1821	Sixth–seventh mag.; 3 in.–4 in. arc	Kater <sup>8</sup>
May 4–6, 1821	< 1 in. arc	Obers <sup>9</sup>
Jan. 27, 1822	Eighth mag.	Ward <sup>10</sup>
May 1, 1824	Ninth–tenth mag.	Baily <sup>11</sup>
April 22, 1825	—	Struve <sup>12</sup>
Dec. 25, 1832	—	Göbel <sup>13</sup>
Dec. 22, 1835	—	Argelander <sup>14</sup>
Jan. 14–16, 1866	Ninth–tenth mag.	Göbel <sup>15</sup>
April 9, 1867	Reddish-yellow	Smyth <sup>16</sup>
May 7, 1867	Seventh mag.	Smyth <sup>16</sup>
March 1–3, 1903	Reddish-yellow	Temple <sup>17</sup>
Feb. 22, 1931	Reddish-yellow	Elger <sup>17</sup>
March 30, 1933	White	Temple <sup>18</sup>
Nov. 26, 28, Dec. 3, 1961	Red and blue	Rey <sup>19</sup>
Oct. 29, 1963	Reddish-orange to light ruby	Gheury <sup>18</sup>
Nov. 27, 1963	Light ruby	Joula <sup>20</sup>
		Douillet <sup>21</sup>
		Kozyrev <sup>22</sup>
		Greenacre <sup>4</sup>
		Greenacre and Boyce <sup>5</sup>

Kopal and Rackham<sup>28</sup> have proposed that the luminescence observed by them in the vicinity of Kepler was excited by streams of corpuscular radiation emanating from a solar flare. Accordingly, Kopal<sup>1</sup> suggests that the luminosity of Aristarchus seen in 1787 as well as in 1963 was likewise due to flare-accelerated particles directed to the crater by interplanetary magnetic fields. In support of this suggestion, he notes that May of 1787 was the peak of an unusually active solar cycle. It therefore seems worthwhile to consider the distribution of the events in Table 1 with respect to solar activity:

Yearly mean sunspot No.	No. of events
0–30	15
30–60	2
60–90	0
90–120	1
120–150	1

Clearly, the 19 events are negatively correlated with solar activity. This fact alone does not necessarily eliminate solar flares as a cause of the luminescence, since major flares have occurred, although infrequently, very near solar minima.

Unfortunately, flare records are incomplete before the last few solar cycles; but none of the flares reported<sup>29</sup> can be associated with the first 16 events in Table 1. Of the remaining three events, only one followed a major flare. This was the first emission observed by Greenacre, which followed by two days the largest flare (Class 3) of 1963. The second event seen by Greenacre a month later, on the other hand, occurred during a period in which the Sun was exceptionally inactive. The three luminescent

emissions reported by Kozyrev—which he, like Herschel, attributed to volcanic activity—fell during a period in which no flares larger than Class 1, and no high-energy solar particle radiation, were recorded. On the basis of this review, then, it appears that luminous spots in the vicinity of Aristarchus are not directly associated with particle radiation from solar flares.

A more quantitative objection to the excitation mechanism proposed by Kopal concerns the radiant energies required to produce the observed red spots. As shown in Table 1, the spots seen on the dark side of the Moon were estimated to vary in brightness from 10 to 4 stellar magnitudes, corresponding to luminous fluxes at the lunar distance of  $10^{13}$ – $10^{16}$  ergs/sec. The areas involved were a few seconds of arc or less, corresponding to dimensions of kilometres. Hence, the radiant energies associated with the spots were of the order of  $10^3$ – $10^6$  ergs/cm<sup>2</sup> sec. Derham and Geake<sup>30</sup> have demonstrated that certain achondritic meteorites luminesce in the red when bombarded with 40-keV protons, the particle energy being converted to light quanta with an efficiency of approximately 20 per cent. Assuming a comparable efficiency for the excitation of lunar luminescence by solar particles, corpuscular streams carrying from  $5 \times 10^3$  to  $5 \times 10^6$  ergs/cm<sup>2</sup> sec to distances of 1 A.U. are required. In order to direct the solar particles to the dark side of the Moon, Kopal postulates that their radius of gyration in the interplanetary magnetic field is large compared with the lunar diameter. Such particles must have energies close to 10 MeV or greater in an interplanetary field of 10γ, taken by Kopal as the average value. The largest particle flux above 10 MeV observed at the Earth so far, corrected for atmospheric absorption and for geomagnetic screening, is somewhat less than  $10^8$  per cm<sup>2</sup> sec (ref. 31). With a mean particle energy of 40 MeV, this particle flux corresponds to an energy flux of less than 3 ergs/cm<sup>2</sup> sec. These figures apply to the peak of the particle event following the 3+ flare of November 12, 1960. Thus, even for the largest event on record, the energies carried to 1 A.U. by solar corpuscular radiation above 10 MeV are only a few ergs/cm<sup>2</sup> sec, from 3 to 6 orders of magnitude too small to account for the emissions listed in Table 1. The solar wind, proposed as an alternative source of lunar luminescence, likewise carries energies of only a few ergs per cm<sup>2</sup> sec at 1 A.U. during peak solar activity. In fact, the entire spectrum of charged particles observed in interplanetary space, integrated over energy and including galactic as well as solar components, has peak intensities well under 100 ergs/cm<sup>2</sup> sec. This is less than the energy of the 'Earthshine', which has been suggested as the source of the phenomenon seen by Herschel. It is also three orders of magnitude too small to account for the luminescence near Kepler observed by Kopal and Rackham<sup>28</sup>.

In summary, luminous emissions, usually reddish in colour, occur in the neighbourhood of the crater Aristarchus whether or not the crater is in sunlight and whether or not the Sun is active. Neither electromagnetic nor charged particle radiation in interplanetary space provides sufficient energy to produce the luminescence by direct excitation unless the flux over a large area is focused on the Moon. Alternatively, the flux might be stored over time to provide sufficient energy for the short-lived lunar emissions. Thus, focusing and/or storage appears to be a necessary feature of any model which attributes lunar luminescence to external energy sources.

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Flamm and Lingenfelter are right in pointing out that the flux of particles accompanying solar events, as recorded so far by ground-based (or Earth-bound) experiments, are too low to provide adequate source of excitation for the visibility of daytime luminescence of the lunar surface; for night-time events it is adequate. Whether or not this is so at the distance of the Moon (that is, sufficiently far from the terrestrial magnetosphere) and in all parts of the energy spectrum remains, however, yet to be seen. Possibly the primary solar particles act only as a trigger of the lunar events<sup>1</sup>. The electromagnetic spectrum of the Sun contains ample energy for excitation; but its responsibility for transient lunar luminous phenomena seems to be contradicted by the time-lag, from several hours to a few days, indicated by the observation.

The stellar magnitudes assigned to lunar night-time events by early observers listed in Table 1 lack quantitative meaning; for no one could make photometric measurements at that time; and the definition of stellar magnitude in use to-day goes back only to 1850 (Pogson). Suffice it to say here that one square second of earthlit lunar surface would appear as a star of +13.5 vis. magn.; and the doubling of its brightness by luminescence would reduce it to only +12.7 magn. An area of 10 square seconds (comparable in size to Herschel's "volcanoes") would then appear as a star of 10.2 magn.

The existence of a positive correlation between solar inactivity and lunar events claimed by Flamm and Lingenfelter would—if confirmed—only deepen the problem, but the residual brightness of eclipsed Moon was found by Danjon<sup>2</sup> (1920) also to change abruptly at the time of the minimum (rather than maximum) of solar activity. At present it should be merely noted that both most conspicuous instances on record of transient lunar luminous phenomena (that is, the Greenacre-Barr and Kopal-Rackham flare-ups of October 30–November 2, 1963; and the Herschel flare-up of April 19–20, 1787) occurred at a time when the Sun was strongly disturbed. No flare observations are available, to be sure, for 1787; but the fact that the Sun must have been greatly disturbed

is attested by the visibility, on both days, of polar aurorae as far south as Padua, Italy<sup>3</sup>.

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## GEOLOGY

### Carbon Isotope Composition of Individual Hydrocarbons from Italian Natural Gases

SINCE the fundamental paper by Craig<sup>1</sup> on the geochemistry of the stable carbon isotopes, much work has been done on the determination of carbon isotope distribution in different materials.

It was found<sup>1,2</sup> that the  $^{13}\text{C}/^{12}\text{C}$  ratio of methanes from natural gases ranges from  $-10.4$  per mil to  $-84.4$  per mil in  $\delta$  units. ( $\delta$  Carbon-13 is determined as follows:

$$\delta^{13}\text{C} = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1,000$$

Results are referred to the PDB standard (ref. 1).) This range of variation is the widest observed within a single class of carbonaceous materials.

Silverman<sup>3</sup> has recently reported the  $^{13}\text{C}/^{12}\text{C}$  ratios of individual light hydrocarbons separated from petroleum by fractional distillation. He showed that ethane and propane are about 11 and 13 per mil isotopically heavier than the associated methane. Apart from these data, referred to a single crude oil,  $^{13}\text{C}/^{12}\text{C}$  ratios relative to ethane, propane and higher hydrocarbons from natural gases are hitherto missing from the literature, in spite of their obvious geochemical importance.

We have developed an experimental technique, based essentially on gas chromatography, which enables the quantitative separation of individual carbon compounds from natural gases. Each hydrocarbon is converted to carbon dioxide, which is then analysed for the  $^{13}\text{C}/^{12}\text{C}$  ratio using a mass spectrometer equipped with double collector. Reproducible results are thus obtained, with an overall accuracy of  $\pm 0.3$  per mil. This technique was applied to the examination of natural gases from nine producing fields, located in southern Italy and Sicily. The chemical composition of such gases was also determined by gas chromatography.

The  $^{13}\text{C}/^{12}\text{C}$  ratio of methane ranges from  $-37.8$  to  $-71.5$   $\delta$  units in a total of 52 samples analysed. It was found that the carbon-13 content of methane decreases with increasing molar ratio  $\frac{\text{methane}}{\text{total hydrocarbons}}$  in the gases.

This trend (Fig. 1) is exhibited by all the samples examined, which were collected from different Italian gas fields.

As expected, ethane, propane and butane hydrocarbons are isotopically heavier than the associated methane. The results reported in Table 1 indicate that the ranges of  $\delta$  carbon-13 for such hydrocarbons are much narrower than that for methane.

The isotopically lightest Italian methanes fall in the same  $\delta$  carbon-13 range as other methanes of alleged bacterial origin<sup>2,4</sup>. Their  $\delta$  carbon-13 values indicate a probable depletion in carbon-13 of approximately 40–50 per mil with respect to the original organic matter.

The isotopically heaviest, among the Italian methanes analysed, are associated with relatively large amounts of ethane, propane and higher hydrocarbons. These heavier methanes indicate probable depletions not greater than 20 per mil, in agreement with the results obtained by Silverman<sup>3</sup> for a methane allegedly produced in the 'maturation' of petroleum.

Therefore, the trend of Fig. 1 could be explained by assuming that each gas results from the admixture of an

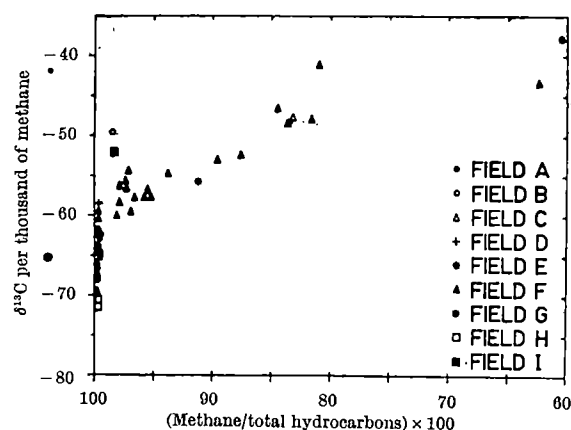


Fig. 1. Carbon isotopic analysis on methane from Italian natural gases. Ages of host-rocks: fields A, D, F, H = Pliocene; fields B, C, G = Miocene; field E = Cretaceous; field I = Triassic

isotopically lighter bacterial methane, and of a heavier methane produced with its homologues in a chemical process similar to the 'maturation' of petroleum.

As an alternative hypothesis to explain the trend of Fig. 1, it could be assumed that the wide isotopic range observed in methanes is essentially due to a fractionation, in the course of the migration, of the natural gases from the source of the reservoirs. Migration should thus be regarded as a complex process, which on one hand causes the segregation of each hydrocarbon and non-hydrocarbon component of the gas according to its mobility, and on the other hand gives rise, within each molecular species, to an isotopic fractionation, the extent of which depends on the molecular mass of the component itself.

Table 1. CARBON ISOTOPIC COMPOSITION OF INDIVIDUAL HYDROCARBONS FROM ITALIAN NATURAL GASES

Sample No.	CH <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	C <sub>3</sub> H <sub>8</sub>	C <sub>4</sub> H <sub>10</sub>
1	-47.7	-30.5	-26.5	-23.4
2	-57.6	-32.2	-26.4	-22.7
3	-59.5	-33.1	-27.0	-25.3
4	-57.6	-32.5	-27.5	
5	-52.9	-32.3	-25.3	
6	-41.1	-30.2	-26.0	
7	-46.4	-31.9	-25.2	-22.8
8	-48.4	-29.6	-25.3	-22.6
9	-56.9	-31.0	-26.1	

This latter hypothesis is corroborated by the isotopic data obtained on 17 methane samples from a single relatively small multi-horizon gas field in southern Italy, where a regular variation of  $\delta$  carbon-13 values as a function of depth was established, with an over-all range of 4.3  $\delta$  units.

Laboratory experiments were performed, in order to measure the isotopic fractionation of methane during chromatographic flow through a column filled with hydrogen-bentonite. Pure methane samples were allowed to flow, in a current of oxygen, through a column 20 m long at a temperature of 25° C with a pressure gradient of 2 atm. A Perkin-Elmer '154 B Vapor' fractometer was used, by which it was possible to split each sample of methane coming through the process into two consecutive fractions of approximately equal volume. An isotopic fractionation of 3.0 per mil was measured, with the isotopically lighter fraction coming through the process first. Similar experiments were carried out with limestone and dolomite columns, resulting in smaller but still detectable fractionations.

These laboratory experiments simulate rather poorly actual geological conditions. Nevertheless, they have shown the possibility of obtaining measurable isotopic fractionations of methane by physical processes, such as effusive flow, diffusion and adsorption, similar to those which may occur in Nature during migration and accumulation of hydrocarbons.

In conclusion, although a two-fold mechanism of origin (bacterial and chemical) of the methane from Italian natural gases cannot be discarded, our findings suggest that isotopic fractionation during migration of the gas from the source to the trap positions might be so important as to become the dominant feature of the over-all isotopic fractionation measured.

Details of the experimental techniques and results obtained will be published elsewhere<sup>5</sup>.

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## GEOMORPHOLOGY

### Permafrost in England during the Last Glacial Period

PERMAFROST has been shown by many authors to have existed in various parts of England during the last glacial period<sup>1,2</sup>. Its full extent and distribution have remained somewhat uncertain but can be deduced from the occurrence of certain fossil ground structures. Ice-wedge polygons, the trough patterns of chalkland<sup>3</sup>, and the largest stone polygons and stripes are generally accepted as requiring permafrost. Less reliable features are involutions, and a deep layer of upturned stones above horizontally-bedded gravels. Preliminary results are reported here from mapping these structures using pit sections and air photographs.

South-west England seems to have been relatively free from permafrost except on high ground. Permafrost structures are much less frequent than in eastern England. For example, in East Anglia and Kent involutions or fossil ice wedges are found in nearly half the sand and gravel pits, and involutions in one in three chalk pits. In Dorset, by contrast, several miles of suitable face were examined in pits in many places on the Tertiary gravels and the Chalk and only one doubtful set of involutions could be found. The only reason why structures should be so rare in Dorset and the south-west generally would seem to be that permafrost was largely absent. Locally in England permafrost structures are rare for other reasons, which, however, have only very limited application to Dorset. In Surrey, for example, many pits on the Chalk are located on the scarp face where periglacial structures, if they ever occurred, would have been destroyed by post-glacial erosion. Furthermore, involutions are apparent in Chalk only when overlying materials are present and cannot be found in bare Chalk country. This does not explain their absence in Dorset, where the Chalk is not markedly less covered by superficial deposits than in other counties. Again, most of the sand pits in Surrey are in the Folkestone Beds which were evidently too dry for structures to develop. The latter is not the case with pits in the Tertiary beds in Dorset. Permafrost seems to have been widely present in Surrey, for where suitable pits occur structures are frequent.

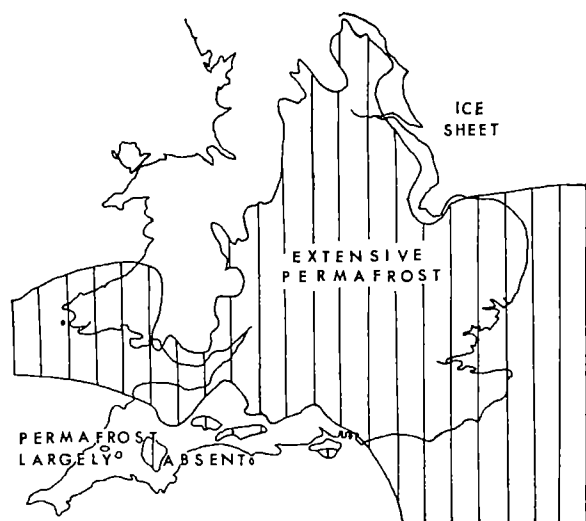


Fig. 1. Distribution of permafrost during the last glacial period

Extensive permafrost probably extended as far west as the Sussex coastal plain, the western Weald and the Oxford district (Fig. 1). Further west, permafrost seems to have been largely absent from chalk and gravels on low ground. Stone stripes and other structures suggest that permafrost extended down to about 900 ft. on Dartmoor. The lower limit of permafrost in east Devon and west Dorset was probably between 400 ft. and 500 ft. There is reason to suppose that some of the few structures which occur in south-western England are old features dating from the early Weichsel or Gipping glaciations.

The Arctic and Subarctic can be divided into zones of continuous, discontinuous and sporadic permafrost which correspond to different mean annual temperatures<sup>3</sup>. Where the permafrost is patchy, its existence depends on the thermal properties of the ground and the direction of slope<sup>4</sup>. Thus, in the Subarctic in the sporadic zone permafrost is found mostly in peaty materials and on a few north-facing slopes.

It is not immediately clear how the British distribution relates to these divisions. The transition from restricted to extensive permafrost is much more abrupt than in the Arctic. However, the sporadic zone is not properly represented on the map because peaty materials of periglacial age have been little preserved. In eastern England continuous permafrost must have existed at least in certain districts to judge from the abundance of structures. In west Norfolk and Suffolk and parts of Kent, permafrost structures underlie an estimated 50 per cent of the land surface. Permafrost was certainly much more extensive than this figure would indicate since the structures show no preference for slopes facing north-east or damp valley bottoms to which permafrost would tend to be restricted if it were discontinuous. Also the structures would only have developed in part of the area occupied by the permafrost. However, in many districts structures are not found frequently enough to show whether the ground was frozen everywhere. The widespread occurrence of asymmetric valleys also fails to decide whether the permafrost was continuous or chiefly restricted to slopes facing north-east, as many other explanations of the asymmetry are possible<sup>5</sup>. The best evidence is provided by fossil ice-wedges. Most in Britain occur in gravels and sand. In Alaska, wedges in the discontinuous zone are ice-filled only in silts. Even these, according to Péwé, are inactive and relics of former cold conditions. Only in the zone of continuous permafrost are wedges forming at the present day, and only in this zone do wedges in gravels and sand contain ice<sup>6</sup>. The critical mean annual temperature necessary for continuous permafrost is widely accepted as  $-6^{\circ}\text{C}$ . The margin of extensive permafrost

in Britain probably corresponds to this isotherm and suggests the relative importance of Atlantic rather than continental climatic influences in the south-west.

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## PHYSICS

### Existence of the Schrödinger and Heisenberg Pictures

IN a recent article in *Nature*<sup>1</sup>, a discussion on the foundations of quantum theory was presented by Dirac. He pointed out that the Schrödinger picture (*S*-picture) does not exist for quantized interacting fields such as quantum electrodynamics. Furthermore, the Heisenberg picture (*H*-picture) is assumed to exist with the *H*- and *S*-pictures not unitarily equivalent. We wish to point out here that there is a natural separable Hilbert space on which the *S*- and *H*-pictures both exist and are equivalent.

Dirac's argument was based on the non-existence of the vacuum state in the *S*-picture. He has introduced a simple model (Dirac, P. A. M., seminar at the Institute for Advanced Studies, Princeton, 1965. We would like to thank Prof. Dirac for communicating this model prior to publication, and for stimulating discussion), in which, by the appropriate representation of the creation and annihilation operators, this difficulty becomes apparent<sup>2</sup>. We wish to show in this model how a suitable representation may be chosen for the *S*-picture to exist.

The Hamiltonian of this model is in terms of a set of Fermion creation and annihilation operators  $N_r, \bar{N}_s$  ( $r = 1, 2, \dots, \infty$ ) satisfying the canonical commutation relations (CCR):

$$[N_r, N_s]_+ = [\bar{N}_r, \bar{N}_s]_+ = 0 \\ [N_r, \bar{N}_s]_+ = \delta_{rs}$$

The Hamiltonian  $H$  is:

$$H = \frac{1}{2} \sum_{r,s=1}^{\infty} (A_{rs} N_r N_s - \bar{A}_{rs} \bar{N}_r \bar{N}_s)$$

$$\text{with } A = \begin{pmatrix} 0 & -10 & 0 & \dots \\ -10 & 0 & 0 & \dots \\ 0 & -10 & 0 & \dots \\ \vdots & \vdots & \vdots & \ddots \end{pmatrix}$$

If we assume  $\bar{N}_r(0)|0\rangle = 0$  for all  $r$ , then it follows that  $\|H|0\rangle\| \rightarrow \infty$ . The conclusion is that the *S*-picture may only be good on some larger non-separable Hilbert space. If instead we choose new variables  $N'_i$  and  $\bar{N}'_i$  (which still obey the CCR) of the form:

$$N'_1 = \frac{\bar{N}_1 - N_2}{\sqrt{2}} \quad N'_2 = \frac{\bar{N}_1 + N_2}{\sqrt{2}} \quad \text{etc.}$$

then  $H = N'_1 \bar{N}'_1 - N'_2 \bar{N}'_2 + \dots$ , which is well defined on the Fock space  $\mathcal{H}$  of the particles described by the operators  $N'_i$  and  $\bar{N}'_i$  provided the number of particles is finite. On this separable Hilbert space  $\mathcal{H}$ , the *S*- and *H*-pictures are well defined and unitarily equivalent.

This model is obviously an over-simplification of the world, but we feel it has at least the essential properties of a full theory which are relevant to the existence question.



When we turn to a more realistic theory such as quantum electrodynamics, we are faced with the problem of diagonalizing a much more complicated Hamiltonian. This is done in principle via the asymptotic in and out fields<sup>3</sup> and will result in practice (in perturbation theory) by inserting the solutions of the field equations as power series expansions in terms of the in or out fields. (From the asymptotic condition,  $H$  is the time translation operator for the in and out fields. The only form of  $H$  expressible as a polynomial in either the in or out creation and annihilation operators must then be the usual diagonal expression for the free energy in terms of these operators.) In the presence of bound states, we do not expect these series to converge and the complete diagonalization will involve a self-consistent type of calculation of these bound states. Even in their absence the convergence is unlikely.

The diagonalization process cannot involve a unitary transformation on the field operators. This is evident for the model discussed earlier and is also true for any relativistic theory as follows from Haag's theorem<sup>4</sup>. Indeed, the Heisenberg operators at finite  $t$  and the corresponding Schrödinger operators must belong to a representation of the CCR<sup>5</sup> which does not possess a no-particle state. These 'strange' representations are very complicated and lead to great difficulties in the  $S$ - and  $H$ -pictures. In fact, all numerical successes of quantum field theory have been obtained by using Green's functions equations (GFE)<sup>6</sup>. These GFE avoid finding the strange representations by considering matrix elements of the field operators and not the operators themselves. We do not know yet whether there exist solutions to these GFE<sup>7</sup>; if we could show that they exist then we would have achieved the diagonalization process outside of perturbation theory.

In conclusion, we see no logical inconsistency in the foundation of quantum field theory provided the non-perturbative solutions of the GFE exist.

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### Precursor Shocks produced by a Large-yield Chemical Explosion

PRECURSOR shocks are phenomena normally associated with surface burst or low-level nuclear explosions. The precursor moves out along the ground ahead of the primary shock, and usually produces a large amount of airborne dust. The precursor has been explained<sup>1</sup> as an effect of the intense thermal radiation produced by a nuclear explosion.

In July 1964, a 500 ton TNT hemispherical surface burst charge was detonated at Suffield Experimental Station in Alberta, Canada. High-speed photographs of the explosion show that in some radial directions dust clouds moved out ahead of the main shock and had reached a height of 50 ft. before its arrival. The dust clouds were enveloped by a shock wave. At ground-level this precursor eventually became downward facing and produced a reflected shock and a Mach stem. Photography from an aeroplane at 19,000 ft. immediately above the explosion

showed that all the precursors were produced along well-compacted roadways running radially from the charge centre. The precursors occurred in the region 250–750 ft. from the centre of the explosion, corresponding to peak overpressure levels of 150 lb./in.<sup>2</sup>–20 lb./in.<sup>2</sup>. A gauge measuring the total density within the blast wave, by means of a  $\beta$ -radiation absorption technique<sup>2</sup>, showed the dust density to be four times that of the peak air density expected in the blast wave at that position. Targets placed in the regions of the precursors experienced considerably more damage than had been expected. From the evidence of seismometer records it seems probable that the precursors were produced by strong ground waves feeding energy into the air ahead of the air shock in a manner similar to that observed by Boys<sup>3</sup> for supersonic missiles penetrating metal plates and by Benioff, Ewing and Press<sup>4</sup> for earthquakes.

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### Does Quantum Mechanics exclude Life?

P. T. LANDSBERG, in a recent paper<sup>1</sup>, reopened a problem discussed earlier by Wigner<sup>2</sup>: the possibility that quantum mechanics might predict and explain the phenomena of life.

More precisely, this problem may be formulated by asking two questions which sum up its essential content. These derive from a recognition that a living organism, together with the environment from which it draws its food, may be considered as a quantum system of very large size, which we may assume to be isolated from any external influence.

Let us start by supposing that such a system contains initially no living organism. What, then, is the probability that it will evolve towards a state in which a living organism is present? This is the problem of the spontaneous generation of life.

Alternatively, let us suppose that our system contains initially one living organism. What, then, is its probability of evolving toward a state in which two such organisms are present? This is the problem of reproduction.

Wigner sought, essentially, to reply to the second of these two questions: he thus assumed reproduction to be a characteristic and definitive property of life. The conclusion he reached, however, on the basis of a statistical treatment, was that quantum mechanics predicts a practically nil probability for the existence of states corresponding to auto-duplication of a part of the system. He therefore suggested that quantum mechanics is not suitable for a complete description of all natural phenomena or that, at the least, it must be modified to include concepts, such as consciousness, which are not necessary for the description of physical phenomena.

Landsberg deliberately avoids giving any precise definition of a living organism, limiting himself to the assumption that the number of dimensions of the region of phase space, corresponding to the presence of  $n$  living organisms in the system, diminishes very rapidly as  $n$  increases. Using less-restrictive statistical assumptions than Wigner, he establishes that spontaneous generation of life and reproduction are not completely ruled out by quantum mechanics, although their probability on average (that is, over a large number of possible initial conditions) remains extremely small. However, favourable initial conditions for self-reproduction can certainly be expected.

I propose to call attention to two facts. First, in accordance with Landsberg, quantum systems which, when placed in a suitable environment, possess the property of reproducing themselves, do exist, and are moreover fairly well known. Secondly, the contentions of Wigner and Landsberg do not in fact justify any statement on the validity of a quantum mechanical description of life.

The first of these statements is easily demonstrated if one considers an atomic nucleus in an environment with a thermal neutron density always different from zero<sup>3</sup>. In such an environment the nucleus enlarges progressively as a result of successive neutron captures and remains stable, so far as the emission of nucleons is concerned, by successive  $\beta$ -disintegrations. At a certain point, however, it reaches the stability limits for nuclear fission and then, following a final neutron capture, it splits into two nuclei of medium atomic weight. Its behaviour is thus wholly analogous to that of a bacterium in a suitable nutrient.

One certainly cannot maintain that this is a rare or contrived quantum system; this example should rather give us grounds for thought whether the capacity to reproduce in a suitable environment is not one common to all matter.

Furthermore, it is clear that an atomic nucleus does not fall, even marginally, within the category of entities which we describe as living. This means that the capacity to reproduce is not wholly peculiar to these entities, and therefore cannot serve as a quality for their definition. This is why Wigner's and Landsberg's contentions have not in fact anything significant to contribute with regard to the applicability of quantum mechanics to a description of life.

The fact that, for each of the functions (reproduction included) which we consider essential for maintaining life, one can find an inorganic model seems then to suggest a rigidly defined set of ideas. This in fact entitles us to suspect that the problem of ensuring this group of functions may have many very different solutions, and that the single solution which we see universally adopted in the world of living organisms, the cell, is after all only one solution, which has effectively displaced all the others, either through historical events, or more probably because it can ensure a much faster reproductive cycle. In this context, it is interesting to note that the period of duplication of an atomic nucleus exposed to a flux of slow neutrons like that produced by cosmic rays in the atmosphere is of the order of  $10^{18}$  years, and in a nuclear reactor this period would be reduced to  $10^5$  years.

Thus it seems, more generally, that a catalogue of functions is not adequate to define what we call a living being: at least a partial description of the structure of the organism seems essential for an effective definition.

There are in addition many facts which confirm the fundamental nature of the concept of structure (by which we mean the nature and arrangement of the parts constituting the system) as regards both definition and maintenance of life. The most striking of these is the effect of ionizing radiations on living organisms. We know that the permanent changes caused by such radiations in the irradiated material consist almost entirely of the breaking of molecular bonds, which may possibly be followed by the reconstruction of other bonds of the same type. What is thus changed is the arrangement of the particles constituting the cell, and it is clear that this change in the order, that is in the structure of the organism, when it involves certain large molecules of fundamental biological importance, causes a change in the functions which these molecules are intended to perform and thus, triggering a sort of relay amplification process, determines the observable macroscopic damage.

Structure is thus seen, in biology, to be an absolutely basic concept, of which functions are nothing other than dynamic aspects.

But when we remember that the structures we are considering are in point of fact molecular, that is, made of

atoms, it is difficult to see why they could not be described by quantum mechanics.

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PROF. AGENO is correct in quoting inorganic examples of self-duplicating states. Living objects can certainly not be defined by this property alone and this was not our intention. Self-reproduction is, however, one of the properties which has to be discussed from the point of view of quantum mechanics in connexion with living objects. Such a discussion seems to be of interest<sup>1</sup>, even though it can only be very speculative (as pointed out in our publications), since a living system is always large by quantum mechanical standards. We were, therefore, interested in dealing with methods of analysing this problem and in developing assumptions which might reasonably be made in this connexion. As our understanding of these questions increases, one may hope that other features of living matter can be introduced into the argument to make it more specific.

Perhaps it is worth while to point also to a concrete error in Prof. Ageno's argument. Neutron multiplication is not a process of extremely small probability according to quantum mechanics, because the state of the neutrons which are emitted is not a highly specialized one. In contrast, if we asked for the probability that a neutron with a definite energy incident on a uranium nucleus should emit two neutrons with the same energy, the probability would turn out to be very small. The essential point which Prof. Ageno disregards is that the living state is a very uncommon state of the matter of which it is composed, that is, that the same matter can be present in many, many other states which are not living.

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## RADIATION CHEMISTRY

### Detection in Denmark of the Sinkiang Nuclear Detonation

MEASUREMENTS of fission products in air at ground level are made regularly in Copenhagen using a high-volume air sampler and a 100-channel  $\gamma$ -spectrometer.

A filter exposed during the period October 23–26, 1964, gave the first reliable indication of new fission-products by the appearance of the 1,596-keV line of lanthanum-140. The sample was a compressed filter containing dust from about 150,000 m<sup>3</sup> air. The measurement position of the filter is close to a 3-in sodium iodide (TI) crystal. The concentration of lanthanum-140 was estimated as  $5 \times 10^{-5}$  pc./m<sup>3</sup>. Filters sampled on October 28 and October 30 show concentrations which are approximately 10 and 100 times greater.

To show the presence of lanthanum-140 clearly in the last-mentioned filter  $\gamma$  -  $\gamma$  coincidence measurement with the 1,596-keV line was made and the 815-, 487- and 329-keV lanthanum lines appeared clearly as shown in Fig. 1.

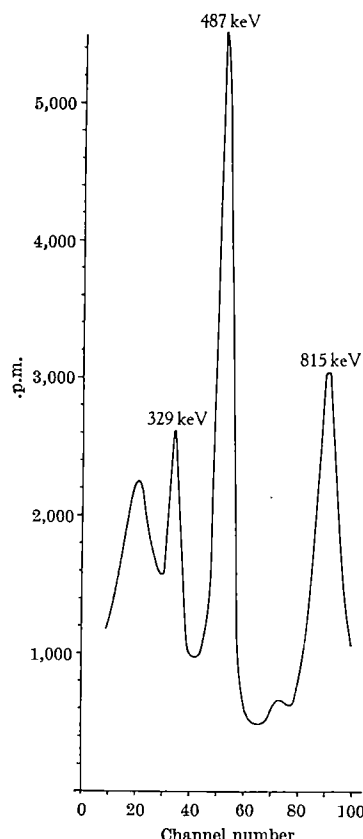


Fig. 1.  $\gamma$ -Coincidences with 1,596-keV line of lanthanum-140

This seems to prove that debris from the Sinkiang explosion reached Copenhagen by transportation in the upper troposphere in less than 10 days.

Later measurements on a rain sample from October 23 finally proved that the transportation time did not exceed 7 days.

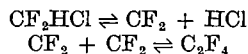
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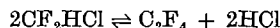
## CHEMISTRY

### Chemical Behaviour of Difluorocarbene, and the Dissociation of the Carbon—Carbon Bond in Tetra-fluoroethylene

**Bond strengths.** The heat of formation of  $\text{CF}_2$  has been estimated through electron impact, thermochemical and kinetic studies. The most recent measurements were obtained through a study of the pyrolysis of  $\text{CF}_2\text{HCl}$  at 530°–750° C, which is believed<sup>1-3</sup> to follow the mechanism:



Either by interpolation from known data, or by assuming the heat of formation of  $\text{C}_2\text{F}_4$  (–151.5 kcal/mole (ref. 4)) and combining this with the thermodynamic data for the equilibrium:



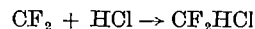
it is possible to estimate the heat of formation of  $\text{CF}_2\text{HCl}$  ( $\sim -112$  kcal/mole); an estimate of the Arrhenius factors of the elementary steps can be derived from the kinetic analysis. Combination of the thermochemical and kinetic data yields  $\Delta H_f(\text{CF}_2) \leq -40$  kcal/mole, and hence  $D(\text{F}_2\text{C}-\text{CF}_2) \leq 70$  kcal/mole. Similar conclusions have been reached by Stull<sup>5</sup>, on the basis of a

study of the formation of  $\text{C}_2\text{F}_4$  from carbon and  $\text{CF}_4$  in a furnace<sup>6</sup>. Majer and Patrick<sup>7</sup> have suggested that higher (less negative) values for  $\Delta H_f(\text{CF}_2)$ , which have been reported on the basis of electron impact studies, result from the production of excited fragments in the primary dissociation.

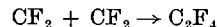
Whatever may be the absolute value of the heat of formation of  $\text{CF}_2$ , there is little doubt that it leads to a very low value for  $D(\text{F}_2\text{C}-\text{CF}_2)$ , probably  $\sim 70$  kcal/mole, and this conclusion is consistent with other evidence to be presented. By contrast the heat of formation of  $\text{CH}_2$ , although not known with absolute certainty<sup>8</sup>, leads to  $D(\text{H}_2\text{C}-\text{CH}_2) \sim 150$  kcal/mole. However, the C—C bond stretching frequency in  $\text{C}_2\text{H}_4$  is  $\sim 250$   $\text{cm}^{-1}$  lower than in  $\text{C}_2\text{F}_4$  (refs. 9 and 10) and the C—C bond length is  $\sim 0.02$  Å longer. Despite the very low bond dissociation energy in  $\text{C}_2\text{F}_4$ , there is no doubt that, in the region of the potential minimum at least, the carbon atoms remain linked by a double bond.

**Decomposition of fluoromethanes and olefines.** The spectroscopic detection of  $\text{CF}_2$  produced in the flash photolysis of many fluorinated methanes and olefines has been reported<sup>11-13</sup>. Evidence has been obtained, indicating that  $\text{CF}_2$  is produced in a 'molecular' split, in the photolysis of  $\text{CF}_3\text{HBr}$  and  $\text{CF}_3\text{Br}_2$  (and  $\text{CF}_3\text{COOH}$ ); gas chromatographic and mass spectrometric analysis of the volatile products of flash photolysis of  $\text{CF}_2$ :  $\text{CCl}_2$  and  $\text{CF}_2$ :  $\text{CFCl}$  have revealed  $\text{CF}_2$ :  $\text{CF}_2$  and  $\text{CCl}_2$ :  $\text{CCl}_2$ , and  $\text{CF}_2$ :  $\text{CF}_2$  and  $\text{CFCl}$ :  $\text{CFCl}$  respectively<sup>14</sup>. The ready production of  $\text{CF}_2$  from these olefines, and from  $\text{C}_2\text{F}_4$ , with light in the quartz ultra-violet, again emphasizes the ease of dissociation at the C—C bond in these molecules. The products of the Hg ( $6^3P_1$ ) photosensitized decomposition of  $\text{C}_2\text{F}_4$  have been interpreted in terms of a primary dissociation at the C—C bond<sup>15</sup>; this places  $D(\text{F}_2\text{C}-\text{CF}_2) < 112$  kcal/mole.

The ultra-violet absorption spectrum of  $\text{CF}_2$  that is observed after flash photolysis is that of its ground state, which is a singlet,  $^1A_1$ . Its decay is slow and the absorption persists for  $> 20$  msec (ref. 12). Evidently,  $\text{CF}_2$  ( $^1A_1$ ) does not rapidly dimerize in the gas phase at room temperature. If  $\text{CF}_2$  is produced from any of the molecules mentioned here, in an atmosphere of oxygen rather than nitrogen, its yield and decay are not sensibly altered; any homogeneous reaction of  $\text{CF}_2$  in its ground electronic state with molecular oxygen cannot be detected at room temperature. Fielding and Pritchard could find no evidence for reaction between  $\text{CF}_2$  and  $\text{O}_2$ ,  $\text{H}_2$ ,  $\text{CO}$ ,  $\text{C}_2\text{H}_4$  or  $\text{C}_2\text{H}_6$  at 250° C (ref. 16). Kinetic data relating to reactions of  $\text{CF}_2$  in the gas phase have been obtained, however, at 530°–750° C. It was found that the pyrolysis of  $\text{CF}_2\text{HCl}$  is inhibited by addition of  $\text{HCl}$  or  $\text{HBr}$ , and that in the latter case  $\text{CF}_2\text{HBr}$  is a product<sup>2,3</sup>. The activation energies for the reactions:



and:



were estimated to be  $\sim 6$  kcal/mole, though in the latter case the uncertainty in the estimate was greater than the estimate itself. However, it cannot be assumed that the dimerization of  $\text{CF}_2$  requires no energy of activation, in view of the very slow decay of  $\text{CF}_2$  after its production in flash photolysis. Of particular note are the Arrhenius factors for the dissociation of  $\text{C}_2\text{F}_4$ , which were found to be  $10^{16.7} \text{ sec}^{-1}$  and 70 kcal/mole.

The slow decay of  $\text{CF}_2$  ( $^1A_1$ ), produced from  $\text{C}_2\text{F}_4$ , indicates that the initial approach of two  $\text{CF}_2$  molecules in their ground singlet states is repulsive. Let us suppose, then, that near the minimum of its ground electronic state  $\text{C}_2\text{F}_4$  is 'attempting' to dissociate into two  $\text{CF}_2$  molecules in their lowest triplet state,  $^3B_1$  (assumed to be non-linear by analogy with  $\text{SO}_2(^3B_1)$ , with which it is iso-electronic<sup>17</sup>). The initial approach of two triplet species would be

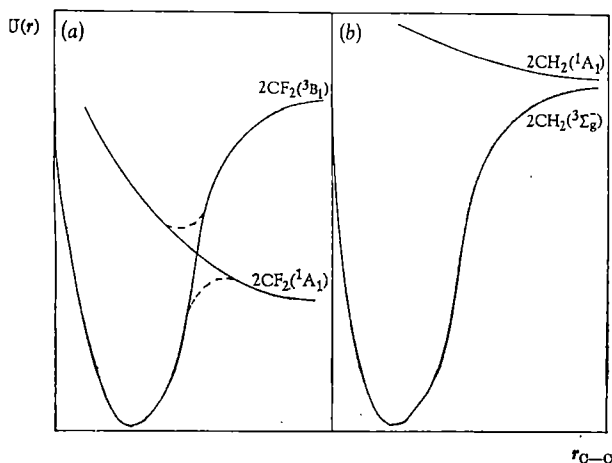


Fig. 1. Potential energy curves for  $C_2F_4$  (a) and  $C_2H_4$  (b)

attractive, and would be represented by a descending potential curve; it would cross the repulsive curve correlating with two normal  $CF_2(^1A_1)$  molecules, since these will lie at a lower energy. The nett result is represented in Fig. 1a, where the potential energies are plotted as a function of the C—C separation (assuming that the remaining bond lengths and valence angles are 'frozen'). In  $C_2H_4$ , the products of dissociation at the C—C bond would be  $CH_2$ , which has a triplet ground state,  $^3\Sigma_g^-$  (ref. 18). As in the case of  $CF_2(^3B_1)$ , these would approach each other along an attractive curve, but one which now cannot cross the curve correlating with  $2CH_2(^1A_1)$ , since this lies at higher energies (see Fig. 1b).

Some consequences of this interpretation can be listed: (i) The dissociation energy of the C—C bond in  $C_2F_4$  will be reduced by an energy approximating to twice the energy of  $CF_2(^3B_1)$  above its ground state. The depth of the perturbed curve representing the ground electronic state of  $C_2F_4$  is  $\sim 70$  kcal/mole below its dissociation limit. Assuming that dissociation to two triplet carbenes requires  $\sim 160$  kcal/mole, as in  $C_2H_4$ ,  $CF_2(^3B_1)$  is estimated to lie  $\sim 45$  kcal/mole above  $CF_2(^1A_1)$ ; (ii) the association of two singlet  $CF_2$  molecules will require an activation energy the magnitude of which will depend on the height of the 'cross-over' point; (iii) the first excited singlet state of  $C_2F_4$  will possess a relatively shallow minimum and a considerably increased equilibrium C—C distance. The longest wave-length ultra-violet absorption of  $C_2F_4$  should be continuous on the basis of the Franck-Condon principle, and lead to dissociation at the C—C bond. In the event of predissociation the primary products would be singlet  $CF_2$  molecules. The ultra-violet absorption is indeed continuous at wave-lengths around  $2000 \text{ \AA}$  (ref. 19), and it is this transition which is excited in the flash photolysis of  $C_2F_4$ ; (iv) the C—C stretching frequency in the ground state of  $C_2F_4$  should show strong anharmonicity—this might be detected if high overtones could be observed in its infra-red spectrum. There is no conflict between the low dissociation energy of the C—C bond and its high stretching frequency, since the former relates to the behaviour of  $C_2F_4$  at its dissociation limit, and the latter to its behaviour around the minimum, where it is unaffected by the perturbation occurring at higher energies; (v) the Arrhenius factors for the dissociation of  $C_2F_4$  should reflect the perturbation of the ground state curve. The high-frequency factor ( $10^{16.7} \text{ sec}^{-1}$ ) is consistent with completely free rotation in the transition state (at the top of the maximum in the ground state curve), as has been proposed in the dissociation of  $N_2O_4$ .

It is of interest to extend the interpretation to the mixed ethylene  $CF_2:CH_2$ . In this case, one may expect the dissociation energy to be reduced by only half the amount in  $C_2F_4$ , since  $CH_2$  has a triplet ground state. The difference between the dissociation energy of  $CF_2:CH_2$  and

$CF_2:CF_2$  should be the energy of  $CF_2(^3B_1)$  above its ground state. The heat of formation of  $CF_2:CH_2$  is  $-77.5$  kcal/mole<sup>4</sup>, and taking  $\Delta H_f(CF_2) \sim -40$  kcal/mole and  $\Delta H_f(CH_2) \sim 86$  kcal/mole,  $D(H_2C-CF_2) \sim 123$  kcal/mole. Subtracting  $D(F_2C-CF_2)$  from this leaves  $\sim 53$  kcal/mole, which is reasonably close to the estimate of  $\sim 45$  kcal/mole for the excitation energy of  $CF_2(^3B_1)$ , considering the approximate values for the numerical data. Flash photolysis of  $CH_2:CF_2$ , using light of wave-length greater than  $1700 \text{ \AA}$ , does indeed result in the transient appearance of  $CF_2$  ultra-violet absorption bands, though with an intensity greatly reduced in comparison with  $C_2F_4$ , consistent with the greater energy required for dissociation.

I thank Prof. A. D. Walsh for his advice.

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## Nuclear Magnetic Resonance Standards for Aqueous Solutions

FOR the nuclear magnetic resonance investigation of interaction in solution, careful consideration must be given to the choice of standard against which changes in chemical shift are to be measured. An external standard is in many ways ideal, but a correction has then to be applied to the observed chemical shift, due to differences in bulk magnetic susceptibilities. The true chemical shift  $\delta$  is given by the equation:

$$\delta = \Delta\nu + g(\chi_{\text{ref}} - \chi_{\text{sol}}) \quad (1)$$

where  $+\Delta\nu$  is the observed upfield shift, in p.p.m., of a line with respect to the reference, and  $\chi_{\text{ref}}$  and  $\chi_{\text{sol}}$  the volume magnetic susceptibilities of the reference and solution being investigated. The value of  $g$  is determined by the shape of the interface of contact between the reference and the solution. Since it is somewhat tedious to measure susceptibilities<sup>2</sup>, many investigators have either ignored any corrections (thereby invalidating the work<sup>3</sup>) or have used internal standards, for which no susceptibility corrections have to be applied.

We used dioxan<sup>4</sup> as an internal standard for an investigation of aqueous resorcinol solutions. It transpired that the effects due to resorcinol-dioxan interactions are much greater than the effect due to resorcinol-water interactions. This highlights the danger of using an internal standard. To avoid interactions involving the reference compound, external standards must be used. The required  $\chi$  values can be determined by the nuclear magnetic resonance method using a stationary pair of coaxial cylindrical tubes<sup>5</sup>. In our hands these tubes proved difficult to make and those that were made had such a large geometrical asymmetry as to give results even less accurate than those previously reported<sup>2</sup>.



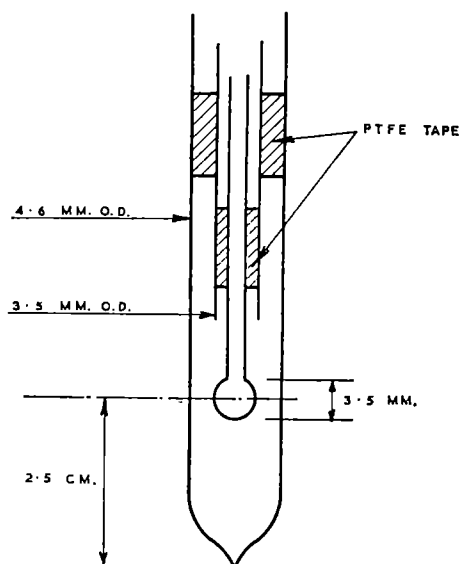


Fig. 1. Sample tube assembly

With cylindrical interfaces,  $g_{\text{cyl}}$  is theoretically  $2\pi/3$ . However, for a spherical contact interface,  $g_{\text{sph}}$  is theoretically zero, in which case susceptibility values would not be required. We made spheres about 3.5 mm in diameter, blown on the end of 1.65 mm (outer diam.) glass tubing. These tubes (Fig. 1) were then mounted, by using PTFE tape, inside tubing (3.5 mm outer diam.) placed inside standard Perkin-Elmer 4.6 mm soda-glass nuclear magnetic resonance tubes containing the system to be examined. The centre of the sphere was arranged to be 2.5 cm above the bottom of the nuclear magnetic resonance tube which, on the Perkin-Elmer R-10 used, brought it in the centre of the detecting coil. Measurements were made on various dioxan-water mixtures using this arrangement. The results were compared with those obtained with the same solutions, but using a cylindrical external standard. The susceptibilities required to correct the latter results were taken from published data<sup>6</sup>.

Table 1. CHEMICAL SHIFTS IN DIOXAN-WATER MIXTURES

% H <sub>2</sub> O by vol	$\Delta\nu_{\text{sph}}$	$\Delta\nu_{\text{cyl}}$	$\chi_{\text{ref}} - \chi_{\text{sol}} \times 10^{-6}$	Calc. cor- rection	$\Delta\nu_{\text{corr}}$	$\Delta\nu_{\text{sph}} - \Delta\nu_{\text{corr}}$	$\Delta\nu_{\text{cyl}} - \Delta\nu_{\text{sph}}$
1.0	1.938	2.172	-0.113	-0.237	1.935	+0.003	0.234
2.5	1.870	2.098	-0.111	-0.231	1.867	+0.003	0.228
5.0	1.587	1.752	-0.106	-0.222	1.587	+0.007	0.215
10.0	1.188	1.387	-0.100	-0.209	1.178	+0.010	0.199
15.0	0.942	1.140	-0.093	-0.195	0.945	-0.003	0.198
20.0	0.780	0.943	-0.086	-0.180	0.763	+0.017	0.163
35.0	0.520	0.648	-0.065	-0.136	0.512	+0.008	0.128
50.0	0.291	0.383	-0.047	-0.098	0.285	+0.006	0.092
65.0	0.170	0.222	-0.031	-0.065	0.157	+0.013	0.052
80.0	0.068	0.100	-0.017	-0.036	0.064	+0.004	0.032
90.0	0.030	0.042	-0.008	-0.017	0.025	+0.005	0.012

The recorded shifts are of the water line with reference to (external) water, in p.p.m. at 60 Mc/s.

The two sets of results (Table 1) are, within our experimental errors, identical. This can also be seen as follows. Combining two equations of the form of (1) leads to equation (2):

$$\Delta\nu_{\text{cyl}} - \Delta\nu_{\text{sph}} = (g_{\text{cyl}} - g_{\text{sph}})(\chi_{\text{sol}} - \chi_{\text{ref}}) \quad (2)$$

A plot of  $\Delta\nu_{\text{cyl}} - \Delta\nu_{\text{sph}}$  against  $\chi_{\text{ref}} - \chi_{\text{sol}}$  should therefore give a straight line passing through zero, of slope  $g_{\text{cyl}} - g_{\text{sph}}$ , or  $\frac{2}{3}\pi - 0 = 2.095$ .

The data in the table lead to such a line with a least means square slope of 2.080 (standard deviation 0.041). This either means that  $g_{\text{cyl}}$  is less than  $2\pi/3$  or that  $g_{\text{sph}}$  is 0.015. At the worst, for a susceptibility difference of  $0.3 \times 10^{-6}$  (and by careful choice of reference compound a much smaller value will ordinarily be possible), then the error introduced by assuming  $g_{\text{sph}} = 0$  should be less than 0.0045 p.p.m., 0.5 c/s at 60 Mc/s.

Although some loss of resolution occurs the present results demonstrate the usefulness of a spherically shaped external standard<sup>7</sup>.

Finally, it should be noted that, at the low-water concentration region, the data presented here differs from that previously given for dioxan-water mixtures<sup>6</sup>. This is due to an error in the concentrations in the earlier work<sup>8</sup>.

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## Unpaired Electrons in Ultra-violet-irradiated Keratin

RECENT work on the electron spin resonance signals observed in ultra-violet-irradiated proteins<sup>1</sup> has stimulated interest in the theory that proteins may exhibit conduction band properties. In experiments with ultra-violet irradiated proteins and amino-acids carried out in this laboratory, results have been obtained which in a particular instance may support such a theory.

Several amino-acids and the proteins gelatin, insulin, casein, silk fibroin, egg albumin and unpigmented keratin were evacuated and sealed in quartz sample tubes. The samples were then irradiated by narrow wave-length bands of monochromated ultra-violet radiation in the spectral range 250–600 mμ to doses of the order of  $10^{20}$  quanta. An X-band electron spin resonance spectrometer, using 110-kc/s magnetic field modulation, was used to detect any free radicals present in the samples before and after irradiation. The keratin samples were the only samples to exhibit electron spin resonance signals before the irradiation treatments. Such a signal (Fig. 1) has previously been observed with wool<sup>2</sup>, and in the specimens examined here the magnitude of the electron spin resonance signal indicated a free radical concentration of the order of  $10^{13}$  per g.

The results observed following the irradiations were generally in accordance with the energy absorptions of the samples. For example, radiation bands of wave-length less than 325 mμ, which are absorbed by chromophoric groups known to be present in proteins<sup>3</sup>, produced significant electron spin resonance spectra, indicating that free radicals, stable in vacuum, were formed as a result of the irradiation-induced photolytic reactions<sup>4,5</sup>. Radiations of wave-lengths greater than 325 mμ, which are not absorbed by any known protein chromophores, produced no significant electron spin resonance signal in any of the proteins with the exception of the keratin samples.

In the case of the unpigmented keratins (various types of wool and rhinoceros horn) a strong electron spin resonance signal (approximately  $10^{14}$  spins/g) was observed



Fig. 1. Electron spin resonance spectrum of keratin before irradiation



Fig. 2. Electron spin resonance spectrum of keratin after irradiation at 360-400 mμ

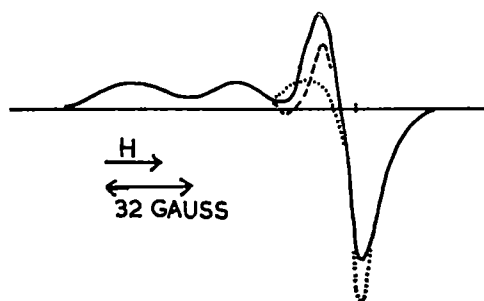


Fig. 3. Electron spin resonance spectrum of keratin irradiated at 300-325 mμ. —, Original spectrum; ---, 5 min aeration; ..., 1 h aeration

After irradiation at the wave-length intervals of 325-360, 360-400 and 400-600 mμ. This electron spin resonance signal (Fig. 2) was quite different from that produced in keratin by irradiation at shorter wave-lengths (Fig. 3). Furthermore, the electron spin resonance signal was stable in air and could be produced in the presence of air at a rate comparable with its production in vacuum, whereas the electron spin resonance signal produced by radiation at shorter wave-lengths decreased on the admittance of air to the sample (Fig. 3) due to radical decay. These results clearly indicate that the radicals produced by long ultra-violet and visible radiation are quite different from those produced by radiation of wave-lengths of less than 325 mμ. The similarity between spectra of the keratin samples before irradiation, and after irradiation at longer wave-lengths, suggests that the signal observed before irradiation (Fig. 1) may have been the result of exposure to radiation during growth.

The electron spin resonance signals observed following the exposure of keratin to long ultra-violet and visible radiation is very similar, in stability to oxygen, line width, line shape and  $G$  value, to the electron spin resonance spectra of melanin and certain carbon samples where unpaired electrons are considered to be highly mobile in some form of conduction band<sup>1</sup>. This suggests that the electron spin resonance spectrum of irradiated keratin is generated by the excitation of electrons into a conduction band in which they are highly mobile. This conduction band may be associated either with the protein molecule or with some non-protein impurity present in the keratin. If the former is the case, then the structure of the  $\alpha$ -keratin must play an important part, since no comparable electron spin resonance signal was observed for silk fibroin—a protein the molecular structure of which could lend itself to conduction band properties to the same extent as  $\alpha$ -keratin<sup>6,7</sup>. Alternatively, the conduction band could be associated with some non-protein impurity in the form of either melanin pigmentation (in minute quantities since the keratin samples appeared to be unpigmented) or some unpigmented precursor to melanin. The fact that the only protein investigated which exhibited the conduc-

tion band properties, namely keratin, was also the only protein of this group likely to be associated with melanin pigmentation leads us to believe that the second alternative is the more likely explanation.

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### Phase Transformation of Thin Caesium Chloride Layers

In connexion with investigations on the structural properties of thin ionic crystal layers, the phase transformation of caesium chloride was examined by means of electron diffraction. The specimens (Merck p.a.) were prepared in a Balzers 350-G vacuum device at  $2 \times 10^{-5}$  torr by evaporation from a molybdenum boat, and then investigated in a hot-stage electron diffraction specimen holder of a Hitachi HU-10A electron microscope operated at 75 kV. In order to prevent sublimation during heating the caesium chloride crystal layers were coated, in the vacuum evaporator, with a thin carbon film. The contrast of the diffraction patterns was only slightly affected by this procedure. The layer thicknesses expected<sup>1</sup> were 20 Å and 300 Å, respectively. The  $L\lambda$  values ( $L$  is the tube length and  $\lambda$  is the electron wave-length) were determined with a thallium chloride standard<sup>2</sup>.

According to the experimental results, the (200), (220) and (420) rings of the  $\beta$ -phase appeared at 703° K. However, the layer structure remained mixed at this temperature since a major part of the rings of the  $\alpha$ -phase was also observed. Above 723° K only the  $\beta$ -phase remained ((200), (220), (311) and (420) rings). The temperature values may not be exact, as allowance should be made for the heating effect of the electron beam.

Fig. 1 shows a typical example for the  $\alpha$ -phase at room temperature and the  $\beta$ -phase above 723° K.

Once the  $\beta$ -phase developed it remained very stable and could be brought down to room temperature. The first rings of the  $\alpha$ -phase ((211), (221), (310) and (400)) only appeared after several hours at room temperature (Fig. 2). The  $\beta$ -phase of the layers with an expected thickness of

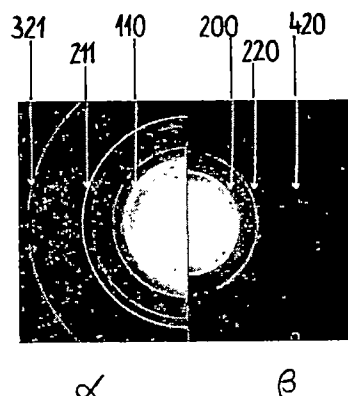


Fig. 1

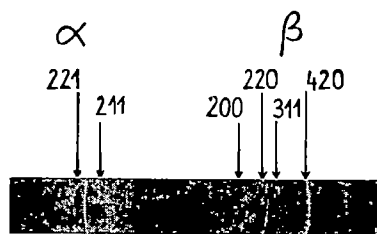


Fig. 2

20 Å proved to be quite stable, whereas thicker layers (300 Å), when cooled to room temperature, readily transformed into the  $\alpha$ -phase.

The lattice constant as determined from the (211) ring of the  $\alpha$ -phase at room temperature was  $4.09 \pm 0.05$  Å, and for the  $\beta$ -phase, as determined by the intensive (200) ring at 723° K,  $7.00 \pm 0.06$  Å. The value of the lattice constant showed an expansion with the temperature.

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### Crystal Structure of Monomethyltriethyl Titanate

In solution most of the titanium orthoesters have been shown to be associated into trimers<sup>1-3</sup>, and a structure has been proposed for the trimeric aggregate<sup>1</sup>. There has been considerable interest in the structure of this aggregate<sup>3-5</sup>. In 1963 Ibers<sup>6</sup> reported a preliminary crystal structure of tetraethyl titanate showing this to be tetrameric, not trimeric, in the solid state. We have now determined the main features of monomethyltriethyl titanate and have found it also to be tetrameric in the solid state. However, large thermal motion and the limited amount of data for the large number of parameters in the cell have prevented our locating all the carbon atoms or obtaining precise bond distances and angles. Low-temperature data are now being collected to complete the structure determination.

Intensity data were collected by the multiple film technique using a Supper Weissenberg camera. 751 non-equivalent reflexions were observed on levels zero through six. Diffraction data showed that the crystal belonged to the triclinic system and gave the following cell parameters:  $a = 12.13$  Å,  $b = 12.14$  Å,  $c = 16.93$  Å,  $\alpha = 76^\circ 56'$ ,  $\beta = 77^\circ 2'$ , and  $\gamma = 74^\circ 15'$ . With eight monomer units/unit cell, the calculated density is 1.236 g/c.c.; the measured density is 1.244 g/c.c.

From a three-dimensional Patterson map the eight titanium atoms were located, showing the molecules to be arranged in tetramer units. The titanium atoms in each tetramer form a planar diamond-shaped pattern, both tetramers lying parallel to the  $ab$  crystal plane, and displaced from each other one-half a unit cell edge along the  $c$  axis, with corresponding titanium atoms in each unit having the same  $x$  and  $y$  co-ordinates. In the early stages of the structure determination it was assumed that the crystal was centrosymmetric. With the titanium atoms thus located, an electron density map was calculated. Due to this arrangement of the titanium atoms, twice the number of expected oxygen peaks appeared on the map since at this point there was nothing to distinguish between the two titanium tetramers. With the restriction

that the co-ordination number of titanium be six, only one chemically reasonable structure could be found, and this was essentially the same as that reported by Ibers<sup>6</sup> for the tetraethyl titanate.

Once the oxygen positions were included the ambiguity between the two tetramer units was removed, and the subsequent electron density maps were normal. Several carbon atoms were located from electron density maps, from minimum function maps, and from difference maps. Fig. 1 indicates the general structure of the tetramer unit.

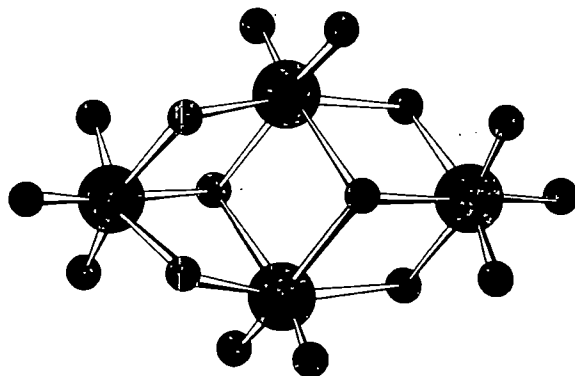


Fig. 1. Structure of  $\text{Ti}(\text{OCH}_3)(\text{OC}_2\text{H}_5)_3$  tetramer. Large circles are titanium atoms; small circles are oxygen atoms; carbons not shown

With only titanium atoms located, the usual discrepancy index was 0.585; when all oxygens were included, the index dropped to 0.32. Refinement was continued using a block-diagonal matrix least squares programme written by van der Helm<sup>7</sup> and modified by George Svetich<sup>8</sup> for card input for the IBM 1620 computer. With half the carbon atoms included, refinement stopped at a discrepancy index of 0.23. Up to this point it had been assumed that the crystal belonged to the centric space group  $P\bar{1}$ .

Consideration of the possible structure of the crystal suggested that even if the titanium and oxygen atoms in the tetramer unit were related by a centre of symmetry, there was no reason to expect the structure to be centric with respect to the carbon atoms. With 46 carbon atoms included a cycle of a full matrix least squares was run on the IBM 709 at the University of Washington. The discrepancy index dropped to 0.136, assuming the crystal to belong to the space group  $P1$ . Many bond distances and angles appeared to be improbable, and temperature factors of some of the carbons ranged from 12 to 19. The remaining ten carbon atoms could not be found. Undoubtedly this inability to complete the structure determination is due to the limited amount of data. With 86 atoms, each with isotropic temperature factors, the number of parameters to be adjusted is 344. The number of observed reflexions used was 746, giving an over-determination of only 2.17.

Although the complete structure was not determined, the following essential features were found. (1) The unit cell is occupied by two tetramer units which are parallel to the  $ab$  plane. The second tetramer may be generated, approximately, from the first by 180° rotation of the first about an axis perpendicular to the  $ab$  plane and passing through the centre of the tetramer, followed by translation one-half the unit cell length along the  $c$  axis. (2) Each tetramer unit is very nearly centrosymmetric, at least with respect to the titanium and oxygen atoms. (3) The oxygen atoms lie approximately in planes above and below the plane of the titanium atoms. (4) There are three types of oxygen atoms: (a) those associated with only one titanium atom; (b) those associated with two titanium atoms; and (c) those associated with three titanium atoms. (5) Titanium-oxygen bond distances vary from about

1.6 Å to 2.4 Å. Generally, the greater the number of titanium atoms associated with an oxygen atom, the longer are the bonds. (6) The distances between adjacent titanium atoms vary from 3.3 Å to 3.5 Å. (7) In each case the co-ordination number of titanium is six. Fig. 1 shows the structure of the tetramer unit without the carbons.

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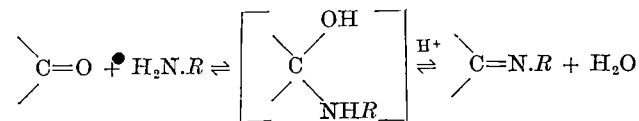
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### Use of 2,4-Dinitrophenylhydrazine for the Estimation of Micro Amounts of Carbonyls

THE use of 2,4-dinitrophenyl hydrazine (2,4-DNP hydrazine) in acid solution as a reagent for the estimation of methyl ketones and other carbonyls is common despite the fact that the reaction is apparently non-quantitative<sup>1-3</sup>. Between 20 and 90 per cent recoveries of methyl ketones from aqueous solution have been reported<sup>4,5</sup>. Wong, Patton and Forss<sup>4</sup> attributed the low recovery of 2-pentanone (25 per cent) from whole milk by steam distillation under reduced pressure to the non-completion of the reaction with the reagent. Haverkamp Begemann and de Jong<sup>5</sup> concluded that the method of shaking had a marked influence on the yield of 2,4-DNP hydrazone, and by use of a 'Celite'/2,4-DNP hydrazine column to ensure more intimate contact between the reagent and the carbonyl compound they achieved practically complete conversion into the 2,4-DNP hydrazone.

In a recent investigation in this laboratory of the oxidation of fatty acids to methyl ketones by the fungus *Penicillium roqueforti* a rapid and accurate method of estimating the methyl ketones formed was required. An investigation was therefore made of the causes of the low recoveries of methyl ketones as their 2,4-DNP hydrazones, in an attempt to devise a method which would be accurate and also less time-consuming than the column method of Haverkamp Begemann and de Jong<sup>5</sup>.

Jencks<sup>6</sup> suggested that in strongly acid solution there is an initial reversible addition of phenylhydrazine, and presumably therefore of 2,4-DNP hydrazine, to a carbonyl compound to give an intermediate that undergoes acid-catalysed dehydration:



In acid solution the concentration of free phenylhydrazine is so low that the first step is considered to be rate-controlling.

In preliminary experiments, 2,4-DNP hydrazones of methyl ketones dissolved in 2 N HCl were found to dissociate slowly in an open vessel, the foregoing equilibrium presumably moving to the left with time as a result of evaporation of the volatile ketones. The extreme insolubility of the 2,4-DNP hydrazones of methyl ketones above

Table 1. RECOVERIES OF METHYL KETONES (0.75 μMOLE) AS THE 2,4-DNP HYDRAZONES FROM STOPPERED TEST-TUBES AND FROM OPEN BEAKERS

Stoppered test-tubes	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>7</sub>	C <sub>8</sub>	C <sub>9</sub>	C <sub>10</sub>	C <sub>11</sub>
30 min	0.73	0.73	0.72	0.75	0.75	0.73	0.74	0.75	0.73
20 h	0.73	0.71	0.72	0.75	0.75	0.73	0.74	0.75	0.75
Beakers									
30 min	0.38	0.45	0.54	0.59	0.58	0.51	0.61	0.68	0.68
2 h	0.17	0.24	0.48	0.51	0.48	0.47	0.54	0.64	0.66
3 h	0.09	0.12	0.45	0.47	0.42	0.43	0.50	0.63	0.66
5 h	Nil	Nil	0.23	0.25	0.23	0.28	0.34	0.53	0.58
20 h	Nil	Nil	0.23	0.25	0.23	0.28	0.34	0.53	0.58
% decrease	100	100	68	66	70	62	54	30	23

1 ml. standard ketone solution added to 2 ml. 2,4-DNP hydrazine reagent

Table 2. RECOVERIES OF n-SATURATED ALDEHYDES (0.75 μMOLE) AS THE 2,4-DNP HYDRAZONES FROM STOPPERED TEST-TUBES AND FROM OPEN BEAKERS

Stoppered test-tube	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>7</sub>	C <sub>10</sub>
15 min	0.75	0.74	0.75	0.75	0.74
20 h	0.75	0.73	0.75	0.75	0.75
Beakers					
5 min	0.57	0.54	0.64	0.68	0.68
2 h	0.37	0.34	0.60	0.65	0.68
20 h	0.15	0.15	0.47	0.50	0.68
% decrease	80	79	37	21	8

1 ml. standard aldehyde solution added to 2 ml. 2,4-DNP hydrazine reagent.

C<sub>6</sub> in 2 N HCl, however, prevented a detailed investigation. As an alternative approach the 2,4-DNP hydrazones were prepared *in situ* by adding 1 ml. aliquots of standard ketone solutions (0.75 μmoles/ml.) to 2 ml. 2,4-DNP hydrazine reagent (2 g/l. 2 N HCl) both in a series of 50-ml. beakers and in stoppered 25-ml. test-tubes. At intervals the 2,4-DNP hydrazones were extracted with 20 ml. hexane from one of each of the vessels as detailed below. The yield of 2,4-DNP hydrazones from the beakers slowly decreased with time, with the greatest decrease in the 2,4-DNP hydrazones of the lower methyl ketones (Table 1). When the stoppered test-tubes were used the recovery of the ketones as their 2,4-DNP hydrazones was virtually quantitative.

Similar investigations showed that n-saturated aldehydes formed more stable 2,4-DNP hydrazones than the corresponding methyl ketones. This was presumably a reflexion of their greater relative additive power with 2,4-DNP hydrazine and, by analogy with the equilibrium constants for the formation of the bisulphite addition compounds from a number of aldehydes and ketones<sup>7</sup>, may be attributed to both steric and electronic factors. The 2,4-DNP hydrazones of the most volatile aldehydes (C<sub>2</sub>-C<sub>4</sub>) were the least stable and those of the higher aldehydes (C<sub>7</sub> and C<sub>10</sub>) the most stable (Table 2). As with the methyl ketones the greatest loss of aldehyde in the open beakers occurred in the first 15 min.

Since it was probable that the low recoveries obtained by other investigators were a result of the volatility of the carbonyls in aqueous solution, a method of estimation was developed which reduced the number of operations leading to the final determination of the 2,4-DNP hydrazones in a spectrophotometer. 1 ml. of the aqueous ketone solution was allowed to stand for 30 min with 2 ml. of 2,4-DNP hydrazine in a stoppered test-tube, 20 ml. of carbonyl-free hexane, prepared according to the method of Schwartz and Parks<sup>8</sup>, were added and the mixture was vigorously shaken. The aqueous and hexane layers were allowed to settle, the upper hexane layer, which contained the 2,4-DNP hydrazone, was poured directly into a Beckman cell and its absorption read at 345 mμ in a Beckman DU spectrophotometer. A 'blank', prepared by shaking 2 ml. of 2,4-DNP hydrazine reagent with 1 ml. of water and 20 ml. carbonyl-free hexane, corrected for the slight solubility of the reagent in the hexane. The concentration of the methyl ketones could then be obtained directly from standard curves relating absorption at 345 mμ to the concentration of 2,4-DNP hydrazone. The recovery of up to 5.0 μmoles/ml. of the higher (C<sub>6</sub>-C<sub>11</sub>) ketones as their 2,4-DNP hydrazones by this method was quantitative. The 2,4-DNP hydrazones of the C<sub>3</sub>-C<sub>5</sub> methyl ketones are less soluble in hexane than those of



the higher methyl ketones, but 3  $\mu$ moles were extracted quantitatively by 20 ml. hexane.

It seems possible that part of the variation and low yields obtained by some investigators<sup>4,9</sup> when distilling carbonyls directly into 2,4-DNP hydrazine reagent results from both the volatility of the carbonyls and the instability of the carbonyl-reagent adduct. When 1 ml. of standard carbonyl solution was added to 2 ml. of reagent and a vacuum applied by means of a water pump, a similar pattern of recovery with time resulted as with the open beakers, despite the much lower surface-to-volume ratio in the vacuum trials.

This investigation has shown that although the 2,4-DNP hydrazones of volatile carbonyls in acid solution are not stable in open systems, under closed conditions the reaction of 2,4-DNP hydrazine with carbonyls up to C<sub>11</sub> is quantitative and rapid, shaking not being necessary. Contrary to another report<sup>10</sup> the reaction was found to be complete within 30 min.

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### Thermal Decomposition of Irradiated Calcium Azide

THE investigations of Andreev<sup>1</sup> and Marke<sup>2</sup> of the isothermal decomposition of calcium azide showed that the decomposition comprised a marked induction period followed by a period of acceleration where the exponential relationship:

$$\log p = k_1 t + c_1 \quad (1)$$

fitted to a limited extent. The decay period of the decomposition was considered to follow the unimolecular decay law:

$$\log (p_f - p) = k_2 t + c_2 \quad (2)$$

where  $p_f$  is the final pressure.

The degree of reproducibility of the pressure-time plots for whole and ground crystals was poor. Marke found no pronounced change in  $k_1$  on grinding the crystals and determined approximate activation energies of 18 kcal mole<sup>-1</sup> and 19 kcal mole<sup>-1</sup> for the acceleratory and decay periods, respectively. Andreev's value for the acceleratory period was 22.8 kcal mole<sup>-1</sup>.

In contradiction, Garner and Reeves<sup>3</sup> concluded that the acceleratory period followed the power law:

$$p^{1/3} = k_3 t + c_3 \quad (3)$$

Reproducibility of results again was not good. A four-fold increase in  $k_3$  was found on grinding the crystals, and exposure to ultra-violet light in air accelerated the reaction and shortened the induction period. Equation (3) still fitted the pressure-time plots for irradiated material. The average activation energy for the decomposition of unirradiated salt during the acceleratory period was 18.2 kcal mole<sup>-1</sup>. It was of interest to determine whether: (1) the reproducibility of the decomposition could be improved; (2) reliable activation energies could be obtained; (3) the uncertainty relating to the correct kinetic expressions for the decomposition could be

resolved. In addition, pre-irradiation with  $\gamma$ - and X-rays could be expected to accelerate the decomposition as was found with barium<sup>4</sup> and strontium<sup>5</sup> azides.

Fig. 1 shows the pressure-time plots and the corresponding mathematical analysis for the decomposition of unirradiated, ground, calcium azide prepared by the method of Marke using materials of high purity. The plots for ground and whole crystal agglomerates were practically identical at a given temperature. Reproducibility of results was excellent. The acceleratory period conformed to the power expression, equation (3), and the decay reaction to the contracting sphere formula:

$$[1 - (1 - p/p_f)^{1/3}] = k_4 t + c_4 \quad (4)$$

The plots of  $\log k_3$  and  $\log k_4$  versus  $1/T(^{\circ}\text{K})$  were good straight lines and the activation energies (ground material) for the acceleratory and decay periods were 27.1 and 18.8 kcal mole<sup>-1</sup>, respectively. The activation energy for processes occurring during the induction periods, as determined from the plot of  $\log$  (induction period) versus  $1/T(^{\circ}\text{K})$ , was 18.2 kcal mole<sup>-1</sup>.

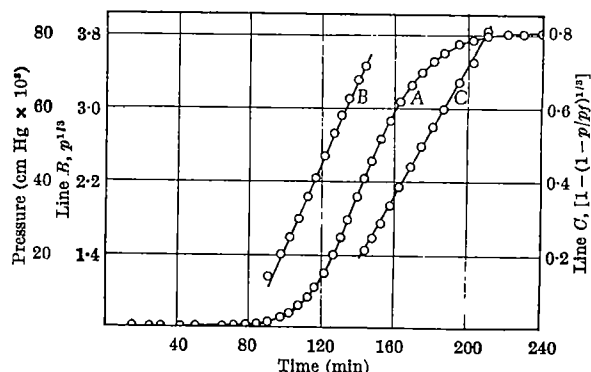


Fig. 1. Curve A, pressure-time plot for decomposition of unirradiated calcium azide at 115°C; line B,  $p^{1/3}$  versus  $t$ ; line C,  $[1 - (1 - p/p_f)^{1/3}]$  versus  $t$ .

Irradiation of calcium azide in air is unsatisfactory since any photolysis or radiolysis on the surfaces of the crystals to give nuclei of, say, calcium or calcium nitride will be accompanied by oxidation processes. Consequently, all irradiation and subsequent handling were done in an atmosphere of nitrogen. The effects of pre-irradiation by X-rays (copper target, unfiltered beam 40 kV and 20 m.amp), ultra-violet light, and  $\gamma$ -rays ( $\sim 1$  MeV) on the subsequent thermal decomposition are shown in Fig. 2. Equations (3) and (4) fitted the pressure-time plots for the decomposition of X-ray, ultra-violet and  $\gamma$ -ray irradiated calcium azide. Activation energies for the decomposition of  $\gamma$ -ray irradiated (8 Mrads) azide were 19.8, 28.1 and 22.5 kcal mole<sup>-1</sup> for the induction, acceleratory and decay periods, respectively.

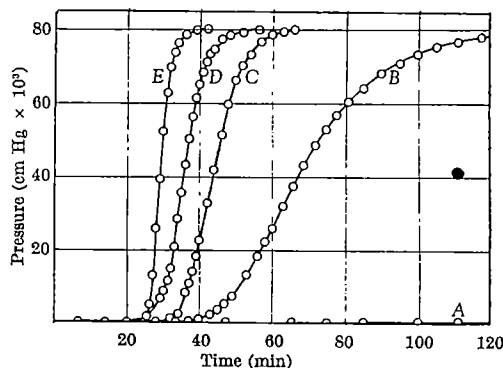


Fig. 2. Pressure-time plots showing the effect of pre-irradiation on thermal decomposition of calcium azide at 100°C. Curve A, unirradiated (induction period ends at 200 min); curve B, pre-irradiated by ultra-violet light for 15 h; curves C and D, pre-irradiated by  $\gamma$ -rays with doses of 0.25 Mrad and 8 Mrads respectively; curve E, pre-irradiated by X-rays for 8 h.

Electrical conductivity measurements of irradiated and unirradiated azide are being made and these and further observations and conclusions arising from this study will be published elsewhere.

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## BIOCHEMISTRY

### Conformational Investigations of Histones

WE have carried out some investigations into the conformations of whole histones and histone fractions under various conditions. The experiments on repression of DNA-dependent RNA synthesis with histones<sup>1,2</sup>, native nucleohistone<sup>3</sup>, and DNA combined with histone fractions<sup>4-10</sup> have led to a renewed interest in the hypothesis of Stedman and Stedman<sup>11</sup> that one of the functions of histones is the regulation of genetic activity. However, the results of the various experiments on the repression of DNA-dependent RNA synthesis are not in agreement with one another, and the inhibitory capacities of the various histone fractions appear to depend on the method of preparation of the recombined nucleohistone, particularly the relative proportions of histone and DNA used for recombination, and perhaps even on the preparation of the histone fractions themselves. These apparently conflicting results indicate the need to investigate the conformations of the histone fractions, prepared by different methods, under conditions used to reconstitute the nucleohistones.

The histones and histone fractions examined were prepared from calf thymus tissue at two laboratories: in the Chester Beatty Research Institute using methods described by Phillips and Johns<sup>12</sup> and Johns<sup>13</sup>, and at the Department of Chemistry, Stanford University, California, according to the procedures given by Satake, Rasmussen and Luck<sup>14</sup>, and also by Murray<sup>15</sup>. In all cases the final product was a histone in the form of a dry powder.

The optical rotations of whole histones and histone fractions have been measured at nine wave-lengths in the range 5780–2967 Å with the spectropolarimeter designed by Malcolm and Elliott<sup>16</sup>, and the rotations were analysed according to Moffitt's equation<sup>17</sup>. The value used for  $\lambda_0$  was 2120 Å since this was found to give the best linear plot of the results in the foregoing wave-length range. In addition to the optical rotatory dispersion measurements, infra-red spectra of insoluble fractions and insoluble portions of fractions were recorded on a Grubb Parsons 'Spectromaster'. Some samples prepared according to the procedure described by Satake, Rasmussen and Luck<sup>14</sup>, which involved freeze-drying, were incompletely soluble in the various solvents. Their infra-red spectra, particularly those of the insoluble residues, showed, in addition to the bands characteristic of helical and random forms, bands at 1,686 cm<sup>-1</sup> ( $\nu$ ), about 1,630 cm<sup>-1</sup> ( $\nu$ ) and about

1,525 cm<sup>-1</sup> ( $\nu$ ). These bands indicate the presence of large amounts of the extended antiparallel  $\beta$ -conformations in these preparations. Elimination of freeze-drying from the preparative procedure resulted in samples which were completely soluble in the various solvents, and their infra-red spectra showed the absence of  $\beta$ -material. It is essential to avoid freeze-drying of histone fractions if they are to be used for physical investigations.

Table 1 shows the values of  $b_0$  obtained for the different preparations of whole histone and histone fractions in water and in aqueous sodium chloride solutions. Also given are values obtained in 2-chlorethanol, a solvent known to favour formation of helices in proteins. Four preparations of histone fractions prepared at the Chester Beatty Research Institute and two preparations from Stanford University were examined. Histone fractions prepared at the former institute are designated F1, F2a, F2b and F3, while similar, though not necessarily identical, histone fractions from Stanford University are designated Ib, Ila, IIb and (III and IV). Assuming that a  $b_0$  value of -630 represents a fully helical protein, it is seen that in 2-chlorethanol the histone fractions have an approximate helical content of about 46 per cent for fractions F1 and Ib, and 65–70 per cent for F2a, F2b, F3, IIb and (III and IV). However, in aqueous solutions the histone fractions are largely in a random conformation, and in the case of fractions F1 and Ib completely so. The addition of salt to these aqueous solutions causes a small increase in helix content, presumably due to a decrease in the repulsion between like-charged side-chains. Measurements were made in salt solution up to 3 M, though a salt molarity of 1.0 was found to be sufficient to produce this increase in helix content of about 10–20 per cent. The low helix content of the histone fractions in salt solutions contrasts with the estimates of 50–60 per cent<sup>18,19</sup> for the helix content of whole histone in native nucleohistones, though it must be emphasized that these estimates need to be confirmed by other techniques. The results presented here suggest that the histone fractions which have been used in the preparation of reconstituted nucleohistone were mainly in a random conformation; whether or not their interaction with DNA in the reconstitution experiments causes the histones to assume their native conformation remains to be determined.

Another noteworthy feature of the results is that histone fractions F1 and Ib are significantly less helical than the other fractions both in 2-chlorethanol and in salt solutions, and this may be correlated with the high proline content (about 9 per cent) of these fractions. In addition, Johns and Butler<sup>20</sup> have shown that in recombination experiments with DNA, histone fraction F1 is appreciably more efficient in precipitating DNA from solution than are the other histone fractions, and Akinrimisi *et al.* have found that the various histone fractions differ in their binding affinity for DNA (ref. 21). It is possible, therefore, that fraction F1 or Ib plays a somewhat different part in nucleohistone than do the other fractions. Bonner and Huang<sup>5</sup> have suggested that the role of the histone fraction Ib is primarily to suppress genetic activity. However, experiments by Hindley<sup>22</sup>, by Murray<sup>15</sup> and by Huang *et al.*<sup>23</sup> on the selective removal of histones from nucleohistone show that fraction Ib is the most easily removed and its removal causes only a small increase in the priming of RNA synthesis by the residual nucleohistone, whereas removal of the fractions of higher arginine content causes a much more marked

Table 1.  $b_0$  VALUES FOR HISTONE FRACTIONS

Solvent	Whole histone	F1	Ib	F2a	F2b	IIb	F3	(III and IV)
Water	-40	0	-5	-47	-28	-100	-70	-93
0.25 M sodium chloride	-118	-43	-28	-112	-127	-133	-135	
0.5 M sodium chloride	-133	-71	-46	-110	-147	-162	-145	-122
1.0 M sodium chloride	-147	-71	-67		-180	-180	-155	-133
2-chlorethanol	-401	-291	-295	-419	-444	-415	-408	-351

increase in the priming of RNA synthesis. These results raise the possibility that the role of histone fraction Ib in native nucleohistone may be a structural one rather than that of a suppressor of genetic activity. In conclusion, we would stress the need for determination of the histone/DNA ratio of nucleohistone complexes (whether reconstituted nucleohistone, or residual nucleoprotein remaining after partial removal of histones) used as primers for DNA dependent nucleic acids synthesis.

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### Amino-acid Composition and Partial Specific Volume of Citrate Oxaloacetate Lyase

STUDIES of the amino-acid composition of citrate oxaloacetate lyase (citrate lyase) from *Aerobacter aerogenes* were made using the pure enzyme prepared by methods previously described<sup>1,2</sup>. The amino-acid composition of the enzyme was determined using the technique of high-voltage electrophoresis to separate the amino-acids in a protein hydrolysate<sup>3</sup>. Hydrolysates of two quantities of enzyme (5.0 mg), by 'AnalaR' hydrochloric acid (6 N) in

Table 1. COLOUR YIELD OF THE AMINO-ACIDS ON A MOLAR BASIS BY THE CADMIUM NINHYDRIN METHOD

Isoleucine has been given a reference value of 100. Absorbancies of solutions were measured at 505 mμ.

Amino-acid	% Colour yield
Alanine	140
Arginine	101
Aspartic acid	52
Cystic acid	98
Glutamic acid	128
Glycine	41
Isoleucine	100
Histidine	84
Leucine	136
Lysine	97
Methionine	96
Methionine sulphone	101
Phenylalanine	75
Serine	118
Threonine	96
Tryptophan	59
Tyrosine	90
Valine	104

Table 2. AMINO-ACID COMPOSITION OF CITRATE LYASE

Amino-acid residue	g %	Moles %	Calculated No. of residues per 315,000 molecular weight
Alanine	5.80	8.12	204
Arginine*	7.79	5.60	141
Aspartic acid	11.50	10.85	273
Cystine	0.99	0.50	13
Glutamic acid	11.01	9.36	236
Glycine	7.43	12.41	312
Histidine	1.44	1.16	29
Isoleucine	7.32	7.00	176
Leucine	8.84	8.45	213
Lysine	4.07	3.50	88
Methionine	1.84	1.53	39
Phenylalanine	5.06	3.86	97
Proline†	2.87	3.12	79
Serine*	4.81	5.72	144
Threonine*	6.23	6.54	165
Tryptophan‡	1.05	0.84	16
Tyrosine§	2.88	2.00	50
Valine	9.00	9.61	242

\* Values taken from extrapolation to zero time of hydrolysis.

† From estimation by cadmium isatin reagent<sup>4</sup>.

‡ From spectrophotometric data and estimations after hydrolysis.

§ Value taken from the 24-h hydrolysis.

sealed tubes at 110° for 24 h and 72 h, respectively, were prepared. The protein in the solution to be hydrolysed was estimated by the method of Hoch and Vallee<sup>4</sup>. The method of amino-acid separation and analysis was similar to that used by Atfield and Morris<sup>5</sup>, using a cadmium ninhydrin reagent. The apparatus was based on the general design of Gross<sup>6</sup>, with longer coiling plates, as favoured by Atfield and Morris<sup>5</sup>. The concentration of each amino-acid, after elution from the strip, was read from concentration-absorbancy graphs previously constructed using similarly treated standard solutions of amino-acids. Absorbancies were read on a Unicam SP 600 spectrophotometer at 505 mμ. The relative colour values of the cadmium ninhydrin complexes with individual amino-acids on a molar basis are given in Table 1; they show wide variations (cf. Helimann *et al.*<sup>7</sup>). Proline was estimated in the hydrolysate, and hydroxyproline shown to be absent, by estimation with cadmium isatin reagent.

From analyses of the two hydrolysates corrections were made for losses of some amino-acids (serine, threonine, arginine) on longer hydrolysis, while valine and isoleucine were incompletely released in the 24-h hydrolysis<sup>8</sup>. Tryptophan was only present in the longer hydrolysis but here showed good agreement (+ 9 per cent) with a spectrophotometric estimation by the method of Goodwin and Morton<sup>9</sup> using the formula derived by Beaven and Holiday<sup>10</sup> (cf. Hotchkiss<sup>11</sup>). The recovery of protein on the basis of amino-acids recovered was 102 per cent and 106 per cent, respectively in 24-h and 72-h hydrolysates. By the methods used no distinction could be made between cysteine and cystine, glutamic acid and glutamine, or between aspartic acid and asparagine. The amino-acid composition of citrate lyase thus determined is given in Table 2. The enzyme contains all the amino-acids commonly found in proteins and no unidentified compounds were evident. The partial specific volume,  $\bar{v}$ ,

of the protein calculated from this amino-acid composition<sup>13</sup> was 0.735, a value in good agreement with that assumed for molecular weight calculations in an earlier paper<sup>2</sup>.

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### Apparent Paradox in the Effects of Ethionine feeding on the Specific Activities of Rat Liver Glucose-6-phosphate Dehydrogenase and Pyruvate Kinase

It is generally agreed that protein and enzyme syntheses are depressed in the liver of ethionine-treated animals and that the mature female preferentially exhibits this diminution<sup>1,2</sup>. We were surprised, therefore (Fig. 1), to find a rise (200–300 per cent) of liver glucose-6-phosphate dehydrogenase activity in both sexes of ethionine-treated rats. On the other hand, 6-phosphogluconate dehydrogenase, which is the next enzyme in the shunt pathway sequence, is unaffected and pyruvate kinase of the Krebs cycle undergoes a decrease (50 per cent) in the liver of the female but not the male rat. In animals receiving both methionine and ethionine, no alterations from normal occurred. Accordingly, these diverse effects on enzyme activity of ethionine are now being reported to emphasize the complexity of enzyme phenomena in ethionine-treated animals.

The experimental conditions were as follows: both male and female Wistar rats, weighing approximately 100 g each, were maintained *ad libitum* on three separate diets. The control group received Purina chow, the ethionine groups received 0.5 per cent ethionine supplement in the chow diet, the ethionine-methionine group received 0.5 per cent ethionine and 5.0 per cent methionine supplement in the diet. Each group consisted of 6–10 animals. After 21 days of treatment, the animals were killed and bled, the livers were removed and chilled, and a 10 per cent homogenate was immediately prepared in 0.25 M sucrose–0.001 M EDTA solution. The supernatant fraction was obtained by centrifuging the homogenate for 30 min at 100,000g in a refrigerated Spinco model L preparative ultracentrifuge, and it was assayed for the activities of pyruvate kinase<sup>3</sup>, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase<sup>4</sup>.

In the case of glucose-6-phosphate dehydrogenase, a sex difference in favour of a higher level in the normal female was observed<sup>5</sup>. However, ethionine-fed animals of both sexes exhibited an obvious and statistically significant ( $P < 0.01$ ) increase (Fig. 1) in activity of

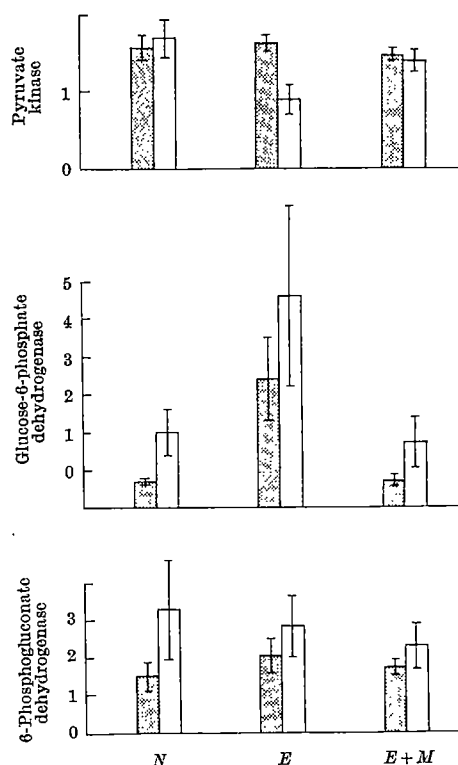


Fig. 1. Alterations in glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and pyruvate kinase in relation to sex and ethionine treatment. The activities of G-6-P and 6-PG dehydrogenases are expressed as  $\mu$ moles of TPNH formed per mg of protein per h. Pyruvate kinase activity is expressed as  $\mu$ moles pyruvate formed per mg of protein per h. Data on male animals are shaded, those on female, unshaded. N, signifies normal control; E, ethionine-fed, and E+M, ethionine + methionine-fed, animals. Standard deviation is indicated by a vertical line in each column.

this particular dehydrogenase. The rise in glucose-6-phosphate dehydrogenase has also been observed in this laboratory<sup>6</sup> in mice on ethionine for only 4 days.

The fact that 6-phosphogluconate dehydrogenase is unaffected in this experiment makes it unlikely that a full activation of the 'shunt' pathway occurred. However, in unpublished experiments of longer duration than 21 days in rats and 4 days in mice, the activity of this enzyme also undergoes a significant elevation. It is reasonable to suggest, therefore, that the rise in glucose-6-phosphate dehydrogenase is the first indication of activation of the 'shunt' pathway, a route of metabolism around which much biochemical interest is now centred, particularly in relation to oncology.

In this connexion, reference should be made to investigations in proliferating and malignant cells<sup>7</sup>, in lymphatic leukaemia and Ehrlich tumour<sup>8</sup>, and in Novikoff hepatoma<sup>9</sup>. In transplanted hepatomata of varying degrees of deviation from normal liver, Weber and Morris<sup>10</sup> noted enhancement most frequently of the activities of glucose-6-phosphate dehydrogenase followed by 6-phosphogluconate dehydrogenase.

A different significance must be assigned to the reduction of pyruvate kinase. This event can be considered to partially explain the fall in liver ATP which has recently been suggested to be the cause of failure in protein synthesis<sup>11</sup>. In brief, since the ability of pyruvate kinase to catalyse the formation of ATP from ADP is greatly impaired, less ATP will accumulate. Moreover, in the female, ethionine is less rapidly metabolized than in the male, which would lead to a more complete trapping of ATP in the form of *S*-adenosylethionine as shown by Stekol *et al.*<sup>12</sup>. The combination of these two circumstances, loss of pyruvate kinase and less-rapid metabolism of ethionine in the female, may explain the sex difference in sensitivity to ethionine.



It may very well be that the activities of those enzymes, like pyruvate kinase, which decline in ethionine-treated females are part of an ATP-associated mechanism and that those enzymes the activities of which increase, such as glucose-6-phosphate dehydrogenase, belong to a group of enzyme proteins which may be undergoing *de novo* synthesis for different reasons. Support for the latter view is provided by the recent evidence for the stimulation of RNA synthesis and the increase of 'soluble' cysteine desulphydrase<sup>13,14</sup> in ethionine-treated rats.

Finally, a possible explanation for the present results is that in the course of time the population of cells in the livers of ethionine-treated animals<sup>1</sup> may be undergoing profound changes in both their type, number and state. Measurements of enzyme activity made on homogenates of such altered tissues may, therefore, be reflexions of these circumstances. Accordingly, it may be desirable in the future to identify the particular enzyme alteration with its cytoplasmic and cellular site.

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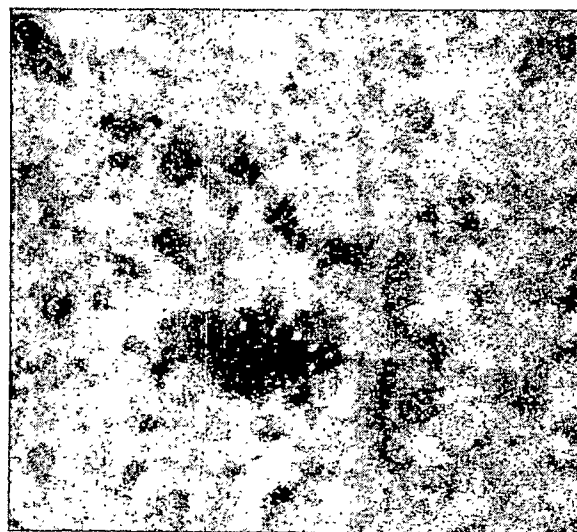
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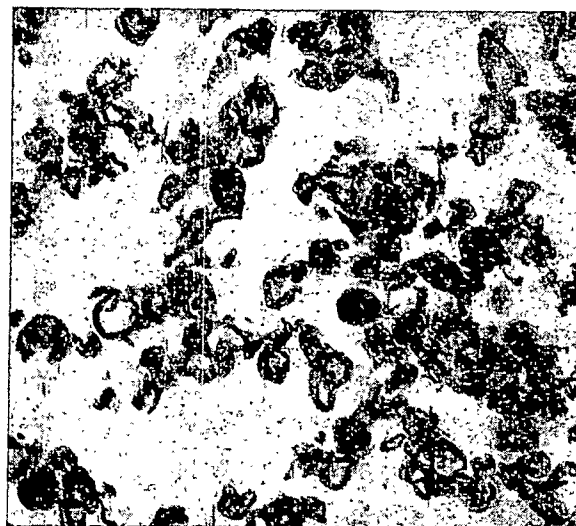
### Death in Mice induced by *Staphylococcus aureus* Protoplasm

THE most rational approach to treatment of an infectious disease is elimination of the infecting organism. Attempts to do this with antibiotics have not always been successful. Therefore, a characterization of the biochemical changes caused by the organism may result in biochemical replacements which can improve treatment. Previous work has shown that a profound derangement of carbohydrate metabolism can be induced in mice by living staphylococci<sup>1,2</sup>. When mice are challenged by a variety of routes, death occurs after a fixed number of cells has accumulated (as determined by total body counts) no matter in which portion of the body they multiply<sup>3,4</sup>. Since the staphylococcal cell is complex it would be desirable to fractionate it and produce death with known components. The first step in this process is to disintegrate the cell without damage to the enclosed chemicals and enzymes. We believe we have achieved this as the protoplasm used in the investigations to be reported was still able to coagulate citrated rabbit plasma at 37° C. The following investigations give some indication of the effect of fractionated *S. aureus* on male Swiss mice.

Coagulase-positive staphylococci were grown overnight in heavy culture on nutrient agar (Difco). The staphy-



A



B

Fig. 1. A, *Staphylococcus aureus* protoplasm (SAP) (phosphotungstic acid staining,  $\times 6,300$ ). B, Cell wall material from *Staphylococcus aureus* (uranyl acetate staining,  $\times 6,300$ ). Electron micrographs made by Dr. J. M. Layton

lococci used were from various clinical sources. They were washed off in distilled water (titre  $10^{10}$  cells/ml.), washed twice by centrifugation and resuspended in 100-ml. distilled water (about 150 mg/ml. dry weight). The Servall-Ribi Refrigerated Cell Fractionator was used to disrupt the cells. In this instrument a pressurized cell suspension is released at the orifice of a needle valve, whereby the cell walls are cracked and the protoplasm is released in a dispersed state<sup>4</sup>. A pressure of 40,000 lb./in.<sup>2</sup> was used and an orifice temperature of 0°–10° C was maintained. About 40–50 per cent of the cells were broken. The effluent was centrifuged at 13,000 r.p.m. at 2° C for 1 h in a Servall RC-2 centrifuge equipped with an SS-34 head to separate the unbroken cells and cell walls from the protoplasmic material. The supernatant was recentrifuged at the same speed and the sediments were discarded. Filtration through a 'Millipore' filter eliminated the last few organisms. All these procedures were carried out at 4° C except where otherwise indicated.

The protoplasmic material obtained by this procedure was a homogeneous, yellow, turbid, somewhat viscous fluid. No growth of micro-organisms in broth culture or on agar could be obtained aerobically or anaerobically. It was

coagulase-positive and  $\beta$ -haemolytic on sheep blood agar. Very few cell walls could be seen (Fig. 1). The material was coarsely granular microscopically. It passed through a HAWP 047.00 white, plain 'Millipore' filter. Heating at 80° C for 30 min destroyed its lethal properties. Preliminary results of analyses of this cytoplasm of *Staphylococcus aureus* (SAP) indicated the presence of 1 g/100 ml. of protein (Biuret method); reducing sugars 25 mg/100 ml. (Nelson-Somogyi method); 'polysaccharides' 10 mg/100 ml. (phenol sulphuric acid method) and total lipids 20–40 mg/100 ml. (gravimetric method). When subjected to free electrophoresis (Klett Tiselius barbiturate buffer pH 8.6, ionic strength 0.1) the protoplasm separated into three components. The major portion migrated at the rate of the  $\alpha$ -globulins, one minor component migrated at the rate of the albumin and a second more rapidly than the albumin of normal human serum. Further fractionation of these components and an examination of their biological activity are in progress<sup>5</sup>.

As little as 0.5 c.c. of the SAP (obtained from 10<sup>8</sup> whole *Staphylococcus aureus* cells) inoculated by the intraperitoneal route killed mice in 5–7 h (Table 1), the time required for death to occur when 10<sup>8</sup> whole staphylococcal cells were injected. We believe this was not due to intracellular mixture with exotoxins as our material was washed three or four times in distilled water before use. The mode of death from SAP was 'clinically' identical to that produced by live cells. That is to say, there was first a ruff-like erection of the neck fur, the animals bunched together, had an unsteady gait and appeared to be walking on tiptoe with a concavity of the buttocks; then they became dyspnoeic, humped their backs and showed spontaneous overactivity. Later they had running fits and a clonic convulsion, lost sphincter control and died. Mice killed by SAP were analysed biochemically and values obtained were essentially similar to those found in mice killed by whole staphylococci and they were significantly different from the biochemical values for normal mouse carcasses (Table 1). No staphylococci were obtained from cultures of the ground-up carcasses of animals killed with SAP. The results were reproduced many times with 5 out of 7 batches made at 40,000 lb./in.<sup>2</sup>. Death did not occur when protoplasm obtained after preparation by higher orifice pressure was used. These results were duplicated using other strains of coagulase-positive staphylococci. Death was also produced by SAP by the intramuscular and subcutaneous routes. In some instances the material was dermonecrotic (Table 2).

The induction of death by non-replicating elements of *Staphylococcus aureus* cells provides a new approach to

investigating the cause of death from staphylococcal infection and to differentiate host from organismal chemical effects<sup>7</sup>.

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## A Haemoprotein from the Lipid of Peanuts

MUCH literature exists on the effects of haematin compounds on the oxidation of unsaturated fats and fatty acids in emulsions and colloidal suspensions<sup>1-3</sup>. We wish to report a naturally occurring haematin protein found in the lipid fraction of peanuts and other seeds.

The absorption spectrum of an intact peanut cotyledon (Fig. 1 A, solid curve) shows a prominent absorption band at 635 m $\mu$ . Similar bands in the 640-m $\mu$  region (635–645 m $\mu$ ) have been found, to a lesser extent, in the absorption spectra of intact lima beans, soybeans, wheat, oats, barley, watermelon seeds, and yeast<sup>4</sup>. Soaking the peanuts in 0.1 M KCN for 15 min causes the loss of the 635-m $\mu$  band and the appearance of a broad absorption band in the 540-m $\mu$  region (Fig. 1 A, dashed curve). The 635-m $\mu$  pigment is distributed throughout the cotyledons so that the cyanide must penetrate the whole seed. The sensitivity of the 635-m $\mu$  band to cyanide distinguishes the material from protochlorophyll, which has an absorption band in the 630- to 650-m $\mu$  region<sup>5</sup>. On the basis of the absorption spectra<sup>4</sup>, the 635-m $\mu$ -absorbing material in intact seeds and yeast was ascribed to a ferric haematin compound. The present paper reports the extraction and partial purification of this material from peanut seeds.

Both dry and imbibed peanuts were ground and extracted with 0.4 M sucrose, and the extract was fractionated by differential centrifugation. The various fractions were examined with a spectrophotometer which could accommodate dense, highly scattering samples such as intact seeds and turbid media<sup>6</sup>. The only fraction that showed an appreciable amount of the 635-m $\mu$ -absorbing material was the white lipid layer collected from the top of the centrifuge tube (Fig. 1 B). The lipid also showed a carotene-like absorption band at 480 m $\mu$ . The 635-m $\mu$  pigment remained with the residue after thorough extraction of the lipid with methanol, acetone, ether, chloroform or butanol and the extraction did not render the pigment soluble to aqueous systems. The absorption spectrum of the residue from the acetone extraction is shown in Fig. 1 C. Removal of the carotene-like pigment unmasked a broad absorption band in the 500-m $\mu$  region. A part of the

Table 1. ANALYSES OF THE MOUSE CARCASS AT DEATH  
(The figures relate to weight/100 c.c. carcass suspension)

	Normal mice	Killed * with <i>S. aureus</i> No. I and X	Killed † with SAP I and X
Glucose (mg per cent)	59	19	25
Total protein (g per cent)	1.8	1.2	1.3
Inorganic phosphorus (mg per cent)	9	23	26
Alkaline phosphatase (S.U.)†	54	25	14
Transaminase (S.F.U.)§	937	1,268	1,840
Cholesterol (mg per cent)	31	13	6

\* 10<sup>8</sup> *S. aureus* intraperitoneal.

† *Staphylococcus aureus* protoplasm.

‡ S.U. Sigma units.

§ S.F.U. Sigma-Frankel units.

Table 2

Batch No.	Staphylococcal strain No.	Dry weight	Pressure (lb./in. <sup>2</sup> )	Deaths in mice 0.1 c.c.	Deaths in mice 0.5 c.c.	Deaths/total 1.0 c.c.
Protoplasm I	2902 and 2929 mixed	150 mg/ml.	40,000	0/5	5/5 5/5	5/5
IV	2902	20 mg/ml.	40,000	—	3/3	5/5
V	3016	18 mg/ml.	40,000	—	5/5	5/5
VI	2902	—	40,000	—	9/10	—
VIII	3016	670 mg/ml.*	40,000	—	0/5	0/5
IX	2902	170 mg/ml.	40,000	0/3	2/3	—
X	2902	145 mg/ml.	40,000	—	3/5	—
Cell wall IX	2902	—	—	0/3	0/3	—
X	2902	—	—	0/5 0/5	0/5	—

\* Wet weight.

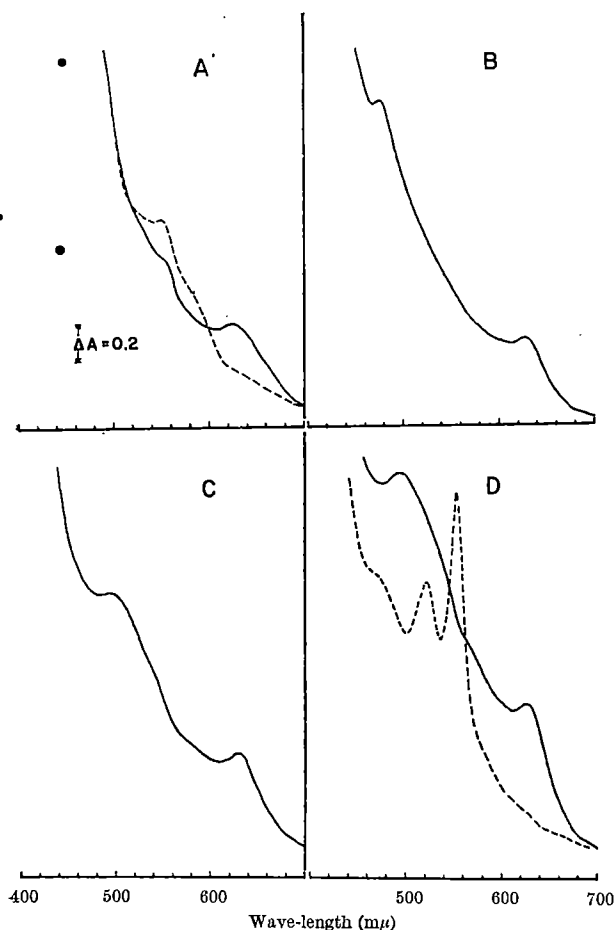


Fig. 1. *A*, solid curve, absorption spectrum of intact peanut cotyledon. Dashed curve, absorption spectrum of same cotyledon after soaking in 0.1 M KCN for 15 min; *B*, absorption spectrum of lipid fraction collected from peanut homogenate; *C*, absorption spectrum of residue from acetone extraction of the lipid; *D*, solid curve, absorption spectrum of digitonin extract (2 per cent digitonin for 24 h) of the acetone powder of the lipid.  $\text{CaCO}_3$  powder was added to the extract to increase light scatter. Dashed curve, absorption spectrum of the same sample after addition of pyridine and sodium hydrosulphite

635-mμ-absorbing material could be solubilized by extracting the acetone powder with 2 per cent digitonin. The solid curve in Fig. 1 *D* shows the absorption spectrum of the digitonin-solubilized material to which enough  $\text{CaCO}_3$  was added to make a thick white paste. The  $\text{CaCO}_3$  makes the light-scatter more comparable to the other samples and increases the intensity of the 635-mμ absorption band 10- to 20-fold by increasing the optical path-length<sup>7</sup>. The pigment could also be solubilized from an acetone powder by 4 M urea, but reprecipitated when the urea was removed by dialysis.

The absorption spectrum of the solubilized material is very similar to that of catalase, peroxidase and methaemoglobin<sup>8</sup>. With the solubilized pigment, cyanide, fluoride and azide (pH 4.2) all form complexes which destroy the 635-mμ absorption band. Carbon monoxide, which combines with ferrous but not ferric haematin compounds<sup>8</sup>, has no effect on the absorption spectrum. The pigment is not reduced by sodium hydrosulphite or potassium borohydride, which is typical of a catalase, but not of a peroxidase or methaemoglobin<sup>8</sup>. The addition of sodium hydrosulphite plus pyridine gives a typical haemochromogen spectrum (dashed curve, Fig. 1 *D*).

The prosthetic group could be extracted from the residue of chloroform extracted lipid with acid acetone (500 acetone : 1 conc. HCl). An equal volume of ether was added to the extract followed by the addition of NaCl to 5 per cent, with shaking. The ether layer was then removed and concentrated by evaporation. Fig. 2 shows

the absorption spectrum of the concentrated ether extract plus  $\text{CaCO}_3$  and the spectrum obtained by adding sodium hydrosulphite and pyridine. These spectra indicate that the prosthetic group is protohaem.

The concentration of the 635-mμ-absorbing material in the lipid fraction was estimated by assuming an extinction coefficient of a typical catalase or peroxidase and determining experimentally the effect of the light scatter on the intensity of the absorption band. The absorption band resulting from the addition of a small volume (10 μl.) of a methanolic solution of chlorophyll to the lipid was intensified 5.6 times by the light scatter in the lipid (assuming that the extinction of chlorophyll is the same in lipid as it is in methanol). Taking the absorbancy of the 635-mμ absorption band of the 6-mm-thick sample of lipid (Fig. 1 *C*) to be 0.3, the scatter intensification factor to be 5.6, and the molar extinction coefficient to be  $10 \text{ mM}^{-1} \text{ cm}^{-1}$ , which is typical for peroxidase or catalase<sup>8</sup>, the concentration of the 635-mμ pigment in the lipid was  $9 \times 10^{-6} \text{ M}$ .

The protein content of a digitonin-solubilized solution, measured by the Biuret test after dialysis and precipitation of the protein with 10 per cent trichloroacetic acid, was 10 mg/ml. The haem content, estimated from the absorption spectrum of the solution after addition of  $\text{CaCO}_3$ , assuming an extinction coefficient of  $10 \text{ mM}^{-1} \text{ cm}^{-1}$  and a scatter intensification of 10, was  $5 \times 10^{-6} \text{ M}$ . This gives a ratio of  $2 \times 10^{-6} \text{ g}$  protein per mole haem, which is probably correct to within an order of magnitude. The strong affinity of the protein for the lipid and the partial solubilization by digitonin suggest that the haem is attached to a lipoprotein. The digitonin-solubilized material showed moderate catalase activity as assayed by potassium permanganate titration<sup>9</sup> and essentially no peroxidase activity by the guaiacol test<sup>9</sup>. The lipoprotein, however, may not catalyse a specific reaction *in vivo*. In general, the action of haematin compounds in the oxidation of unsaturated fats is quite non-specific<sup>1,2</sup>.

In order to ascertain how germination affected the 635-mμ pigment, absorption spectra were measured on intact peanut cotyledons from seedling plants which were germinated in the dark. For the first 6 to 7 days' imbibition, the magnitude of the 635-mμ absorption band remained constant to within the limits of sample reproducibility. During this time, there was no shrivelling or visible deterioration of the cotyledons even though the plants had grown to a height of 10-15 cm. Estimates of the lipid content of the cotyledons, made by grinding 10-g samples of cotyledons in 0.4 M sucrose and collecting the lipid by centrifugation, also indicated little if any loss of lipid during this period. (The lipid decreases much earlier in peanuts that are germinated in the light, but the

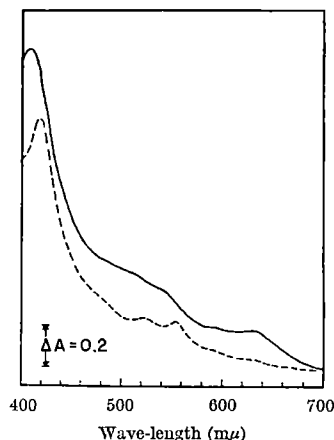


Fig. 2. Absorption spectra of the prosthetic group split from the residue of a chloroform extract of the lipid: solid curve, absorption spectrum of extracted prosthetic group; dashed curve, absorption spectrum after addition of pyridine and sodium hydrosulphite

accumulation of chlorophyll in the cotyledons obscures the 635-m $\mu$  absorption band.) After 7 days, however, the lipid content of the dark-germinated cotyledons decreased abruptly, the cotyledons began to shrivel, and the magnitude of the 635-m $\mu$  absorption band decreased. Thus, the haemoprotein appears to be broken down as the lipid is utilized. It should be noted that when haematin compounds catalyse the oxidation of unsaturated fatty acids they are destroyed<sup>1,2</sup>, presumably through reactions with lipid peroxides.

Lewis and Wills<sup>3</sup> showed that haemoglobin could either catalyse or inhibit the oxidation of linoleic acid, depending on the ratio of the two compounds. At a concentration of  $1.2 \times 10^{-6}$  M, haemoglobin inhibited the oxidation of 0.017 M linoleic acid, while at a concentration of  $4 \times 10^{-6}$  M it was a strong catalyst. With  $5 \times 10^{-3}$  M linoleic acid, however,  $4 \times 10^{-6}$  M haemoglobin inhibited the oxidation. The relatively high concentration of haemoprotein in peanut fat suggests that it could have a protective effect, particularly in preventing the peanut from becoming rancid during long periods of storage. However, if the degree of lipid unsaturation increased during germination, the haemoprotein might change from being an inhibitor to being a catalyst of lipid oxidation.

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## Changing Base Ratios in RNA of Developing Tissues of *Xenopus laevis*

THE new forms of RNA synthesized during embryonic development of the South African clawed toad, *Xenopus laevis*, have come under investigation recently by Brown and Gurdon<sup>1</sup> and are discussed more fully by Brown and Littna<sup>2</sup>. Since their work was concerned only with events in the embryo as a whole, we have undertaken a study of possible differences in the new forms of RNA synthesized in different tissue primordia of this species. In the preliminary work to be reported here, the nucleotide composition of the total acid-insoluble RNA extracted both from whole embryos and from ectoderm undergoing three different courses of differentiation—into neural plate, neural crest and epidermis—has been analysed. Although data published by Yeas<sup>3</sup> indicated that different tissues of the adult rat do not show any significant differences in their RNA composition, there have been several other reports<sup>4-7</sup> of altered base ratios in RNA of tissue cells undergoing special phases of modified protein synthesis in response to external stimuli. It seemed likely, therefore, that embryonic tissues responding to stimuli such as neural induction might also show some alterations of RNA base ratios. As will be reported here, RNA extracts both from whole embryos and from the isolated ectoderm do, in fact, exhibit some changes in base ratios, which are of general interest in view of the protein syntheses known to be occurring at these stages of development.

For the analyses, samples of 12 whole, demembrated embryos or 80 ectoderm pieces were collected in cold 80 per cent ethanol, then extracted for 1 h in 200  $\mu$ l.

0.3 N perchloric acid, followed by a minimum of 4 h in two changes of 400  $\mu$ l. of cold 3:1 ethanol:ether<sup>8</sup>. The residues were hydrolysed for 16–24 h in 200  $\mu$ l. 0.35 N potassium hydroxide solution at 37° C (ref. 9). The pH of the hydrolysate was adjusted to 1.0 with 6 N perchloric acid and the resultant precipitate removed by centrifugation. The supernatant, after pH adjustment to 3.5 and volume adjustment to equivalence with the other samples in the same experiment, was applied in 30- $\mu$ l. aliquots to strips of Whatman No. 1 filter paper, 4  $\times$  28 cm., for electrophoretic separation of the mononucleotides. 0.15 M formate buffer, pH 3.5, was used<sup>10</sup> and a current of 3.5 m.amp/cm width was applied for 10 h. After marking the positions of the absorption bands under ultra-violet light, they were cut out and eluted into 0.01 N hydrochloric acid. Absorption spectra of the eluates were plotted between 220 and 300 m $\mu$ . The spectra corresponded to those published by Beaven, Holiday and Johnson<sup>11</sup>, and these authors' absorptivity coefficients were used to estimate the concentrations of the nucleotides and hence their molar ratios.

Table 1 gives the results of analyses of RNA from whole embryos, and Table 2 gives the analyses of RNA from the different types of ectoderm at stages from gastrula to tailbud. Fig. 1 shows diagrammatically how the embryos were dissected to obtain presumptive neural plate, neural crest and epidermis. The vertical lines in Tables 1 and 2 indicate statistically significant changes in base ratios.

Table 1. MOLAR RATIO OF NUCLEOTIDES IN RNA OF WHOLE EMBRYOS

Stage	AMP	GMP	CMP	UMP
Uncleaved	22 $\pm$ 1.0	32 $\pm$ 1.2	[25] $\pm$ 1.8	21 $\pm$ 1.2
Morula	20 $\pm$ 1.4	32 $\pm$ 2.1	[28] $\pm$ 3.6	20 $\pm$ 0.2
Early blastula	21 $\pm$ 1.7	29 $\pm$ 1.7	[31] $\pm$ 2.3	19 $\pm$ 1.1
Mid-blastula	22 $\pm$ 0.7	29 $\pm$ 0.1	27 $\pm$ 1.1	22 $\pm$ 2.6
Late blastula	23 $\pm$ 1.7	[30] $\pm$ 2.7	[25] $\pm$ 3.2	22 $\pm$ 0.5
Early gastrula	22 $\pm$ 1.0	24 $\pm$ 2.8	[31] $\pm$ 1.6	[23] $\pm$ 0.5
Mid-gastrula	21 $\pm$ 0.8	33 $\pm$ 0.4	28 $\pm$ 2.1	18 $\pm$ 0.5
Late gastrula	23 $\pm$ 1.7	[33] $\pm$ 1.5	26 $\pm$ 0.8	18 $\pm$ 1.5
Early neurula	23 $\pm$ 0.7	[29] $\pm$ 1.1	[30] $\pm$ 1.8	18 $\pm$ 0.5
Late neurula	[24] $\pm$ 0.4	29 $\pm$ 0.8	29 $\pm$ 0.6	18 $\pm$ 0.8
Tailbud stage	[18] $\pm$ 1.9	28 $\pm$ 2.8	29 $\pm$ 2.5	25 $\pm$ 3.8
Hatching larva	16 $\pm$ 1.9	28 $\pm$ 2.4	32 $\pm$ 3.7	24 $\pm$ 3.8

Data are expressed as moles/100 moles, and are means, plus standard errors, from six experiments.

Table 2. MOLAR RATIOS OF NUCLEOTIDES IN PARTS OF EMBRYOS

Tissue	AMP	GMP	CMP	UMP
Neural ectoderm				
Early gastrula*	18 $\pm$ 1.8	25 $\pm$ 1.5	31 $\pm$ 0.9	26 $\pm$ 1.2
Late gastrula*	[24] $\pm$ 1.1	27 $\pm$ 2.2	[28] $\pm$ 0.9	21 $\pm$ 1.1
Early neurula	14 $\pm$ 0.6	25 $\pm$ 0.9	[40] $\pm$ 1.3	21 $\pm$ 2.5
Late neurula	18 $\pm$ ...	31 $\pm$ ...	33 $\pm$ ...	18 $\pm$ ...
Tailbud stage	23 $\pm$ 1.5	30 $\pm$ 1.3	25 $\pm$ 2.0	22 $\pm$ 0.7
Epidermis				
Early gastrula	19 $\pm$ 1.1	[17] $\pm$ 1.2	31 $\pm$ 0.3	[33] $\pm$ 1.3
Late gastrula	19 $\pm$ 0.5	[27] $\pm$ 1.6	33 $\pm$ 1.8	[21] $\pm$ 0.7
Early neurula	19 $\pm$ 0.9	31 $\pm$ 2.5	29 $\pm$ 1.2	21 $\pm$ 0.9
Late neurula	21 $\pm$ ...	25 $\pm$ ...	32 $\pm$ ...	22 $\pm$ ...
Tailbud stage	21 $\pm$ 1.2	25 $\pm$ 1.2	31 $\pm$ 2.3	23 $\pm$ 0.8
Neural crest				
Early neurula	20 $\pm$ 1.9	27 $\pm$ 1.2	30 $\pm$ 1.7	23 $\pm$ 0.9
Late neurula	20 $\pm$ ...	24 $\pm$ ...	33 $\pm$ ...	[23] $\pm$ ...
Tailbud stage	15 $\pm$ 0.5	26 $\pm$ 0.2	29 $\pm$ 0.8	[30] $\pm$ 1.6

Moles/100 moles. Means, with standard errors, from 5 samples.

\* Includes neural crest.

† Only one sample available for analysis.

The data of Table 1 show that there are marked relative increases in cytidine (CMP) at the onset of gastrulation and neurulation, respectively, and also smaller increases during cleavage stages. At gastrulation and early neurulation, the guanine ratio decreases. It is interesting that such marked changes should occur at these particular stages when, according to Brown and Littna's recent investigations<sup>2</sup>, new ribosomal RNA is synthesized. Brachet *et al.*<sup>12</sup> and Denis<sup>13</sup> have also concluded from an entirely different approach, using actinomycin D treatment, that these two phases, as well as that of late cleavage, are critical for the synthesis of DNA-dependent RNA. However, the very high cytidine content of RNA featured here has not been observed by any of these workers. From the data on isolated ectoderm (Table 2)



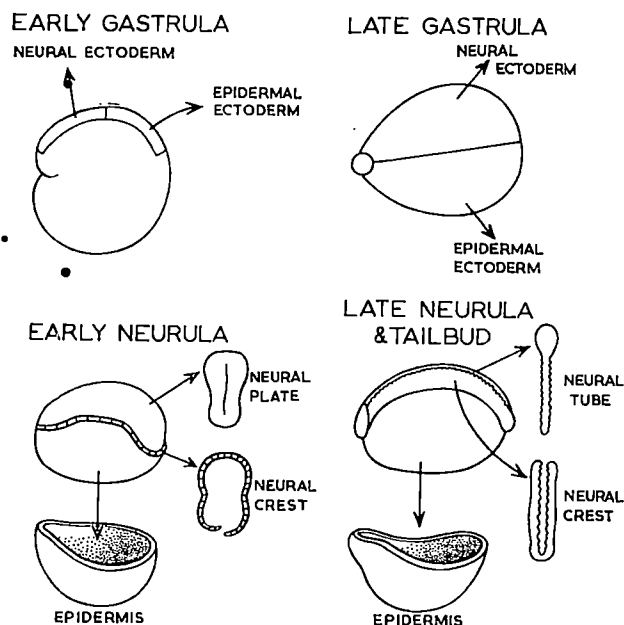


Fig. 1. Diagram to show the origin of tissues analysed

it is clear that the neural plate, in particular, exhibits a marked increase in molar concentration of cytidine at the early neurula stage. There is at the same time a marked decrease in relative concentration of adenylic acid in this tissue. The epidermis, by contrast, shows a slight relative decrease in cytidine and increase in guanidine, at the early neurula stage. The most striking base ratio changes in epidermis occur between early and late gastrula stages, however: there is a very large increase in guanidine, matched by a decrease in uridine. In the neural crest ectoderm, the uridine ratio becomes higher than in the other two tissues at the tailbud stage, and one is led to speculate whether this may be due to an increased proportion of uridine triplets coding for phenylalanine uptake which is known<sup>14</sup> to be essential for the differentiation of neural crest cells and for melanin synthesis.

These findings are of general interest since they show that certain clear differences in the ribonucleotide composition of total RNA already arise in the ectoderm as it diverges along three different paths of differentiation in the early embryo. The significance of these differences, in terms of the kinds of RNA being synthesized in these tissues, is now under investigation using <sup>32</sup>P-labelling and sucrose density-gradient separation methods.

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## PHYSIOLOGY

### Effect of $\alpha$ -Melanocyte-stimulating Hormone and a Minute Trauma on the Ciliary Processes of the Rabbit's Eye

MELANOCYTE-stimulating hormone (MSH) and other hypophyseal polypeptides have been shown to exert an effect on the permeability of the blood aqueous barrier of the rabbit's eye<sup>1,2</sup>. This effect has certain similarities to a localized trauma on the eye (such as the increase in flare, fluctuation in intra-ocular pressure), but it is not, unlike the traumatic effect, diminished by local anaesthetics<sup>3</sup>. Considerable interest is attached to a comparison of the histological changes after MSH and trauma. In a group of 18 animals, a minute trauma was induced in 5 of them by infra-red irradiation<sup>4</sup> on the upper part of the iris, a subcutaneous injection of 10  $\mu$ g synthetic  $\alpha$ -MSH (Schwyzer) was given to 7, and 6 acted as controls (as also did the contralateral eyes of the first group). The eyes were enucleated in the trauma group 30 min after the trauma, in the MSH group 90 min after

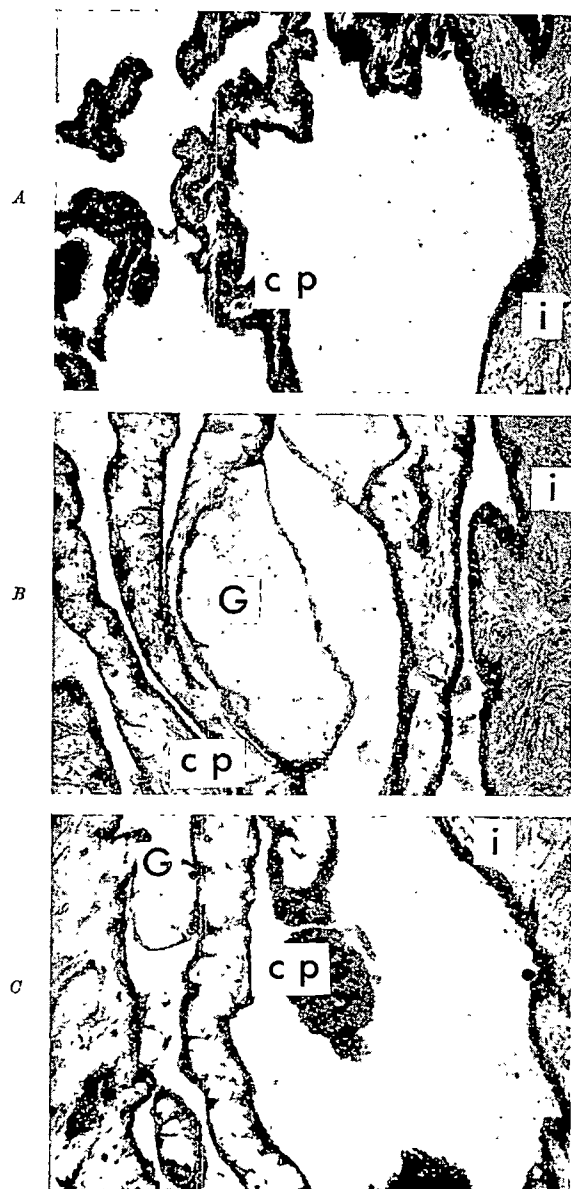


Fig. 1. (A) Ciliary processes (cp) and iris (i) from normal eye; (B) from traumatized eye, with 'Greeffsche Blasen' (G); (C) from MSH-treated animal ( $\times 86$ )

the injection. Following freeze-drying and fixation in Carnoy's solution the tissues were embedded in paraffin, sectioned<sup>5</sup>, and stained with haematoxylin-eosin.

The following are the results of our histological examinations (see Fig. 1): (1) In both the trauma and MSH groups there was, without exception, a swelling of the anterior ciliary processes, an interstitial oedema and possibly a dilatation of the small intraciliary vessels. Typical 'Greeffsche Blasen' with splitting of the two layers of the secretory epithelium<sup>6</sup> were observed to a similar extent in both groups. In the 17 normal eyes no such changes were seen.

(2) In 7 eyes from MSH-treated animals, where no flare effect occurred, there was still a marked swelling of the ciliary processes, but no 'Greeffsche Blasen' were found.

(3) The trauma effect was observed around the whole circumference of the ciliary body but more excessively on the upper part, that is, behind that part of the iris treated with infra-red irradiation.

(4) No changes in the iris, not even in its irradiated part, or in the melanocytes of the iris and ciliary body could be observed in any of the eyes.

Although recent investigations<sup>7</sup> have shown that MSH causes a series of general effects in the rabbit, for example, a decrease in the blood pressure and dilatation of the peripheral vessels, there is still no evidence that the permeability disturbance should be a generalized effect.

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## Permeability Effects of $\alpha$ -Melanocyte-stimulating Hormone

$\alpha$ -MELANOCYTE-stimulating hormone ( $\alpha$ -MSH) induces an increased permeability of the blood-aqueous barrier of the rabbit eye<sup>1,2</sup>. It has been shown in the preceding communication that  $\alpha$ -MSH produces a swelling of the ciliary processes with an interstitial oedema in this species. Since the permeability disturbance might be part of a more generalized effect, it was considered worthwhile investigating whether or not there was also an increased leakage of proteins through other barriers.

<sup>125</sup>I-labelled human serum albumin (Radiochemical Centre, Amersham) was given intravenously 30 min after a subcutaneous injection of 10  $\mu$ g/kg synthetic  $\alpha$ -MSH (ref. 3) (supplied by Ciba, Basle) in 0.9 per cent sodium chloride to 12 pigmented rabbits and after the same volume of 0.9 per cent sodium chloride had been given to 5 pigmented rabbits (controls). Blood samples were collected at 30-min intervals. The aqueous flare response<sup>2</sup> was taken as proof of the local permeability disturbance of the eye after the  $\alpha$ -MSH and was estimated roughly by means of focal illumination before and 2 h after the injection. 2.5 h after the  $\alpha$ -MSH injection the rabbits were killed, their eyes enucleated and dissected essentially as described previously<sup>4</sup>. Small tissue samples were also collected from various organs. The radioactivity of the blood and tissue samples was determined.

The radioactivity in counts per minute of the tissue samples was divided by the radioactivity in c.p.m./ $\mu$ l. plasma in the blood sample obtained immediately before the animals were killed. This gave a figure for the apparent

Table 1. THE RELATIVE FALL IN BLOOD RADIOACTIVITY AFTER AN INJECTION OF <sup>125</sup>I-ALBUMIN

In animals showing an aqueous flare response after the injection of  $\alpha$ -MSH, R; in animals not responding with aqueous flare, NR; and in controls, C. Arithmetic means  $\pm$  the standard error of the means. The radioactivity of the first blood sample is 1.00

Time after <sup>125</sup> I- alb. inj.	4 min	30 min	60 min	90 min	120 min
R	1.00	0.90 $\pm$ 0.0086	0.83 $\pm$ 0.0114	0.79 $\pm$ 0.0125	0.77 $\pm$ 0.0191
NR	1.00	0.87 $\pm$ 0.0114	0.79 $\pm$ 0.0187	0.75 $\pm$ 0.0196	0.71 $\pm$ 0.0200
C	1.00	0.89 $\pm$ 0.0205	0.82 $\pm$ 0.0249	0.77 $\pm$ 0.0232	0.74 $\pm$ 0.0190

Table 2. THE APPARENT PLASMA CONTENT OF THE ANTERIOR UVEA AND THE CHOROID PREPARATIONS AND IN 1 ML. AQUEOUS HUMOUR

In animals responding with increased aqueous flare to  $\alpha$ -MSH, R; in non-responsive animals, NR; and controls, C. Arithmetic mean  $\pm$  the standard error of the mean

	The apparent plasma volume ( $\mu$ l.)		
	Anterior uvea	Choroid	1 ml. aqueous humour
R	33.5 $\pm$ 2.83	6.03 $\pm$ 0.66	265.2 $\pm$ 33.9
NR	7.1 $\pm$ 0.64	4.40 $\pm$ 0.32	3.4 $\pm$ 0.70
C	7.1 $\pm$ 1.27	4.81 $\pm$ 0.26	6.6 $\pm$ 2.90

plasma content, which included 'plasma' both from intravascular and extravascular compartments.

In 6 of the 12 rabbits which had been given MSH there was a pronounced increase in aqueous flare. In the remaining 6 and in the control group no aqueous flare was detected.

We found no increased rate of loss of <sup>125</sup>I-albumin from the intravascular compartment (Table 1). In the 6 rabbits where the aqueous flare was prominent, the albumin content of the aqueous humour was very much increased as compared with the 6 test rabbits in which no flare was observed and with the controls (Table 2). The apparent plasma content of the anterior uvea was greatly increased, while no such effect was observed in the choroid as shown in Table 2. No similar change in apparent plasma content could be found in the urine, the gall, the brain, the liver, the kidneys, the lung, the spleen, the intestines, the suprarenals or the muscles.

It thus seems that the permeability increasing effect of the melanocyte-stimulating peptides is rather specific for the eye. However, it should be pointed out that a rise in the vasomotor tone of the small veins might be of importance in the increased protein transfer.

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## The Ussing Relationship and Chemical Reactions: Possible Application to Enzymatic Investigations

It may not be widely appreciated that the Ussing flux ratio relationship<sup>1,2</sup> is applicable to chemical reactions in general. It is possible that measurement of such flux ratios may yield information about reaction intermediates, particularly in enzymatic processes, but perhaps also in catalytic, heterogeneous, and other chemical reactions.

To demonstrate the relationship of the Ussing criterion to a typical chemical reaction it is convenient to use an argument based on induced transport<sup>3</sup>. Consider the reversible reaction:



and let  $A$  and  $C$  be labelled in such a way that all the labelling from  $A$  appears in product  $C$  and vice versa. Then, the free energy change for the conversion of  $\Delta m$  moles of radioactive  $A$  into  $C$  is:

$$\left[ RT \ln \frac{f_C k_C C_C f_D C_D}{f_A k_A C_A f_B C_B} + nEF + RT \ln K \right] \Delta m$$

where  $C_A$ ,  $C_B$ ,  $C_C$  and  $C_D$  are the concentrations and  $f_A$ ,  $f_B$ ,  $f_C$  and  $f_D$  are the activity coefficients of  $A$ ,  $B$ ,  $C$  and  $D$ .  $k_A$  and  $k_C$  are the specific activities of  $A$  and  $C$ .  $E$  is the electrical potential difference between  $A$  and  $B$ , and  $C$  and  $D$ ,  $n$  is the net number of electrical charges transferred from left to right in equation (1) and  $RT$  and  $F$  have their usual significance. If  $A$ ,  $B$ ,  $C$  and  $D$  are present in the same solution  $E$  is, of course, zero, but the electrical potential may be relevant in an electrode reaction or if  $A$  and  $B$ , and  $D$  are separated by a membrane.  $K$  is the equilibrium constant. The expression follows from a consideration of the entropy changes based on the probabilities of the systems and the assumption that the total energies are unaffected by the isotopic composition. Let there be equilibrium with respect to the isotopic molecules of  $A$  and  $C$ . Then:

$$RT \ln \frac{f_C k_C C_C f_D C_D}{f_A k_A C_A f_B C_B} + nEF + RT \ln K = 0 \quad (2)$$

If  $m_{A \rightarrow C}$  and  $m_{C \rightarrow A}$  are the fluxes for the conversion of  $A$  into  $C$  and vice versa, it is also possible to write:

$$\text{flux of radioactive } A \text{ to } C = k_A m_{A \rightarrow C}$$

and:

$$\text{flux of radioactive } C \text{ to } A = k_C m_{C \rightarrow A}$$

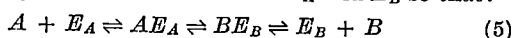
Since there is equilibrium between the radioactive  $A$  and  $C$  molecules the two fluxes should be equal so that:

$$k_A m_{A \rightarrow C} = k_C m_{C \rightarrow A} \quad (3)$$

From equations (2) and (3):

$$\frac{m_{A \rightarrow C}}{m_{C \rightarrow A}} = \frac{f_A C_A f_B C_B}{f_C C_C f_D C_D} \cdot \frac{1}{K} e^{nEF/RT} \quad (4)$$

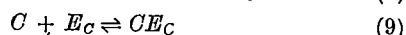
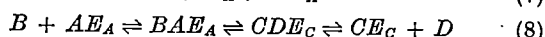
If equation (4) is not obeyed there must be directly or indirectly some interaction between the radioactive and non-radioactive molecules of  $A$  and  $C$  in such a way that some energy is exchanged. If the left-hand side of equation (4) is less than the right-hand side the situation is equivalent to 'exchange diffusion'<sup>1</sup> and either the conversion of one  $A$  molecule to a  $C$  molecule must facilitate the conversion of another  $C$  molecule to an  $A$  molecule, or the conversion of an  $A$  molecule to a  $C$  molecule must interfere with the conversion of another  $A$  molecule to a  $C$  molecule (but not at the same time interfering with the back reaction). A molecular 'shuttle' could give rise to 'exchange diffusion' by either of these processes. Consider an enzyme catalysing a reaction  $A \rightleftharpoons B$  and suppose that the enzyme exists in two forms  $E_A$  and  $E_B$  so that:



and:

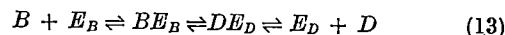
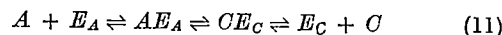


Then it may be shown that, if reaction (6) is at all rate-limiting, exchange diffusion will occur: this is most likely when the concentrations of  $A$  and  $B$  are high. With the bimolecular reaction shown in equation (1) the situation is more complicated. Let the two forms of the enzyme be denoted by  $E_A$  and  $E_C$  then one possible sequence is:



If reaction (10) is at all rate-limiting the interconversions of  $A$  and  $C$ , and  $B$  and  $D$  will both show 'exchange diffusion' and this will be most marked when the concentrations of  $A$ ,  $B$ ,  $C$  and  $D$  are high. If reaction (10) is not rate-limiting, 'exchange diffusion' will not occur between

$A$  and  $C$ ; but if either or both of the reactions (7) and (9) are rate-limiting, exchange diffusion will occur between  $B$  and  $D$  and will be most apparent when the concentrations of  $B$  and  $D$  are high. When  $A$  and  $B$  combine with the enzyme in random order it is necessary to consider all possible pathways: overall exchange diffusion will occur if one or more pathways show the phenomenon. Other reaction sequences are possible, for example:



where  $E_A$ ,  $E_B$ ,  $E_C$  and  $E_D$  are different forms of the enzyme. If reactions (12), (13) or (14) are at all rate-limiting there will be exchange diffusion between  $A$  and  $C$ ; and exchange diffusion will, of course, occur in extreme form if  $B$  and  $D$  are omitted.

If the left-hand side of equation (4) is larger than the right-hand side the situation is equivalent to the 'long pore effect', and the conversion of an  $A$  molecule to a  $C$  molecule must either facilitate the conversion of another  $A$  molecule to a  $C$  molecule or interfere with the conversion of a  $C$  molecule to an  $A$  molecule. Two molecular mechanisms might show this effect. Thus it might occur if more than one molecule of substrate had to become attached to the enzyme before reaction could occur to give more than one molecule of product (cf. polyvalent carriers)<sup>2</sup>. Alternatively there might be a sequence of reaction sites on the enzyme that the substrate had to traverse, and the intermediate products might be unable to leave the enzyme. The effect in the latter case would be apparent at high concentrations and would be the equivalent of diffusion through a long pore<sup>4</sup>.

The test proposed in this communication is related to the measurement of isotope exchange rates at equilibrium<sup>6-9</sup>. The interpretation of exchange rates, however, may be complicated by inhibition (which may be non-specific) of the enzyme by the substrates whereas such inhibition will not affect the flux ratio. The flux ratio should give some indication of the rate-limiting steps and the order of addition of the reactants. If the reaction steps are known some estimate of the rate constants and affinity constants should also be possible.

It will be noted that the enzymes have been depicted as existing in two or more forms. The assumption that the free enzyme exists in only one form (or that the different forms rapidly interconvert) does not necessarily seem to be justified. It is conceivable that an activation energy for the interconversion of the free forms of the enzyme might allow the activation energy for the other reaction steps to be smaller. To show 'exchange diffusion' for an isomerizing enzyme (reaction (6)) would be good evidence of the existence of two forms of the enzyme unless special reactions purely for exchange<sup>9-12</sup> are postulated.

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## Reverberatory Inhibitory Circuits in the Lateral Geniculate Nucleus of the Rat

A PROMINENT feature of synaptic transmission through the lateral geniculate nucleus (LGN) in the cat<sup>1-3</sup> and in the rat<sup>4</sup> is the large and prolonged depression of transmission which occurs following a single shock to the optic nerve. In the work reported here an attempt was made to discover the cause of this depression.

Extracellular records have been obtained from single cells of the LGN in the rat, anaesthetized with paraldehyde or pentobarbital, using glass microcapillary electrodes filled usually with 3 M KCl. Electrical stimuli were applied to the contralateral optic nerve or the ipsilateral visual cortex. Cells were recognized by the criteria of Bishop, Burke and Davis<sup>5</sup>. The cells fall conveniently into two groups:

(1) *P* cells, believed to be the principal cells of the LGN, constitute 88 per cent of the 255 cells investigated, and have the following characteristics. In response to a single shock to the optic nerve they discharge once at short latency (mode 3.2 msec, 207 cells; Fig. 1*a*) and usually later also in a series of bursts (up to 6), occurring at more or less regular intervals (Fig. 1*b*). The number of discharges per burst is usually 2-4 and the interval between bursts ranges from 100 to 1,360 msec. The total duration of the discharge is usually 1-2 sec, but some cells continue to fire for as long as 5 sec. In many cells the longer intervals are multiples of the shortest interval, suggesting an underlying rhythm which does not always fire off the cell. *P* cells are also discharged by cortical stimulation and in most cases either at short latency or with a wave-form characteristic of antidromic invasion<sup>6</sup> or both. Furthermore, the single antidromic response is then followed by a series of bursts in a pattern which is closely similar to that produced by orthodromic activation. Finally, it is an important point that the bursts can usually be elicited by stimuli, either orthodromic or antidromic, which are sub-threshold for the early response.

(2) *I* cells, believed to be inhibitory interneurons, represent 12 per cent of all cells, with the following characteristics. In response to a single shock to the optic nerve they discharge at short latency with a burst of about 10 spikes (Fig. 1*c*). The mode of the latencies of the first spike is 4 msec (30 cells). The first burst is then followed by similar bursts at intervals comparable with those in the pattern of *P* cell firing (Fig. 1*d*). *I* cells are also discharged by cortical stimulation, but in no case does the extracellular wave-form indicate antidromic invasion<sup>6</sup>.

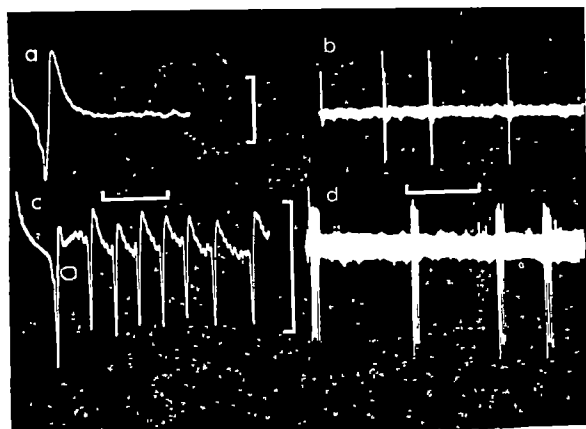


Fig. 1. Extracellular records from single LGN cells in the rat. *a* and *b*, *P* cell discharge; *c* and *d*, *I* cell discharge, both in response to a single shock to optic nerve. *a* and *c*, short latency discharge (time calibration 5 msec between the records). *b* and *d*, short latency discharge followed by later bursts, on slower timebase (time calibration 200 msec between the records). Voltage calibration 1 mV, upper applies to *a* and *b*, lower to *c* and *d*.

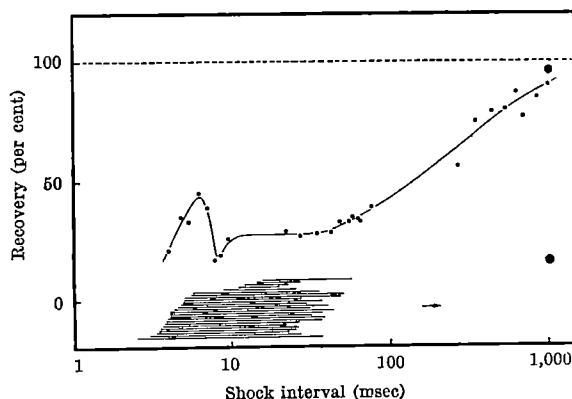


Fig. 2. Recovery curve of postsynaptic field (multineuronal) response in LGN of rat. Two stimuli were applied to the optic nerve, the first (conditioning) supramaximal for the fast group of optic fibres (ref. 4), the second (test) about 50 per cent maximal, and the interval between the two systematically varied. The points on the graph plot the amplitude of the conditioned test response as a percentage of the unconditioned test response. The bars in the lower part of the figure indicate the durations and times of occurrence of the short latency response in 25 *I* cells. Arrows at the end of some bars indicate that the burst continued for an undetermined time. The larger arrow to the right indicates the earliest commencement of a second burst in any *I* cell.

A typical example of the time-course of depression of the field response in the LGN following a single shock to the optic nerve is shown in Fig. 2. A similar curve may be obtained if the conditioning stimulus is applied to the cortex and the test stimulus to the optic nerve. Curves have also been obtained for single *P* cells in which the proportion of times the cell fired was plotted against the interval after the conditioning shock; in some cells inhibition was demonstrated after a conditioning stimulus insufficiently strong to evoke the response of the cell itself. The curves show considerable variation in time-course from one cell to another, a few cells recovering fully in about 40 msec, others only after 5 sec. 10 per cent of *P* cells show an early recovery, between 3 and 10 msec, and subsequent depression similar to the field recovery curve. These curves show that the *P* cell recovers at or somewhat before the time of the first burst and is usually again depressed (even if the first burst fails to appear), although to a smaller extent, before recovering once more. Several oscillations in the recovery curve are frequently seen. In the field recovery (Fig. 2) the oscillations due to individual *P* cell recovery tend to disappear because of asynchrony. Recovery curves of *I* cells are remarkably similar to each other; almost all cells recover fully in about 160 msec and thereafter the curves show no oscillation.

It is unlikely that this inhibition is due to presynaptic inhibition<sup>7,8</sup> because (a) the excitability of the optic nerve terminals in the LGN is increased only to a very small degree following strong repetitive stimulation of either optic nerve or cortex (testing by the method of Wall<sup>9</sup>); (b) a synaptic potential<sup>10</sup> appears in response to a second shock at the shortest interval that can be measured (about 3.2 msec) and reaches a maximum height in less than 20 msec, thereafter not significantly altering in magnitude until the spike reappears; (c) the inhibition is equally effective on the injury discharge of a cell caused by mechanical stimulation from the electrode.

These facts, and some others to be mentioned below, are most easily explained by supposing that the *P* cells are monosynaptically activated from the optic nerve and project to the visual cortex (that is, are principal cells), that collaterals of these cells synapse with the *I* cells which in turn project back on to the *P* cells; that the prolonged discharge of the *I* cells causes a hyperpolarization of the *P* cells. This recurrent inhibition would be identical with that produced on the spinal motoneurone via the Renshaw cell<sup>11</sup>. It is further postulated that, as the hyperpolarization of the *P* cell wanes, the cell tends to



discharge again, possibly because of post-anodal exaltation. This second discharge of the *P* cells in turn re-excites the *I* cells and so the process continues. However, initially the *P* cells discharge synchronously and so the discharge of the *I* cells is also fairly synchronous, producing a deep depression. The relationship of the *I* cell discharge to this inhibition is shown in Fig. 2. The second and succeeding bursts become more and more asynchronous so that the hyperpolarization of the *P* cells presumably becomes smaller although more prolonged, with the result that the *P* cells cease to discharge and then in turn so do the *I* cells. As the *I* cell discharge becomes asynchronous and individual *I* cells cease to discharge, the overall level of inhibition falls off, as shown in the field recovery curve (Fig. 2).

This hypothesis is very similar to that proposed by Andersen and Eccles<sup>12</sup> for the ventrobasal complex of the thalamus, the only difference being that in this nucleus the postsynaptic inhibition appears to be due to a single inhibitory postsynaptic potential (IPSP)<sup>13</sup>. It would seem also that our explanation would apply to the LGN in the cat since recurrent collaterals have been described there<sup>14-16</sup>, rhythmic bursts may be elicited either from optic nerve<sup>17,18</sup> or cortex<sup>18</sup>, and after-positivity and associated inhibition follow both methods of stimulation<sup>19</sup>. A grouping into *P* and *I* cells may also be possible<sup>20</sup>, although the distinction is not so clear-cut in the cat because the *P* cells commonly discharge 3 or 4 times at short latency while the proportion of *I* cells appears to be lower than in the rat. Recurrent inhibition of this type has also been described in the cerebral cortex<sup>21</sup> and hippocampus<sup>22</sup>.

Since the recovery of the *I* cells to optic nerve stimulation is presumed to be dependent on the recovery of the *P* cells, the fairly uniform recovery of the *I* cells at about 160 msec suggests that they all receive a fairly similar innervation and therefore probably a strong convergence from the *P* cells. This conclusion is supported by the fact that strong inhibition can be produced in the LGN even by weak stimulation of the optic nerve or visual cortex.

As suggested by Andersen and Eccles, this type of reverberatory circuit may provide the basis for slow rhythms throughout the brain. We agree also with their suggestion that the inhibitory discharge is widely distributed to the principal cells, and would add the suggestion made in the preceding paragraph that there is also a strong convergence of *P* cells on to *I* cells. This circumstance would provide an explanation for spindles in the EEG record. If we suppose that in a period of quiescence one or a few *P* cells can be excited, their discharge will trigger off a comparatively large number of *I* cells, which in turn will hyperpolarize a still larger number of *P* cells and these will discharge on their recovery phase, leading to still more widespread activation of *I* cells. In this way the process could wax to a maximum and then wane again, for the reasons given earlier. This scheme might also explain why Bishop and Davis<sup>18</sup> obtained rhythmic slow waves in the LGN of the cat to optic nerve stimulation even when synaptic transmission had been blocked. If only a few *P* cells remain unblocked the process could 'spindle'. It is supposed, of course, that slow brain waves are made up of alternating phases of hyperpolarization and spike discharge, the latter appearing as a 'spike envelope'.

The hypothesis also explains other observations: (1) in the cat, the reduction in the duration of the short latency burst as the stimulus to the optic nerve is increased<sup>20,23</sup> could be due to the earlier onset or, more probably, the steeper rise of the summing IPSP's; (2) in the rat, the fact that the short latency response of the *P* cell is a single spike whereas later bursts contain 2-4 spikes could be due to the steeper onset of the first inhibitory phase; (3) in the rat, the existence of some 'labile' *P* cells which do not fire consistently at short latency even with stimuli

well above 'threshold', as well as *P* cells which do not fire early at all, for the same reason.

Finally, we now have extracellular and intracellular recordings from *P* cells showing summing IPSP's, the oscillations corresponding in number and frequency to the pattern of discharge of *I* cells. These findings will be reported in more detail later.

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### Absence of Bile Acids in the Digestive Juice of the Swamp Crayfish (*Procambarus clarkii*)

ACCORDING to our present knowledge, bile salts are excreted in bile as the major metabolic product of cholesterol in vertebrates, whereas there has not yet been found any kind of steroid bile salt in invertebrates. Although Vonk<sup>1</sup> suggested that taurodeoxycholic acid might be present in the digestive juice of some crayfish, his experimental data were not entirely convincing.

Vonk's suggestion, if confirmed, would be of great interest for comparative biochemistry, and we have therefore examined it experimentally. The materials investigated were the digestive juice (*material I*) and the whole intestinal tract (*material II*) obtained from the swamp crayfish, *Procambarus clarkii*.

**Material I:** After each crayfish had been starved for 1 day, the digestive juice was sucked up from its stomach with a syringe and pooled in a vessel containing an aliquot of alcohol. The juice (20-30 ml.) obtained from 100 animals was worked up as one run. The alcoholic extract was hydrolysed (15 per cent w/v potassium hydroxide solution, 130° C, 3 h); and, after being freed from neutral lipids, it was extracted with ether after acidification.

The acidic fraction thus obtained was examined by our enzymatic method<sup>2</sup> using C-3-hydroxysteroid dehydrogenase<sup>3</sup>. Repeated experiments showed that the amount of NAD-reducing substance in each juice sample was only within experimental error.

**Material II:** Pieces (total wt., 3.22 g) of intestinal tract obtained from 50 crayfish were homogenized together with

their contents and extracted with methanol. After being freed from neutral lipids as usual, the acidified extract was extracted with butanol. This extract (*material II<sub>1</sub>*), after evaporation, was hydrolysed in the same way as *material I*, giving *material II<sub>2</sub>*.

One of us had already reported<sup>4</sup> that the digestive juice of swamp crayfish contains taurochenodeoxycholic acid instead of taurodeoxycholic acid, because the alcoholic extract of that juice showed an ultra-violet absorption maximum (at 308 m $\mu$ ) similar to that of chenodeoxycholic acid, when treated with concentrated sulphuric acid (Kazuno *et al.*<sup>5</sup>). This finding was definitely confirmed by our repeated experiments using both *materials I* and *II*. It is, therefore, certain that both samples contained the substance that Hozumi assumed to be taurochenodeoxycholic acid. *Material II<sub>2</sub>* (as acetyl methyl ester), however, showed a quite different curve from that of the diacetyl methyl ester of chenodeoxycholic acid when treated with 70 per cent sulphuric acid solution, as shown in Fig. 1. It must be noted here that *material II<sub>2</sub>*, even when not treated with sulphuric acid, showed a strong absorption maximum near 270 m $\mu$ .

Decisive evidence that the digestive juice does not contain any kind of common bile acid was given, not only by the aforementioned enzymatic determinations, but also by thin-layer chromatography and by gas chromatography.

As shown in Fig. 2 (a), the butanol extract of *material II<sub>1</sub>*, once purified by reversed phase chromatography, showed a single spot running at a different rate from taurochenodeoxycholic acid. This spot might correspond to that obtained by paper chromatography in our early experiments, which had been scarcely distinguished from the spot given by taurochenodeoxycholic acid. The

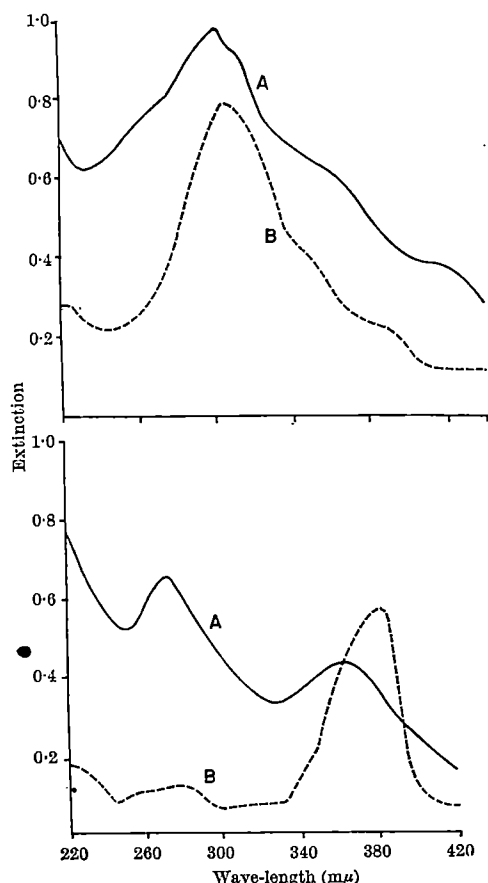


Fig. 1. The ultra-violet absorption curves of *material II<sub>2</sub>* (acetyl methyl ester) (A) and diacetyl methyl chenodeoxycholate (B). Above: treated with conc.  $H_2SO_4$ ; below: treated with 70 per cent w/v  $H_2SO_4$ : water

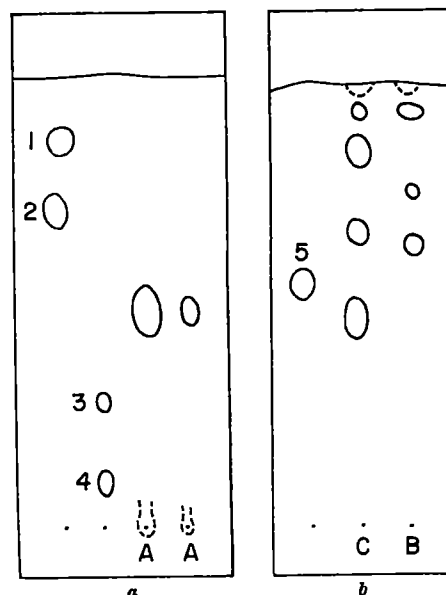


Fig. 2. a, Thin-layer chromatogram of *material II<sub>1</sub>* (A). Solvent system: ethyl acetate:glacial acetic acid:methanol (80:10:10, by vol.). b, Thin-layer chromatogram of *material II<sub>2</sub>* (B). B: methyl ester; C: acetyl methyl ester. Solvent system: benzene, ethyl acetate (40:60, by vol.). Standards for both chromatograms: glycochenodeoxycholic acid (1); glycocholic acid (2); taurochenodeoxycholic acid (3); taurocholic acid (4); methyl lithocholate (5)

acidic fraction obtained from the hydrolysate (*material II<sub>2</sub>*), and the acetyl methyl ester of this, however, showed unexpectedly 3 or 4 spots instead of a single spot on a thin-layer chromatogram; these spots were found to be quite different from the corresponding spot given by chenodeoxycholic acid and its esters (Fig. 2 (b)).

It must be noted that there was found, among these, a spot near that given by lithocholic acid acetyl methyl ester, but not near that given by diacetyl methyl chenodeoxycholate.

Since both the methyl ester and acetyl methyl ester of *material II<sub>2</sub>* had an aromatic odour, the latter ester was subjected to steam distillation. It was found that the non-distillable fraction showed a single spot on the chromatogram near the spot given by the acetyl methyl ester of lithocholic acid.

The foregoing fraction was further subjected to gas chromatography (type: Shimadzu A-2; column: 2 per cent SE-30, 2.5 m in length; temp. 215° C), in comparison with the acetyl methyl ester of lithocholic acid. The results indicated that this fraction was not a single material but developed at least three peaks, all having retention times much less than that of acetyl methyl lithocholate.

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### An Action Spectrum for the Fire Ant (*Solenopsis saevissima*)

EVALUATION of the visual system of ants is important for a comparative analysis of photoreceptor processes in animals. The honeybees have been the subject of numerous visual studies, but ants have been virtually ignored. We have begun an extensive investigation into the spectral responses, eye structure, and visual pigments of ants, and here report our results on the action spectrum.

In previous attempts to obtain an action spectrum for ants, poorly controlled stimuli and filters with broad transmission bands precluded accurate measurements. However, ants were shown to react strongly to ultra-violet light and to be insensitive to red light<sup>1,2</sup>.

The ants used in the present study were from a laboratory colony of fire ants (*Solenopsis saevissima*), generously supplied by Dr. E. O. Wilson of Harvard University. After feeding in the same location for several days, with continuous illumination (control light) on one side of the nest, the ants learned to use the light as a source for orientation in the fashion of the Sun compass reaction. When the control light was turned off, then turned on again, foraging ants became alarmed and began running towards the nest. When the light was moved to the opposite side of the nest (stimulus light), the ants would reverse direction.

Although foragers following odour trails would reverse direction along the trail when the control light was changed directly to the stimulus light, so many of them became alarmed that the alarm response so evoked was more easily and accurately observed. All the alarmed foragers reversed direction when the stimulus light was the same intensity as the control light. As the intensity of the stimulus light was reduced, the percentage of ants reversing direction decreased. The intensity response curve illustrated in Fig. 1 was sigmoid, and the percentage of response was a nearly direct function of log intensity over the greater part of the curve.

For each monochromatic test light, the maximal intensity which produced just less than 10 per cent

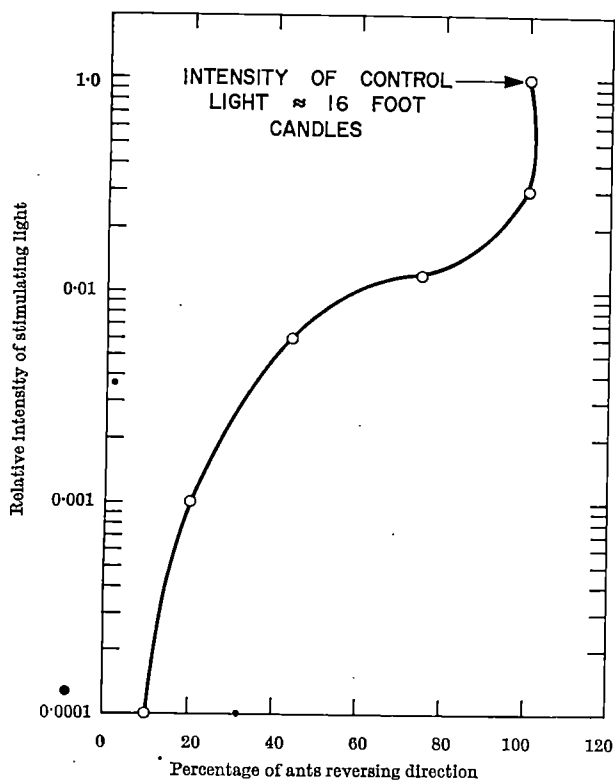


Fig. 1

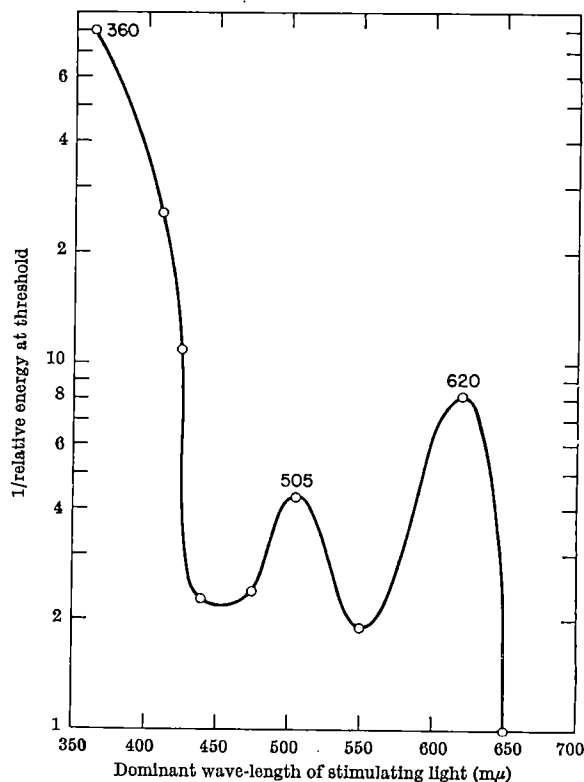


Fig. 2

positive responses, or fewer than 3 out of 25 ants reversing direction, was chosen as threshold.

The stimulus and control light were 60-W General Electric lamps encased in dark boxes with a 2.5-cm<sup>2</sup> opening. They were placed on opposite sides of the nest, which was on a glass platform covered with white paper. These were the only lights in a dark room except for a dim red overhead light.

The wave-length of the stimulus light was varied with combinations of Corning interference filters which pass relatively narrow bands of monochromatic light. Intensity was adjusted with Kodak neutral density filters. Determination of the dominant wave-length and calculation of the relative energies of the different monochromatic lights are discussed by Wolken *et al.*<sup>3,4</sup>.

Since the turning response is a clear and direct function of light intensity (see Fig. 1), we are justified in assuming that different response thresholds for various wave-lengths represent differences in physiological intensity.

Fig. 2 illustrates the action spectrum for the fire ant. The major response was found for ultra-violet light near 360 mμ. This confirms the results of earlier studies of ants and other insects, such as the honeybee, housefly and cockroach<sup>5</sup>. In the visible region of the spectrum, the response peak at 505 mμ is similar to the spectral sensitivity peak found in a wide variety of insects<sup>5,6</sup> and other animals. Sensitivity in this region of the visible spectrum has been associated with absorption characteristics of vertebrate rhodopsins.

While previous studies suggested that ants were not sensitive to red light, we found that the fire ant had an additional response peak near 620 mμ. In a study of colour preference of the odorous house ant, *Tapinoma sessile*, a similar response peak was found in the near red region. Such responses in the near red have been found in honeybees as well as in such lower invertebrates as protozoa and coelenterates. Whether this reflects receptor sensitivity or is due to decreased absorption at lower frequencies by the ommochromes or pigment granules that surround the retinula cells is not clear at present. Preliminary microspectrophotometric studies of the absorption spectra of

ommochromes suggest that they function as cut-off filters that pass some near-red but no other visible light.

The action spectrum must be distinguished from photoreceptor sensitivity. Response peaks cannot be directly equated with either receptor types or the absorption characteristics of the photosensitive eye pigment. However, the action spectrum seems to be a close reflexion of the summated sensitivity of all receptor types. Those wave-lengths where the response is greatest are the wave-lengths where the total sensitivity is greatest but not necessarily characteristic of the sensitivity peak of any one type of photoreceptor<sup>7</sup>.

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## Acid Secretion and Electrogenesis in Isolated Rat Stomach

THE physiological aspects of acid secretion have been investigated for many years in the dog<sup>1</sup>, while Teorell's work in the cat has provided much information on the relation to CO<sub>2</sub> of various ions involved in secretion<sup>2</sup>. There have been extensive *in vitro* investigations of the transport mechanisms in frog tissue<sup>3</sup>, and it has been shown that electrical phenomena in these preparations are substantially dependent on chloride ions<sup>4,5</sup>. In contrast, more recent *in-vitro* experiments with rat tissue<sup>6</sup> show that gastric bioelectric activity depends on sodium ion movement. Because it was possible to characterize a sodium-dependent mechanism in the rat by substitution and ionic flux determinations<sup>7</sup>, we decided to investigate the relation between acid secretion and the sodium-specific transmural potential. The evidence, described here, suggests that changes in potential and in acid secretion are essentially independent of each other.

Electrical measurements were based on the method of Ussing and Zerahn<sup>8</sup>, using a chamber modified for rat stomach<sup>9</sup>. Acid or base formed by the stomach was directly titrated potentiometrically in the chamber, with simultaneous recording of the transmural potential. The titration end-point was a null electrometer reading between glass and calomel electrodes. Electrical balance was adjusted by means of a Zener stabilized power supply, a method shown by others to measure very small pH changes<sup>10</sup>. The medium was maintained at 34° C and at pH 6.3. Eighty ml. of a Krebs-Ringer type solution was added on each side of the tissue. The solution consisted of: 148 mM Na<sup>+</sup>, 5.6 mM K<sup>+</sup>, 1.4 mM Mg<sup>++</sup>, 3 mM Ca<sup>++</sup>, 157 mM Cl<sup>-</sup> and 7 mM glucose. The medium was gassed with pure oxygen. In the substitution

experiments, the appropriate ion was replaced by an osmotically equivalent amount of choline (for sodium) or sulphate (for chloride). Lactate was determined enzymatically<sup>11</sup>.

Fig. 1 compares both titratable acid and base production of rat stomach to the transmural potential of the unstimulated stomach. Both acid and base production continue to increase linearly with time, whereas potential rises to a peak at 60 min and declines thereafter. This indicates a difference in the source of the electrical and acid parameters. The average value obtained for acid secretion amounts to 10  $\mu$ equiv./h, a value similar in magnitude to that reported for the undisturbed rat stomach *in vivo*<sup>12</sup>. More recently, higher rates have been reported<sup>13</sup>, but the pH of titration was not stated. Since we observe markedly higher rates of acid secretion at pH 7.4 and greater, in part reflecting the titration of other groups as indicated by others<sup>14,15</sup>, no direct comparison can be made between these reports.

It is not surprising that base is formed on the serosa, since Davies and Longmuir<sup>16</sup> have shown that base is formed near the mucosa during acid secretion. That there is not a stoichiometric amount of base produced may be due to a limited diffusion of base through the muscular layers of the excised tissue, or to hindrance of base migration caused by the tying of part of the serosa on the lip of the holder. Furthermore, some of the base formed may be neutralized by lactic acid, which can amount to 20 per cent of the total titratable acid (Table 1).

Table 1. EFFECT OF SELECTIVE SUBSTITUTIONS AND HISTAMINE ON ACID SECRETION AND BIOELECTRIC POTENTIAL

Medium	n	Time <i>in vitro</i> of measurements (min)	Titrateable acid (mucosa) ( $\mu$ equiv./h)	Titrateable base (serosa) ( $\mu$ equiv./h)	Potential at 60 min (mV)
Krebs-Ringer (control)	8	30-90	9.8	4.7	19
Choline Ringer	4	30-90	10.4	5.3	-1
Sulphate Ringer	3	30-90	10.2	3.4	21
Krebs-Ringer + histamine $5 \times 10^{-4}$ M	4	60-120	13.3	7.0	*20
			Lactate ( $\mu$ equiv./h)	Lactate ( $\mu$ equiv./h)	
Krebs-Ringer	4	30-90	2.1	1.0	—

\* Value at 70 min, 10 min after addition of histamine; not significantly different from control.

Table 1 shows the effect on acid secretion of substituting choline for sodium. Potential can be eliminated by this substitution, as was demonstrated previously<sup>4</sup>, but the amount of acid or base formed is not affected (Table 1). This result indicates that the secretion of acid is not electrogenic in the sense of being directly related to potential. Substitution of sulphate for chloride did not affect the rate of acid secretion, although it did lower the rate of base formation. This may be due to a restriction of base migration across the tissue by decreasing exchange.

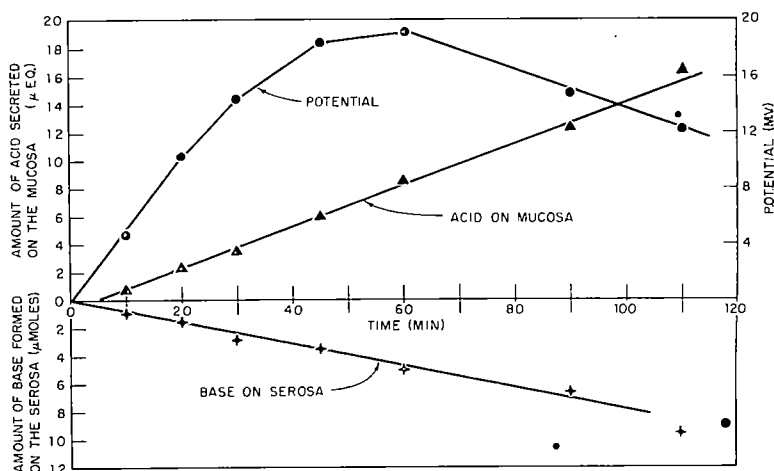


Fig. 1. Comparison of bioelectric potential with acid and base formation in rat stomach. Titration was performed directly in the chamber as described in text and potential was simultaneously recorded.



able chloride ion, as indicated by Teorell<sup>2</sup>. Durbin found that acid secretion is lowered in frog gastric mucosa in sulphate media<sup>17</sup>; it follows that frog tissue differs from rat tissue in the ionic requirements for acid secretion, as well as in transmural potential.

Histamine ( $5 \times 10^{-4}$  M) increases acid secretion from 10 to 13  $\mu$ equiv./h and base formation from 4 to 7  $\mu$ equiv./h (Table 1). Lower concentrations of histamine are less effective. In addition, the increment in acid secretion will vary from 0 to 6  $\mu$ equiv./h, an inconsistency also noted in frog<sup>18</sup>. Unlike frog, the degree of stimulation in rats is never dramatic, perhaps reflecting the continuous acid secretion in rat. Histamine does not affect the potential; therefore, acid production can be affected without changing the sodium-dependent electrogenic mechanism.

Most investigations on acid secretion *in vitro* have been performed on frog tissue, but the experiments described here show that acid and base production can easily be determined in the rat. Both chloride and bicarbonate have been eliminated from the medium without affecting mucosal acid production, and it appears that there is no inter-relationship between acid secretion and transmural potential. The results suggest that further work on hydrogen ion movement in rat tissue, in relation to the mechanism and control of acid secretion, may be profitable.

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## Actinomycin D Inhibition of the Adaptation of Renal Glutamine-deaminating Enzymes in the Rat

PREVIOUS investigations have shown that metabolic acidosis leads to significant increases in the activities of glutaminase and glutamine transaminase- $\omega$ -amidase (GTA) in kidneys of rats<sup>1,2</sup> and guinea-pigs<sup>3</sup>. These changes are associated with, and thought to be related to, the adaptation of renal ammonia excretion to acidosis in the rat<sup>2,4</sup> and guinea-pig<sup>3</sup>. The response of renal glutaminase to metabolic acidosis is not mediated by the adrenal gland<sup>5</sup> or substrate, glutamine<sup>6</sup>, and is restricted to the kidneys<sup>6</sup>. Goldstein and Kensler<sup>5</sup> found that administration of ethionine to guinea-pigs inhibited the acid-induced rise in renal glutaminase activity, and we suggested that metabolic acidosis stimulates the synthesis of glutaminase by renal cells. The manner in which this stimulation occurs is unknown. Kroeger<sup>7</sup> has recently reported that alterations in the electrolyte balance of the medium bathing isolated salivary glands of the insect *Chironomus thummi* produced marked and specific changes in the gene activities

(RNA synthesis) of the giant chromosomes found in these glands. Metabolic acidosis and the accompanying changes in electrolyte balance might have similar effects on RNA synthesis in renal cells. Actinomycin D, an antibiotic which interferes with the DNA-dependent synthesis of RNA in bacterial and animal cells<sup>8</sup>, has been shown to inhibit the synthesis of RNA in rat kidneys<sup>9</sup>. The effects of this antibiotic on the responses of renal glutaminase, GTA and ammonia excretion to metabolic acidosis were therefore investigated in the rat.

Male Sprague-Dawley rats, 250–300 g, were kept in individual cages and maintained on Purina 'Lab Chow' and water *ad libitum*. The animals were divided into four experimental groups: (1) control rats injected with propylene glycol; (2) rats injected with actinomycin D; (3) acidotic rats injected with propylene glycol; (4) acidotic rats injected with actinomycin D. Rats were made acidotic by intragastric administration of 40 m.moles ammonium chloride/kg given in two equally divided doses over a period of 24 h. Actinomycin D (obtained from Dr. George E. Boxer, Merck, Sharp and Dohme Research Laboratories), 200  $\mu$ g/kg, was dissolved in 0.1 ml. propylene glycol and injected intraperitoneally in two equally divided doses at the same time that ammonium chloride was administered. All animals were in good condition at the end of the 24 h and the only significant changes observed were haemoconcentration and low rate of urine flow in group 4. Attempts to lengthen the experimental period to 48 h were unsuccessful, however, because of the high mortality rate in group 4 on the second day of treatment.

Rate of renal ammonia excretion was measured at the end of the 24-h period of treatment. Since urinary pH has a marked effect on renal ammonia excretion in the rat<sup>10</sup>, groups 1 and 2 were given 20 m.moles ammonium chloride in 20 ml. water/kg by stomach tube immediately before collection of urine in order to lower the pH of the urine of these two groups to the level of that observed in groups 3 and 4 (approximately pH 5.5). Groups 3 and 4 received an equal volume of water by stomach tube. The rats were then placed in metabolism cages and urine was collected for 4 h. Urine pH was measured by use of a Beckman 'Expanded Scale' pH meter and urinary ammonia concentration was determined by a microdiffusion-colorimetric technique<sup>5</sup>. Immediately after the period of urine collection, the rats were killed, and slices of renal cortex were removed and homogenized in 19 volumes of ice-cold water. The homogenate was centrifuged for 10 min at 500 g and 2° C to remove unbroken cells and nuclei. The supernatant fraction was removed from the precipitate and analysed for protein, glutaminase and GTA activities. Protein concentration was determined by use of the biuret reagent. Glutaminase activity was assayed by the procedure of Rector *et al.*<sup>4</sup> and GTA by the method of Goldstein *et al.*<sup>3</sup>

The results of these investigations are shown in Table 1. Metabolic acidosis produced a 55 per cent increase in renal cortical glutaminase activity and approximately an 80 per cent elevation in renal glutamine transaminase- $\omega$ -amidase activity within 24 h with no significant effect

Table 1. EFFECT OF ACTINOMYCIN D ON THE RESPONSES OF RENAL GLUTAMINASE AND GLUTAMINE TRANSAMINASE- $\omega$ -AMIDASE ACTIVITIES AND RENAL AMMONIA EXCRETION TO METABOLIC ACIDOSIS IN THE RAT

Group	Protein (mg/g cortex)	Glutaminase ( $\mu$ moles NH <sub>3</sub> /g protein min)	Glutamine transaminase- $\omega$ -amidase ( $\mu$ moles NH <sub>3</sub> /g protein min)	Ammonia excretion ( $\mu$ moles/100 g body-wt. h)
Control	170 $\pm$ 11	184 $\pm$ 27	17 $\pm$ 2	37 $\pm$ 4
Actinomycin	156 $\pm$ 7	173 $\pm$ 16	15 $\pm$ 1	27 $\pm$ 2
Acidosis	180 $\pm$ 9	287 $\pm$ 24*	31 $\pm$ 1†	58 $\pm$ 5*
Acidosis and actinomycin	180 $\pm$ 11	177 $\pm$ 12	21 $\pm$ 2	57 $\pm$ 7*

Values significantly different from control group are marked \* ( $P < 0.05$ ) or † ( $P < 0.01$ ).

Rats were made acidotic by intragastric administration of 40 m.moles ammonium chloride/kg in two equally divided doses over a 24-h period. Actinomycin D, 200  $\mu$ g/kg, was injected intraperitoneally in two equally divided doses at the same time that ammonium chloride was administered. Values are mean  $\pm$  S.E. of five rats per group.

on protein concentration. Administration of actinomycin D completely suppressed the acid-induced rise in renal glutaminase activity and produced a marked depression in the response of renal GTA activity. The antibiotic had no significant effect on basal protein concentration and enzyme activities (Table 1). The degree of acidosis was similar in rats receiving ammonium chloride plus actinomycin as compared with those receiving ammonium chloride alone, since the pH of the urine 20 h after administration of the final dose of ammonium chloride was  $5.5 \pm 0.1$  (mean  $\pm S.E.$ ) in the former group and  $5.7 \pm 0.1$  in the latter group. The inhibition of the adaptations of renal glutaminase and GTA by actinomycin appears, therefore, to be a specific effect of the antibiotic on the kidney. These results support the hypothesis that metabolic acidosis stimulates the production of specific ribonucleic acids by the renal cells which are involved in the synthesis of glutaminase and GTA. Other investigators<sup>11</sup> have shown that actinomycin blocks the glucocorticoid-induced rises in a number of hepatic enzymes, and Karlson<sup>12</sup> has suggested that some hormones may produce their effects by alteration of nuclear RNA synthesis. It may be that a number of general stimuli that have marked effects on cellular metabolism in higher organisms, such as hormones, diet and electrolyte balance<sup>13</sup>, exert their effects by means of the nucleus.

As shown in Table 1, metabolic acidosis produced approximately a 55 per cent increase in rate of renal ammonia excretion. Administration of actinomycin had a slight depressive effect on basal ammonia excretion but had no significant effect on the response of ammonia excretion to metabolic acidosis. The latter result was unexpected, since the rises in renal glutaminase and GTA activities, which are thought to play a part in the adaptation of renal ammonia excretion in the rat<sup>3,4</sup>, were suppressed by the antibiotic. These results indicate that adaptation of renal ammonia excretion can occur without a corresponding increase in activities of renal glutamine deaminating enzymes, but it is possible that actinomycin produced a secondary change(s) in acidotic rats that might have increased the rate of ammonia excretion. Two factors which might have been altered by actinomycin, blood ammonia and glutamine concentrations, were found to be similar in all four groups. In support of the theory that an increased production of urinary ammonia can occur during metabolic acidosis without changes in renal enzyme activities is the observation that the acid-induced adaptation of renal ammonia excretion observed in the dog<sup>14</sup> is unaccompanied by increases in renal glutaminase and GTA activities. It remains to be seen whether other examples of divergence between renal ammonia excretion and glutamine deaminating enzyme activities will be found in the rat.

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## PHARMACOLOGY

### Significance of Worm Shifts in Experimental Schistosomiasis mansonii, with Emphasis on the Action of Anaesthetics

ALL schistosomicidal drugs eventually cause a change of location or 'shift' of the worms from the mesenteric veins, where they are normally found in infected animals, into the liver. There the parasites ultimately die and are ensheathed and phagocytosed by the protective mechanisms of the host. These worm migrations may be very rapid in onset and short in duration, as occurs with antimonials<sup>1</sup>; or they may be slow in onset and permanent in duration, as occurs with diphenoxylalkanes<sup>2</sup>. Various other schistosomicidal drugs produce 'hepatic shifts' with intermediate characteristics. The cause of the worm migrations differs with the different drugs, but essentially it results in paralysis of the worms which are thereafter swept back by the blood stream into the liver. Where the course of treatment is inadequate, the parasites regain their muscle control and return to the mesenteric veins.

It should be emphasized that whereas all schistosomicides sooner or later induce a hepatic shift, not all drugs that cause such a change of worm locality are of any use as schistosomicides. This paper deals with some drugs of this sort, which are pharmacologically active, yet of no clinical importance in schistosomiasis. Of these, perhaps the most worthy of mention is the group of general anaesthetics.

Table 1. EFFECT OF ETHER ON WORM DISTRIBUTION

	Av. No. worms/mouse	Per cent distribution in:		
		Mesenteric veins	Portal vein	Liver
Autopsy after killing with ether	11	0	6	94
Recovery after anaesthesia:				
0.5 h	11	0	7	93
2 h	11	59	20	21
Control	12	76	16	8

When ether is administered to mice infected with *S. mansonii*, a major shift of the worms from the mesenteric veins into the liver occurs more or less immediately as anaesthesia sets in (Table 1). The 'hepatic shift' lasts as long as the anaesthesia itself, and if the animal is allowed to recover, the parasites slowly migrate back. If anaesthesia is taken to a lethal level, a 100 per cent shift of the worms is seen at autopsy. These observations hold true for other inhalation anaesthetics, for example chloroform, as well as for the group of barbiturate drugs.

The mechanism of action of these drugs in inducing this worm behaviour is uncertain, but it could be due to a depressant action on the central ganglion of the parasite. In experimental schistosomiasis some earlier workers<sup>3,4</sup> killed their animals for autopsy using ether or chloroform, a procedure which must have undoubtedly compromised their results with regard to the worm distribution after drug treatment.

The clinical significance of this action of anaesthetics and central depressants should not be neglected. Bilharzial patients are apt to take these or other pharmacologically active drugs during everyday life; such drugs may keep the worms going to and fro in the hepatic portal system with possible undesirable complications. Portal block, which may have serious consequences, may follow the use of such drugs in heavily infected patients.

Testing a new drug for anti-schistosomal activity in experimental animals will invariably involve observation of worm distribution within the hepatic portal system after treatment. In such investigations the test animals should always be killed by cervical dislocation, or by some means other than anaesthesia. If the use of the latter is unavoidable, then its effects should be taken into consideration, not neglecting the fact that, although the worm shift to the liver is indicative of an action on the parasites, it is not necessarily evidence of schistosomicidal properties.

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## HAEMATOLOGY

### Two Populations of Platelets

It has been suggested that the tail frequently observed in a platelet survival study could be explained by a young population of platelets having a longer life span than the remainder<sup>1</sup>. The following experiments demonstrated the presence of two morphologically different populations, following the exposure of human platelets to osmotic stress.

Platelets were obtained from whole blood collected in 2 per cent ethylenediamine tetraacetic acid (EDTA) by differential centrifugation and then centrifuged at 2,000 r.p.m. for 10 min; after removal of the plasma the platelets were re-suspended in 0.85 per cent NaCl and re-centrifuged.

The platelets were then equally divided; one half was placed in distilled water and the other in 0.85 per cent NaCl to act as a control. At various intervals ranging from 10 sec to 1 h, samples were taken from both and, in the case of the distilled water treatment, were added to an equal volume of 1.8 per cent NaCl to make the samples isotonic. The platelets were then centrifuged and fixed as a pellet in 1 per cent osmium tetroxide in Palade's buffer for 1 h. After dehydration through graded alcohols they were embedded in 'Araldite' and sections were cut on an L.K.B. 'Ultratome'. A Philips *E.M.* 200 electron microscope was used to examine the sections.

Fig. 1 shows the changes present in the platelets after 10 min in distilled water. It can be seen that they behaved in one of two ways: either they appeared pale, round and slightly expanded, frequently showing breaks in the cell membrane with an apparent loss of cytoplasmic organelles; or they became darker and slightly contracted, having a continuous cell membrane with many pseudopodia and the usual cytoplasmic organelles. The paler group of platelets became evident after 10 sec in water and the darker group after 60 sec. By 10 min no 'normal'

platelets remained and the two distinct groups were clearly evident. No further morphological change could be noted at 30 and 60 min. Similarly, two types of platelets have been observed in a preliminary study using hypertonic saline.

Serial sectioning was performed to prove that two distinct platelet populations were present. It seems likely that these changes may correspond to the 'spread' and 'sword' forms seen in whole platelets subjected to hypotonic solutions in studies of osmotic fragility<sup>2,3</sup>.

The difference in response to hypotonicity that we have observed probably reflects biochemical differences in the platelet population. This may be due to (i) platelet age, (ii) two biochemically distinct but normally morphologically similar groups of platelets, or (iii) artefact induced in handling. The possibility of an artefact, however, is unlikely, as one patient with severe thrombocytopenia showed only one type of platelet, the 'paler' type, when tested in this way. Further studies are being undertaken in an attempt to determine whether or not these changes are due to age. The possibility that there are two platelet populations has obvious importance in studies of platelet physiology and pathology.

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### Efficiency of Searching Gm Antigens for Identification of Blood-stains in Forensic Medicine

THE recent communications of Brocteur and Moureau<sup>1</sup>, and Nielsen and Henningsen<sup>2</sup>, as well as the interest found in seric groups, lead us to report here a forensic investigation illustrating the importance of the Gm antigens for identification of blood-stains.

In September 1961, a suitcase containing a sum of money was stolen, and it was recovered on waste ground; its lid had been cut, probably with a sharp knife. A thorough examination revealed two very small brown-red stains, looking like blood, on the lid. A suspect arrested soon afterwards had a wound on his thigh, and his trousers were torn at the very spot where the knife must have wounded him.

The police reconstructed the facts as follows: the man had stolen the suitcase, had put it on his lap and, trying to cut it open with his knife, had implanted the blade in his thigh. To verify this hypothesis, we had to prove that the brown stains on the suitcase could have come from the man's blood and that they were similar to the blood-stains found on the trousers.

The difficulty in this investigation was that the stains were very small, making it impossible to determine the antigens of the ABO system or, of course, those of the P or Rh systems. So, after having shown the presence of peroxidase and human proteins in these stains, we tried to identify them simply by searching Gm (a) and Gm (x) antigens.

In fact, the subject was A<sub>1</sub> Rh + Gm (a + x -). Because of the lack of specificity of some anti-Gm (b) sera, we had refrained from using this system in a forensic investigation.

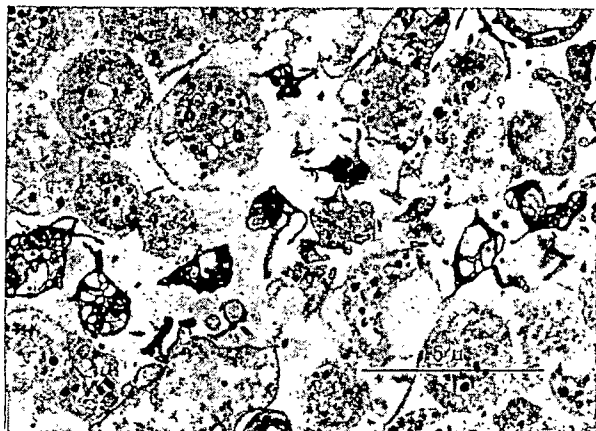


Fig. 1. Platelets after 10 min in distilled water

Table 1

	Anti-Gm (a)						Anti-Gm (x)					
	1/1	1/2	1/4	1/8	1/16	1/32	1/1	1/2	1/4	1/8	1/16	1/32
Stain Gm (a+ x+)	—	—	—	—	—	—	—	—	—	—	—	—
Stain Gm (a- x-)	+++	+++	+++	++	+	—	+++	+++	++	+	—	—
Blood-stain from trousers	—	—	—	—	—	—	—	—	—	—	—	—
Unstained cloth from trousers	+++	+++	++	+	(+)	—	+++	++	+	(+)	—	—
Blood-stain from suitcase	—	—	—	—	—	—	+++	++	+	(+)	—	—

A maceration was prepared by scraping the stains with a bistoury blade and then dissolving the particles thus obtained in physiological serum. This maceration was then brought into contact with anti-Gm (a) serum in one tube, and with anti-Gm (x) in another tube. After incubation for 2 h at laboratory temperature, the titration of the anti-Gm (a) sera was determined by the usual techniques. At the same time, similar preparations were carried out on the trousers stains and on the controlling blood-stains. Table 1 shows the precision of the results obtained which permitted us to say that the stains found on the suitcase might well have come from the suspect's blood. So far as we know, this is the first forensic investigation which has been conducted with the Gm antigens in blood-stain identification.

Since then, we have carried out several investigations to assess the place of Gm antigen determinations in blood-stain identification<sup>3-5</sup>.

The Gm antigens are particularly useful for minute blood-stains<sup>6</sup> because they are often the only antigens that can be used, at least when the absorption techniques are preferred. This kind of investigation can always be relied on: the absorption is always total, even when the stain is very old; we have thus been able to determine the Gm phenotype of experimental stains made 10 years ago.

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## HISTOLOGY

### Autofluorescence of Eosinophils: a Bone Marrow Study

DURING a fluorescent immuno-histological investigation of bone marrow, we observed that certain granulocytes of the unaltered control series showed intense yellow autofluorescence of their granules instead of the expected light yellow-green autofluorescence of cytoplasm<sup>1</sup>. These cells were identified as eosinophils by Wright's stain and phase contrast microscopy. We considered this finding an original observation until we recently found a previous reference<sup>2</sup>. Our report is intended to confirm the reference and quantitate the autofluorescence with reference to individual patients.

Bone marrow smears from sternal aspirations of twenty unselected patients were immediately fixed in 95 per cent alcohol and stored at room temperature. Preliminary work indicated that the alcohol had no effect on the autofluorescence. The slides were mounted with glass coverslips and low-fluorescent buffered (pH 7.2) glycerine. The total white blood cell counts, with two exceptions (chronic lymphatic leukaemia), were within the normal

range. Eosinophilia (10-20 eosinophils per 100 white blood cells) was present in the two cases of intestinal parasitism. The patients were receiving a variety of medications. A Leitz 'Ortholux' microscope with combined tungsten and ultra-violet light (Osram 'HB' 200 W) was used with a monocular photo-tube. Although several combinations of the commonly used exciter and barrier filters produced strong fluorescence of the granules, we used the BG12 exciter filter and the Blau Absorbing (OG1) barrier filter for the entire study. With this system the autofluorescence of the eosinophilic granules was an intense yellow. The cytoplasm of neutrophils often produced a faint yellow-green autofluorescence. A dark field, variable phase, or bright field condenser was used when indicated with the appropriate objectives. Photographs were taken with a Mikas attachment and a 35 mm Leica camera back using Kodak 'Pan X' film. Each eosinophil was first identified under dark field examination by its coarse granules which often produced a white glow; then under ultra-violet light the amount of fluorescence was graded zero to four plus. A cell was graded as one plus when the autofluorescence enabled the granules of the eosinophil to be identified from the background fluorescence, and as four plus when a very intense fluorescence produced a glow beyond the edge of the granule (Fig. 1). The grades two plus and three plus were arbitrary intermediate levels. Twenty eosinophils were identified and graded on each smear of bone marrow. The dark field identification was checked by maintaining the eosinophil in position and changing to phase-contrast microscopy.

Significant autofluorescence of the eosinophilic granules was noted in most preparations. The great majority of eosinophils in each slide showed autofluorescence. (Average 97.5 per cent, range 75-100 per cent.) There was surprisingly little variation of intensity within each preparation,

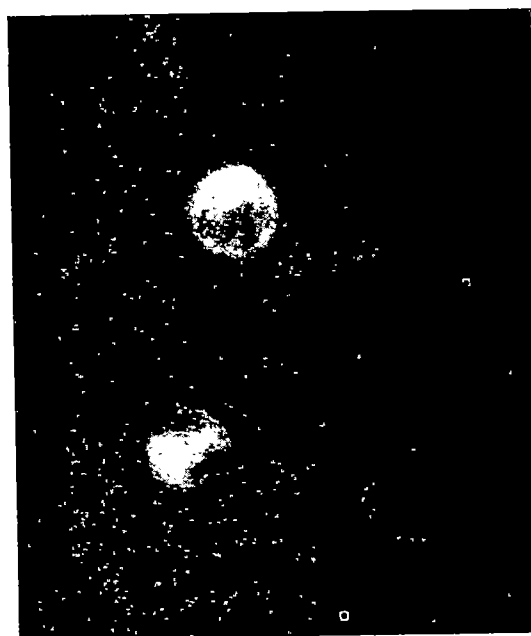


Fig. 1. Two eosinophils with intense yellow autofluorescence of the granules. Other cells of the granulocytic series show a very slight yellow-green autofluorescence of the cytoplasm ( $\times 1,000$ )



most of the eosinophils from each patient showing the same level of autofluorescence. Table 1 illustrates the autofluorescence according to principal diagnosis.

Although the eosinophil has been the subject of countless investigations, its remarkable autofluorescence in the range of the commonly used fluorescent microscopy systems has received scant attention. This study confirmed the previous report in which Grossi and Zaccheo described the autofluorescence of eosinophilic granules but made no comparisons between individuals. Our attempts to quantitate the autofluorescence revealed marked individual differences but apparently no relation to principal diagnosis (Table 1). We also found that no correlation was

Table 1. AUTOFLUORESCENCE OF EOSINOPHILS FROM 20 PATIENTS ACCORDING TO PRINCIPAL DIAGNOSIS

Diagnosis	Autofluorescence
Lymphoma (2), multiple myeloma (2)	1+
Iron deficiency anaemia (3)*	1+–2+
Arteriosclerotic heart disease, cirrhosis, chronic lymphatic leukaemia (2), malabsorption, prostatic hypertrophy, rectal carcinoma	2+
Intestinal parasitism (2), pneumonia	3+
Bronchogenic carcinoma (2), prostatic hypertrophy and uraemia	4+

\* Variation occurred in the eosinophils of three patients with iron deficiency anaemia.

possible between the drug therapy and the intensity of autofluorescence. A correlation may exist in the case of certain drugs, but a much larger series of well-controlled data would be necessary to reveal it. Many organic chemicals are fluorescent within the range of the equipment used in this study and many factors are probably important in producing the autofluorescence of eosinophilic granules demonstrated in this report. The phenomenon certainly warrants further investigation, but it is proper to direct attention to the need for caution in interpreting results of fluorescent staining techniques applied to bone marrow. Changes in pH, temperature, viscosity, ionic strength, heavy metals, and fixation can either increase or decrease fluorescence emission, and these factors must be meticulously controlled in all experiments. In the case of eosinophils the added factor of remarkable autofluorescence must also be carefully considered.

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### Mast Cells of the Pituitary Gland

THERE is a marked similarity in the morphological appearance of mast cells and the parenchymatous glandular cells of the infundibular process (neural lobe) of the pituitary gland described by Gersh<sup>1</sup>. It has been suggested by Rennels and Drager<sup>2</sup> that the parenchymatous glandular cells may play a part in the final release of the hormones of the posterior pituitary into the general circulation. Although Gray<sup>3</sup> described mast cells in the pituitary gland of man, ox and cat, the presence of these cells seems to have been largely ignored by more recent workers.

In the present investigation an attempt has been made not only to identify mast cells in a variety of mammalian pituitary glands, but also to determine their relationship to the parenchymatous glandular cells.

The pituitary glands of man, bullock, calf, rat and mouse were investigated. Of a variety of fixatives employed, formol-Zenker gave the clearest detail. Mast cells were identified following staining with 2 per cent new methylene blue in 0.5 per cent lithium carbonate or

with 1 per cent toluidine blue at pH 3. For the identification of the parenchymatous glandular cells the method described by Gersh<sup>1</sup> was used. For each specimen examined, control tissues, known to contain mast cells, were removed and processed with the glands. These controls were mounted on the same slides as the pituitary sections. In this way identical control staining was obtained.

**Mast cells.** These were present in great number in the infundibular process of the bullock and the calf; as many as 150 per low power field were seen. A few were found in the pars intermedia and occasional ones in the pars distalis. The infundibular stem of the bullock was particularly rich in mast cells and they could be traced proximally to the region of the para-ventricular and supra-optic nuclei of the hypothalamus. More were found in the para-ventricular than in the supra-optic nucleus. None was found in sections of brain taken from a variety of sites other than the hypothalamus. Only occasional mast cells were observed in the pituitary gland of the rat, and many sections were completely devoid of them. Although an occasional mast cell has been seen in the capsule of the pituitary gland in the mouse, none was found in the parenchyma of the gland. In the human the mast cell population was variable. Occasional mast cells were found in the pars intermedia and pars distalis in all glands examined. In the infundibular process of one gland many mast cells were present, but in another no mast cells were seen. The distribution in the remaining glands examined lay between these extremes.

**Parenchymatous glandular cells.** In the pituitary gland of the rat and the mouse typical parenchymatous glandular cells were observed in all the sections examined. The mast cells in the control tissues failed to reduce osmic acid and could not be positively localized by this method alone. In the human, bullock and calf, no cells containing the typical black granules found in the rat and mouse were seen in the infundibular process, nor were these cells found in the control tissues. Cells containing refractile granules were seen, but they were identified as mast cells when stained with new methylene blue.

It is known that the mast cell distribution in the tissues varies from species to species, and the present findings indicate that this is true of the pituitary gland. The pronounced variation in the mast cell population of the human pituitary gland may be related to the physiological state of the body at the time of death. Variation with age cannot be a factor in the present survey, for all human glands examined were removed from cadavers of the same age group.

While it is possible to differentiate the parenchymatous glandular cells of the pituitary from mast cells in the rat and mouse, this is not the case in the human, bullock or calf. In these it is apparent that the cells described by Gersh<sup>1</sup> which contained refractile granules that did not reduce osmic acid are, in fact, mast cells.

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### RADIOBIOLOGY

#### Dependence of DNA Synthesis on Irradiation Dose Rate

THE following report of work on the relationship of dose-rate depression of DNA synthesis is part of a more comprehensive investigation of the effects of irradiation on the biosynthetic mechanism of DNA replication. One of the specific reasons for the investigation was to determine whether one of the major criteria (dose-rate independence)

for utilization of the target theory was fulfilled for radiation effects of DNA synthesis in the biological test system used<sup>1,2</sup>.

Lewis strain rats (200 g) were used in these experiments. The irradiation was given using a 200-kVp. X-ray machine of half value layer, 1 mm copper. A 3-cm cone was used to deliver the irradiation locally to the liver. The experiments were designed so that all periods of radiation, with variations in dose rate, ended at 19 h after partial hepatectomy. The time, 19 h after partial hepatectomy, was chosen because it was previously found to be near the mid-point of the 8-h period of DNA replication in the rat hepatocyte. A total dose of 1,500 r. was used at dose rates of 300 r./min, 50 r./min, 25 r./min and 12.5 r./min. The fractionated dose-rate experiments were as follows: 500 r. at 17, 18 and 19 h after partial hepatectomy, and 750 r. at 17 and 19 h after partial hepatectomy. The total dose and dose rates were a compromise between the sensitivity of the detecting methods of DNA depression, the range of dose rates feasible, and the fraction of time of the DNA synthetic period over which the animals were irradiated.

50  $\mu$ c. tritiated thymidine, 3 c./mM mole, were given at the end of the period of irradiation. The animals were killed 1 h later and squashes made from random samples of finely minced liver. These were fixed by freeze substitution before the application of Kodak 'AR-10' stripping film.

Anaesthesia was necessary in order to keep the animals stationary during the varying periods of irradiation. Four groups of animals were anaesthetized with ether for the same periods used at the four different dose rates. These results are given in Table 1. No significant differences were found in the relative rates of DNA synthesis at 5, 30, 60 or 120 min of ether anaesthesia.

Table 1. THE EFFECT OF VARYING TIMES OF ETHER ANAESTHESIA ON DNA SYNTHESIS

Duration of ether	5 min	30 min	60 min	120 min
Mean grain counts per nucleus	32.4	32.8	26.4	26.7
S.E.	$\pm 7.5$	$\pm 7.9$	$\pm 5.5$	$\pm 2.1$
No. animals	6	5	6	11

Table 2. THE EFFECT OF VARYING IRRADIATION DOSE RATE ON THE DEPRESSION OF DNA SYNTHESIS (TOTAL DOSE 1,500 R.)

Dose rate	300 r./min	50 r./min	25 r./min	12.5 r./min	Controls
Time of delivery	5 min	30 min	60 min	120 min	
Mean grain counts per nucleus	25.1	19.5	15.6	9.7	37.7
S.E.	$\pm 2.2$	$\pm 1.5$	$\pm 1.3$	$\pm 1.4$	$\pm 1.8$
No. animals	10	8	7	7	41

Table 3. THE EFFECT OF DOSE FRACTIONATION GIVEN AT THE RATE OF 300 R./MIN ON THE DEPRESSION OF DNA SYNTHESIS (TOTAL DOSE 1,500 R.)

Dose fractionation	1,500 r. at 19 h after partial hepatectomy	750 r. at 17 and 19 h after partial hepatectomy	500 r. at 17, 18 and 19 h after partial hepatectomy
Mean grain counts per nucleus	25.1	21.5	28.8
S.E.	$\pm 2.2$	$\pm 4.3$	$\pm 3.7$
No. animals	10	6	5

The depression of DNA synthesis at the different dose rates is given in Table 2. The 1,500 r., given at the rate of 300 r./min, reduced the rate of DNA synthesis to approximately two-thirds of the control values. There is a consistent depression of the rate of DNA synthesis with the lowering of dose-rate and the increasing time of radiation. This becomes significant at the 0.05 per cent level when the rate of 2.5 r./min is reached (Fig. 1).

There was no significant difference in the depression of DNA synthesis if the total dose of 1,500 r. was given in 5 min, or in fractionated doses of 500 r. and 750 r. over a 2 h period (Table 3). This would suggest that the intensity of radiation given at a dose rate of 300 r./min is in excess of that needed to provide the optimum depression of DNA synthesis. The continuous depression of DNA synthesis with decreasing dose rates indicates that the optimum dose rate for depression of DNA synthesis has not been reached at 12.5 r./min.

Beltz, Van Lancker and Van Potter<sup>3</sup> found that decreasing the dose rate caused less depression of DNA synthesis rather than greater depression. They found

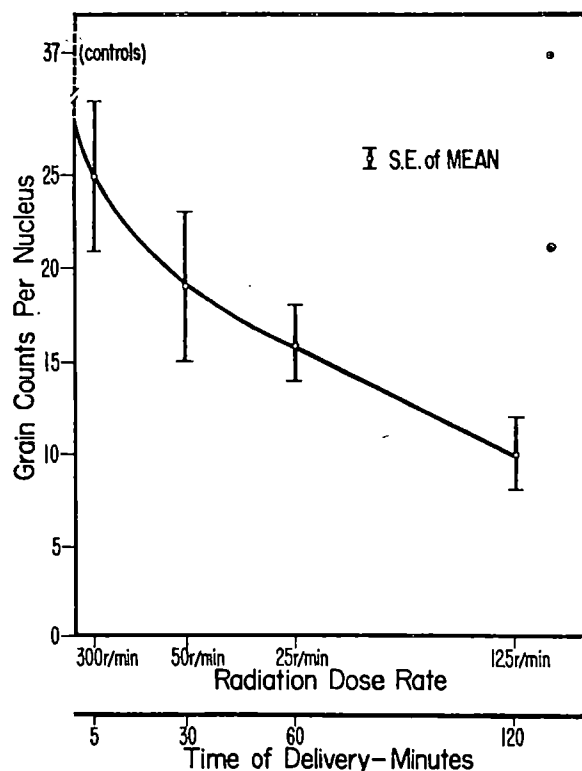


Fig. 1. Relation of radiation dose rate to the depression of DNA synthesis (total dose 1,500 r.)

that a dose rate of 10 r./min gave a concentration of  $2,156 \pm 462$  c.p.m./mg DNA, 19 r./min gave  $1,300 \pm 334$  c.p.m./mg DNA, and 39.5 r./min gave  $463 \pm 209$  c.p.m./mg DNA. Whole-body irradiation was used rather than local irradiation of the liver. The irradiation was started 24 h after partial hepatectomy. Orotic acid-6-<sup>14</sup>C was given 30 h after partial hepatectomy and the animal was killed 36 h after partial hepatectomy. The fact that the findings of Beltz *et al.* are contrary to the results presented here is, in all probability, due to the differences in the time of irradiation, injection of labelled precursors of DNA, and killing of the animal. The possibility that whole-body irradiation resulted in a greater depression at a higher dose rate from the indirect effects of irradiation seems less likely. Irradiation 24 h after partial hepatectomy would be irradiating the last part of the first cycle of hepatocytes synthesizing DNA, as well as the beginning of the second cycle of hepatocytes. Injection of orotic acid 30 h after partial hepatectomy would, in all probability, be taken up by the second cycle of hepatocytes in the controls and a mixture of first- and second-cycle hepatocytes in the irradiated animals. Differences in the time sequences in the irradiated and control animals could therefore lead to differences in the rate of uptake of labelled orotic acid.

The more probable explanation is that radiation affects one or more rate-limiting processes in such a way that the higher dose rate provides an excessive number of ionizing events per unit of time. If this were true, then the lower dose rate would be more efficient in producing the ultimate depression of DNA synthesis. This preliminary finding might be interpreted as follows: the rate of formation of the radiation-sensitive biological units is small, so that only a small number of ionizing events are needed to inactivate them. The results of the investigations of fractionated doses would suggest that effects, if any, on the formation of new biological units are of short duration, since the depression of DNA synthesis is essentially the same, whether the dose is given in 5 min, or given in divided doses over a 60- or 120-min period. Further investigations are in progress to elucidate the relation

ship between the rate of delivery of the radiation dose and the rate of depression of DNA synthesis.

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## BIOLOGY

### Distribution of Indolyl-3-acetic Acid labelled with Carbon-14 in *Avena*

THE active transport of hormones in plants has been under investigation for a number of years. Speeds of transport have been reported in the literature, but there does not appear to have been any attempt to observe experimentally whether a well-defined front exists for the moving hormone stream. This lack of experiment is surprising since, as Canny<sup>1</sup> has pointed out, a speed of transport of a substance in a plant can only be simply defined if there exists a definite front to the stream of that substance. Such a front does not exist for translocated photosynthate nor would it be expected for hormone transport if the movement of the hormone were a diffusion-like process. Goldsmith and Thimann<sup>2</sup> find an exponential profile of a hormone in a section of *Avena* through which it had been passing for several hours. They assumed from this that no definite front exists and therefore they did not attempt an accurate measurement of transport speed.

From electric measurements on the *Avena* coleoptile Newman<sup>3,4</sup> has suggested that there is a definite front to the stream of indolyl-3-acetic acid (IAA) moving down a decapitated coleoptile from an IAA-agar block applied at the top. The basis of this suggestion was the observation that IAA induced an electric wave to move down the coleoptile at a constant speed and with little dispersion.

This communication describes an experiment in which the profile of the moving IAA in the *Avena* coleoptile was determined by application of radioactively labelled hormone to the cut top. It will be seen here that the results confirm the existence of a sharp front to the IAA stream. It is hoped that these results may assist in the development of a more complete description of the distribution and transport of IAA in the *Avena* coleoptile.

Oat seedlings were grown and prepared as previously described<sup>4</sup>. The experiment was performed under weak green light in a room kept at 25° C and 90 per cent humidity. Agar blocks of 5 mm<sup>3</sup> volume containing <sup>14</sup>C-carboxyl-labelled IAA (activity: 96 µc./mg) at a concentration of 0.03 ± 0.01 mg/l. were placed on the cut tops of the coleoptiles and renewed at 10-min intervals to ensure that a constant concentration of IAA was in contact with the top of each coleoptile. After 32 or 64 min the coleoptiles were cut into a number of 1 mm-long sections. All the sections from a particular level in the coleoptiles were placed upright on a small piece of agar for 7 min. During this time most of the moving component of the radioactive IAA in the sections moved into the agar block. No attempt was made to estimate how much of the hormone had become in any way bound in the tissue or how much unlabelled IAA may have moved in the stream. This may be the subject of further experiment. The agar blocks in which the transported IAA had been collected from the 1-mm sections were melted and spread over the surfaces of planchets to reduce self-absorption. They

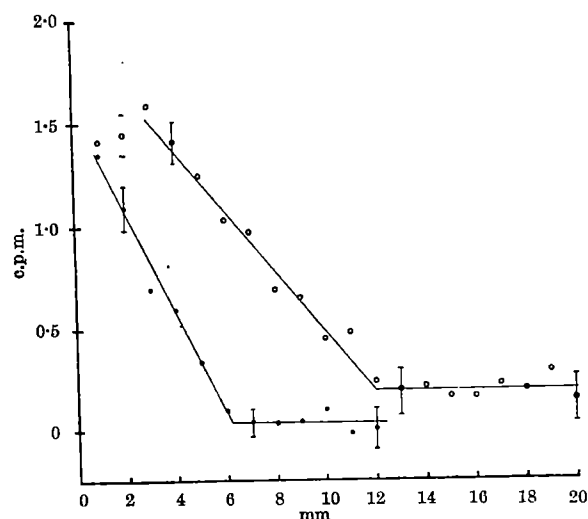


Fig. 1. Counts per min against distance in millimetres below the cut top of the coleoptiles. ● 32-min experiment; ○ 64-min experiment. 95 per cent confidence limits of the measured count rates are shown for 3 positions on each graph

were dried and counted using a thin end-window Geiger-Müller counter with an anti-coincidence array which reduced the background to 1.6 c.p.m.

Fig. 1 shows the activity (c/min, corrected for background) of the hormone that diffused out of the sample of sections at each level in the 7-min period. This is plotted against the distance of the base end of each 1-mm section from the point of application of the hormone. The sample sizes were 11 coleoptiles for the 32-min experiment and 18 coleoptiles for the 64-min experiment.

Two clear deductions (valid for the concentration of IAA used) can be made from the results: the first is that, at a chosen time after application of the hormone, its concentration in the stream moving down the coleoptile falls approximately linearly with distance below the top. It is estimated that in each case the average IAA-<sup>14</sup>C concentration moving in the tissue 1 mm below the top is about 0.01 mg/l.; that is, about 1/3 the applied concentration. However, for the two results it is not useful to compare the intersection points of the lines with the activity axis since the concentration of IAA applied in the two cases was only fixed to within 30 per cent of 0.03 mg/l.

At a certain distance from the top the activity reaches zero or sharply levels off to a small positive value. This greater-than-background activity could have been caused by some contamination during the experiment or perhaps caused by a very small quantity of the hormone being transported much faster. Further experiment should elucidate this. In any event, the concentration of IAA detected in this region is less than 0.001 mg/l. and is probably physiologically insignificant. The second deduction from the results is then that the abrupt change of slope of the line shown in Fig. 1 for each time may reasonably be taken to define the position of the front of the IAA stream at that time. This front moves from 6.2 mm at 32 min to 12.0 mm at 64 min. The average speed of movement is therefore 11 mm/h. This is the same as the speed at which an electric wave has been observed to move down the *Avena* coleoptile under the same experimental conditions<sup>4</sup>, thus confirming that the electric wave is associated with IAA transport.

The results described show, therefore, that IAA, when applied to the top at a concentration of 0.03 mg/l., moves down the *Avena* coleoptile in a stream with a nearly linear profile. The front of this stream advances at 11 mm/h. The results are now being extended to other concentrations and other transport times.

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### *In vitro* Production of Neomorphs in *Anethum graveolens* L.

IN an earlier communication Johri and Sehgal<sup>1</sup> reported the chemical induction of polyembryony in *Anethum graveolens*. The work recorded here is an extension of that investigation.

In Nature the fruits mature approximately 25 days after pollination and the seeds show a copious fatty endosperm and a single minute embryo.

Young ovaries, three days after pollination (Fig. 1), were grown under aseptic conditions on a modified White's medium containing 4 per cent sucrose. This would be referred to as the basal medium. Casein hydrolysate, yeast extract and indolyl-3-acetic acid were used as supplements.

At the time of inoculation the ovules showed the zygote and a free nuclear endosperm. The ovaries implanted on basal medium and basal medium + indolyl-3-acetic acid

(1, 5, 10 p.p.m.) produced only monoembryonate seeds, while basal medium + casein hydrolysate (100, 1,000 p.p.m.) and basal medium + yeast extract (100, 1,000 p.p.m.)—with or without the addition of indolyl-3-acetic acid (1 p.p.m.)—induced polyembryony in 6–20 per cent cultured ovaries. In 13- to 14-week-old cultures the seeds germinated *in situ* and gave rise to normal seedlings. However, in a few cultures grown on basal medium + casein hydrolysate or basal medium + yeast extract, with or without indolyl-3-acetic acid, the seedlings were chlorotic and fasciated. Eight to 12 months later (cultures transferred through 4–6 passages of 8 weeks each) the fasciated tissues differentiated into embryo-like structures (Figs. 2 and 3) which finally developed into 1–2-cm-long thalloid (Fig. 4) or coralloid (Fig. 5) seedlings. These vegetatively reproducing plantlets, termed neomorphs, resemble the cotyledonary stage of a normal seedling. Further proliferation and multiplication of the neomorphs occurred in cultures maintained for 10–12 months (Figs. 3–5). On sub-culturing in basal medium, basal medium + casein hydrolysate or basal medium + yeast extract (with or without indolyl-3-acetic acid) for 6–8 weeks, they produced normal seedlings.

Earlier, Waris<sup>2,3</sup> reported that the mature seeds of umbellifers like *Cicuta virosa*, *Daucus carota*, *Oenanthe equatica* and *O. lachenalii* grown on a liquid medium (for detailed composition of this medium see ref. 3) containing 1 per cent sucrose and 0.1–0.4 per cent glycine produced more or less similar neomorphs. Miettinen and Waris<sup>4</sup> noted that the neomorphs contained a low concentration of sugars but had free amino-acids in a 10-times higher concentration than normal individuals. They therefore concluded that the accumulation of soluble amino-acids in the neomorphs indicated a disturbance in protein synthesis. They further suggested that the neomorphic condition of seedlings is a physiological adaptation to a higher concentration of glycine and does not involve any irreversible change of genotype.

It is possible that in *Anethum graveolens* also the amino-acids present in casein hydrolysate and yeast extract accumulate in the seedlings which produce the neomorphs, but this has to be confirmed by chemical analysis. In any case, like the induction of zygotic polyembryony, the production of neomorphs can be exploited by plant breeders for raising a large population of seedlings with desired characters.

We are grateful to Prof. P. Maheshwari for his keen interest in the progress of this work.

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### Giant Chromocentres and Nucleoli in *Lumbricus*

FURTHER observations on intranuclear aspects of giant nuclei found in the pharyngeal epithelium in *Lumbricus* have been made. A previous report was given concerning these nuclei<sup>1</sup>. Not only were the overall dimensions of these nuclei enlarged when compared with the more typical nuclei, but also on examination of the intranuclear morphology it became apparent that giant chromocentres (Figs. 1, 4 and 6), giant nucleoli (Figs. 1, 2, 3, 4, 5 and 6), and even giant nucleolar organizers (Figs. 3 and 4) were present.

Endopolyploidy could explain these features if it were assumed that not only nucleolar fusion exists but also

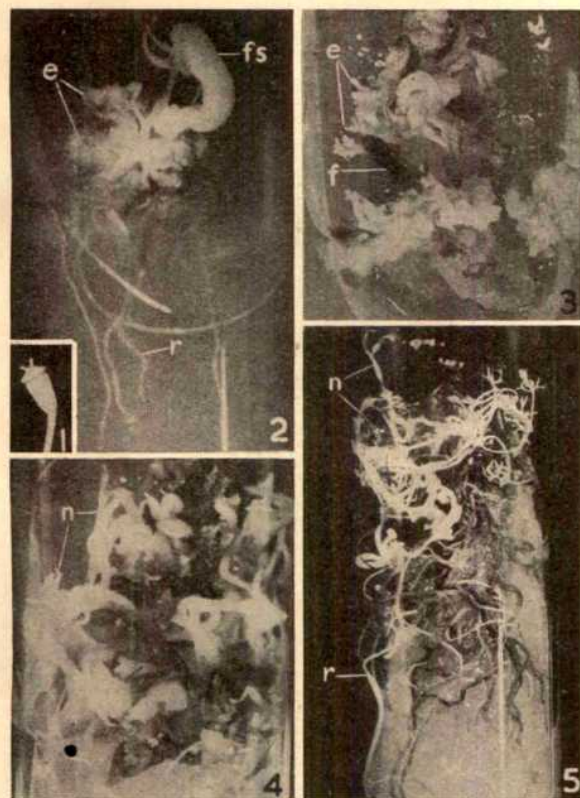


Fig. 1. Ovary (stage of inoculation) 3 days after pollination ( $\times c. 2.6$ ).

Fig. 2. 32-week-old culture on basal medium + yeast extract (1,000 p.p.m.) with a fasciated seedling and several undifferentiated embryo-like structures ( $\times c. 1.66$ ).

Fig. 3 ( $\times c. 1.7$ ) and Fig. 4 ( $\times c. 1.5$ ). 43- and 46-week-old cultures on basal medium + casein hydrolysate (1,000 p.p.m.) + indolyl-3-acetic acid (1 p.p.m.) and basal medium + casein hydrolysate (1,000 p.p.m.) with numerous thalloid neomorphs.

Fig. 5. 40-week-old culture on basal medium + casein hydrolysate (500 p.p.m.) showing coralloid neomorphs ( $\times c. 1.7$ ).

e, Embryo-like structures; f, fruit; fs, fasciated seedling; n, neomorphs; r, root.



Fig. 1

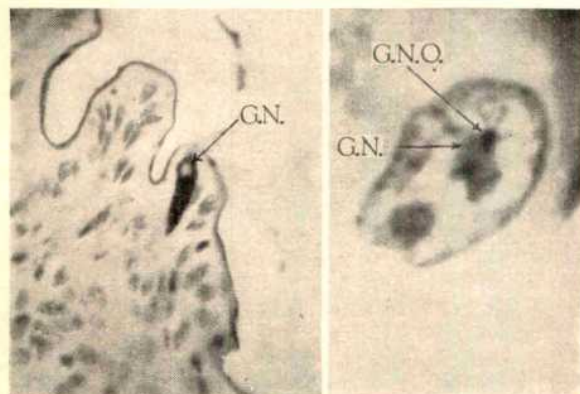
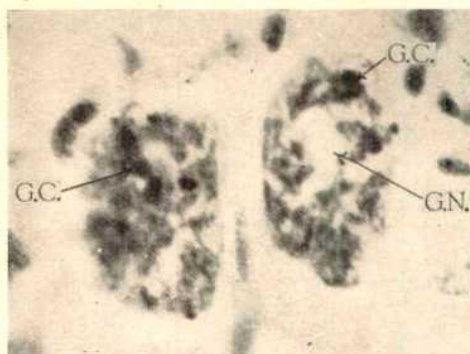


Fig. 2

Fig. 3

Fig. 1. Two giant nuclei in the pharyngeal epithelium containing giant chromocentres (G.C.) and giant nucleoli (G.N.). Feulgen stained. ( $\times c. 960$ )

Fig. 2. A giant nucleus possessing a giant nucleolus. Feulgen stained. ( $\times c. 320$ )

Fig. 3. A giant nucleus containing two giant nucleolar associated chromatin masses considered to be nucleolar organizers (G.N.O.) on the periphery of a giant nucleolus. Feulgen stained. ( $\times c. 960$ )

chromocentre fusion. In *Drosophila* such fusion occurs with heterochromatic portions of the giant polytene chromosomes of the salivary gland. Large nucleoli and 'coarse-chromatin' (large chromocentres?) are terms commonly used in the description of nuclei of various neoplastic cells. In this respect it is of interest that giant nuclei of the same description presented in the previous report<sup>1</sup> have been given for nuclei in a pharyngeal tumour in *Lumbricus* by Stolk<sup>2</sup>.

Synthetic capacities and other functional qualities attributable to nucleolar organizers, and possibly chromocentres in general, may be greatly altered by a fusion process. These nuclear components because of their increased size should lend themselves admirably to autoradiographic experiments, designed to ascertain biochemical aspects of heterochromatin and nucleoli.

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### Deoxyribonucleic Acids of Sperm, Eggs and Somatic Cells of the Sea Urchin, *Arbacia punctulata*

A SEARCH of the literature reveals no report of the comparative base composition of deoxyribonucleic acids isolated from the two gametes and the somatic cells of the same species. Yet the molecular events whereby genetic information from egg and sperm is integrated on fertilization are of fundamental importance in our understanding of the molecular basis of heredity and development in higher animals. These facts prompted the work recorded here, which is concerned with properties of the DNA isolated from sperm, eggs and adult somatic cells of the sea urchin *Arbacia punctulata*. Sperm and eggs were obtained either by the mild electric shock technique of Harvey<sup>1</sup> or by the injection of 0.5 M KCl<sup>2</sup> into the perivisceral cavity of adult sea urchins. The diploid tissues were obtained from spent adults, the gonads of which were discarded. The DNA from sperm and diploid tissues was isolated by a modification of the procedure described by Marmur<sup>3</sup>. High-molecular-weight DNA from eggs could not be prepared in this way unless the nuclei were first isolated by the method of Went and Mazia<sup>4</sup>. The hot phenol method<sup>5</sup> was also effective in the isolation of DNA from whole eggs. Failure to isolate egg DNA by mild procedures might be due to the presence of potent deoxyribonucleases, which in turn could account for the inability of some investigators<sup>6</sup> to obtain highly polymeric DNA from this source.

The buoyant densities in caesium chloride of the DNA preparations before and after heating in a boiling water bath, followed by quick chilling, are shown in Table 1 and Figs. 1 and 2. It is known that heating and quick chilling of double-stranded DNA molecules renders them single-stranded<sup>7</sup>. This is accompanied by an increase in

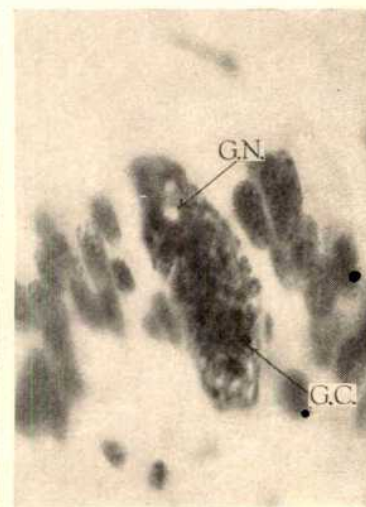
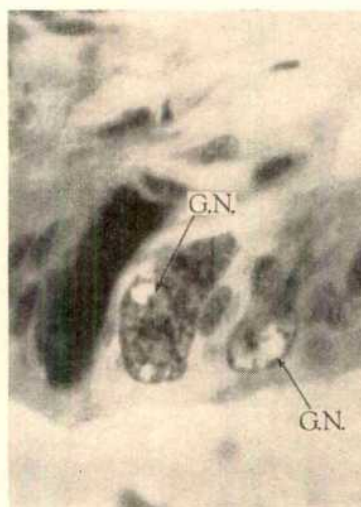
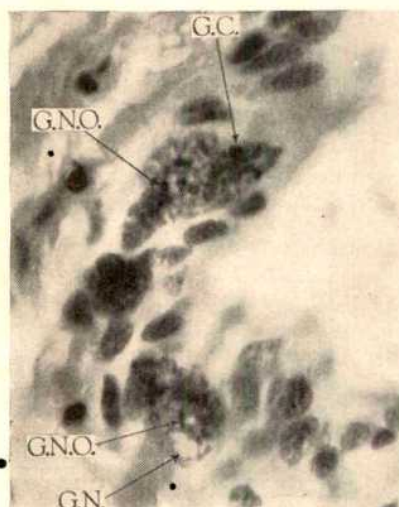


Fig. 4

Fig. 5

Fig. 6

Figs. 4-6. Giant nuclei in the pharyngeal epithelium possessing giant nucleoli and chromocentres. Feulgen stained. ( $\times c. 470$ )

Table 1. PROPERTIES OF THE DEOXYRIBONUCLEIC ACIDS ISOLATED FROM *Arbacia punctulata*

Specimen	Density (g/cm <sup>3</sup> )	% Guanine + cytosine*	Structure
Diploid, prep. I†	1.702	43	Double-stranded
Diploid, heated	1.718	—	
Diploid, prep. II†	1.702	43	
Diploid, prep. III†	1.702	43	Double-stranded
Sperm, prep. I†	1.700	41	
Sperm, heated	1.716	—	
Sperm, prep. II†	1.700	41	Double-stranded
Sperm, heated	1.714	—	
Eggs, prep. IV‡	1.700	41	Double-stranded
Eggs, prep. V§	1.700	41	
Eggs, heated	1.714	—	

\* The guanine-cytosine contents were estimated from the buoyant densities on the assumption that the DNA contained no unusual bases or substituents. A previously described relationship was used (ref. 8).

† Prepared by a detergent procedure (ref. 3).

‡ Eggs pre-treated with dithiodiglycolate, versene and dextrose (ref. 4).

§ Prepared by a hot phenol procedure (ref. 5).

buoyant density of approximately 0.015 g/cm<sup>3</sup>. It can thus be seen that the deoxyribonucleic acids isolated from both eggs and sperm are double-stranded and have identical buoyant densities. If these nucleic acids should be free of unusual bases, it can be calculated<sup>8</sup> that this density corresponds to a guanine-cytosine content of 41 per cent.

Sea urchin eggs have been reported to contain 100 times more DNA in the cytoplasm than in the nucleus<sup>9</sup>. It is therefore noteworthy (Table 1) that the DNAs extracted by the phenol procedure from whole eggs and from isolated nuclei have the same buoyant density, thus indicating, by the criteria used, the identity of the nuclear and cytoplasmic DNA of *Arbacia punctulata*.

The buoyant density of the DNA isolated from diploid cells, although double-stranded, was consistently higher

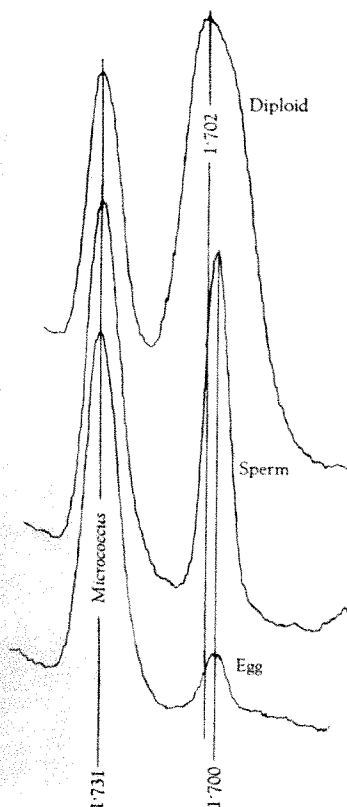


Fig. 1. Buoyant densities of deoxyribonucleic acids isolated from the sperm, egg and somatic cells of the sea urchin *Arbacia punctulata*. The specimens from the sperm and diploid cells were isolated by a detergent procedure (ref. 3) whereas the DNA from the eggs was isolated by the hot phenol method (ref. 5). Portions of the specimens (2–5 µg) together with a reference sample (*Micrococcus lysodeikticus* DNA, 1.731 g/cm<sup>3</sup>) were placed in a caesium chloride solution of density 1.70 g/cm<sup>3</sup>. The samples were spun at 44,770 r.p.m. for 24 h and photographs were taken. The pictures were scanned with a Joyce-Loebel Mark III B densitometer and the densities of the specimens were calculated by comparison with the position of the reference DNA.

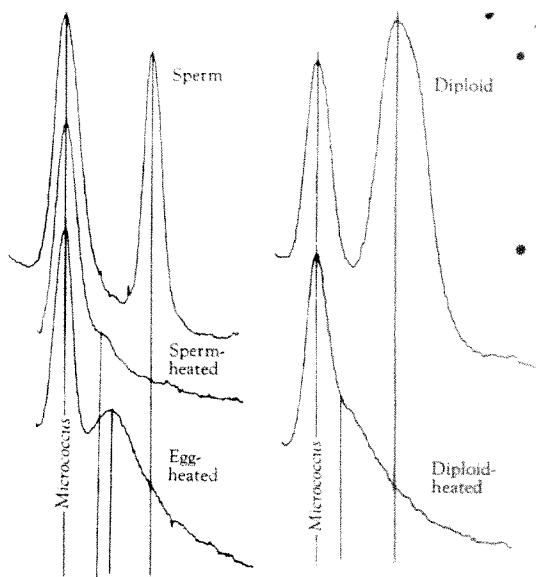


Fig. 2. Effect of heating and quick chilling on the buoyant density of DNA extracted from *Arbacia punctulata*. The DNA (30 µg/ml, 0.15 M NaCl in 0.015 M sodium citrate) was placed into a boiling water bath for 10 min and was then chilled by immersion in an ice-bath.

than the density of DNA derived from the gametes (Fig. 1 and Table 1). With the same reservations mentioned here, the buoyant density of this DNA can be calculated to correspond to a guanine-cytosine content of 43 per cent. An examination of Fig. 1 reveals that the buoyant density of DNA from somatic cells does not have a perfectly gaussian distribution, and this may account for the increased density. In this respect it is interesting that other investigations have also shown the DNA from adult somatic cells to be more heterogeneous with respect to density than is the nucleic acid isolated from haploid<sup>10</sup> or embryonic cells<sup>10,11</sup>.

The relationship of this increased density to development and cellular differentiation is now being investigated in this laboratory. In order to gain a better understanding of the mechanisms operative immediately after fertilization, sequence homologies between these nucleic acids will be looked for.

This work was supported by a grant from the U.S. Public Health Service (AI-05111) and by a contract (Nonr 266 (89)-NR 103-574) between the Office of Naval Research, U.S. Department of the Navy, and Columbia University.

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## MICROBIOLOGY

## Association of Bacterial L-Phase Organisms in Chronic Infections

THE pathogenicity, in the classic sense, of bacterial L-phase cells has yet to be conclusively demonstrated. The association of the L-phase of certain bacteria with diverse clinical states has been noted in the past, and this association is being reported more frequently as knowledge and interest in these forms have increased<sup>1-3</sup>. Such reported investigations lead to the assumption that host resistance mechanisms tend to create the L-phase of a bacterium. Experiments designed to determine the possible pathogenicity of the bacterial L-phase have usually ignored the host. It is not unreasonable to assume that the L-phase is the bacterial form most readily handled by normal host resistance mechanisms.

In regard to these assumptive possibilities, it was thought that chronic staphylococcal infections may be associated with L-phase bacteria. Patients were chosen for their history of chronic infection, usually associated with staphylococci. Blood samples, 10 ml., were drawn after the remission of clinical symptoms of disease brought about by antibiotic therapy. 5.0 ml. of each sample was injected immediately into 5.0 ml. double-strength L-phase minimal medium prepared according to the Mattman modification of the method of Medill and O'Kane<sup>10</sup>. The remaining 5.0 ml. was inoculated into 50 ml. 'sloppy' brain heart infusion medium (BHI plus 0.1 per cent agar). The inoculated L-phase medium was then incubated anaerobically until a change was obvious. Sometimes the only change was a slight haemolysis of the blood layer. Growth generally appeared by the end of the 5th-7th day of incubation. The 'sloppy' BHI was incubated aerobically for a minimum of two weeks.

When L-phase medium cultures were 5-7 days old, they were removed from the anaerobic chambers (95 per cent nitrogen; 5 per cent carbon dioxide) and two 0.5 ml. samples were inoculated into 4.5 ml. fresh medium. They were incubated either aerobically or anaerobically. Growth appeared in both tubes, but generally was greater in the tube incubated in the absence of air. At the time of maximal development (3-5 days) the organisms were again transferred to fresh medium and incubated aerobically. Adequate growth of the organism occurred in 3 days. These L-phase cells were then reverted to classic bacteria by daily transfers in BHI and on to 'Difco 110' medium slants. The cultures on the 'Difco 110' medium usually underwent a colour change from white to bright yellow during the successive transfers. The 'sloppy' BHI medium remained negative for any culture, even though the reverted L-phase cells were able to grow in this medium. Medium controls remained aseptic.

From a total of 11 patients selected for their history of recurring boils and staphylococcal problems, positive L-phase cells were obtained without the appearance of the classic bacterial forms. The L-phase cells all reverted to staphylococci after continued sub-culture.

As a control of the methodology, blood samples were taken from 22 apparently uninfected persons from the hospital staff. These blood samples were cultured in the routine process, and of these, 20 were negative. On re-testing samples of blood from the two whose blood had given positive cultures, no culture was obtained. The possibility of sub-acute infection in these two persons may be a reasonable explanation.

When large numbers of L-phase bacterial cells are injected into a healthy animal, no disease occurs and the L-phase cells are rapidly cleared. This may indicate merely that the animal is normal and therefore capable of efficiently handling the intruding cells. The classic bacterial growth form is not handled as well, if at all, and thus causes the symptomatic onset of disease. However, if the animal host resistance is interfered with by mucin

injection, then the L-phase cells survive, revert to the classic form and cause overt disease (unpublished results). Unfortunately, mucin is so non-specific in its interference with resistance that many factors only indirectly involved in host resistance are undoubtedly affected. The usual interpretation of this sequence of events is that the L-phase cells are non-pathogenic and are therefore not involved in the infectious process or the disease state. This interpretation may not be correct. We speculate that the inability to handle L-phase bacteria may be related to an incomplete host resistance system caused by stress or inherited deficiencies. The consequence of survival of the L-phase in tissues not amenable to therapy would, therefore, be chronic disease. During therapy no overt disease state may exist. If therapy should be discontinued before the host resistance system is functioning efficiently, the surviving L-cells may slowly revert to classic growth forms and eventually give rise to overt disease. It would be expected that during therapy, however, the host defence mechanisms would become more active or that the physical or biochemical stress would have disappeared. This would allow complete recovery. Complete recovery may be primarily dependent on the reacquisition of host resistance factors rather than on the course of chemotherapeutic treatment.

A more extensive investigation of chronic staphylococcal L-phase infection should be initiated. Other chronic disease states should be examined during various phases of treatment and non-treatment for possible L-phase association. Rheumatic fever comes to mind as a most logical chronic disease that could be associated with streptococcal L-phase survival, perhaps localized in heart valve tissue.

The association of L-phase cells in cases of chronic infections of humans which have been treated with antibiotics requires greater consideration than has been outlined in this report. The possible involvement of the bacterial L-phase in this type of infection should no longer be ignored.

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## CYTOLOGY

## A New Basic Chromosome Number in the Family Fagaceae

CHROMOSOME numbers have been determined previously for some 58 species in the family Fagaceae, covering the three genera of the northern temperate zone, *Quercus*, *Castanea* and *Fagus*<sup>1-3</sup>. These determinations are uniformly  $2n = 24$ , the Fagaceae resembling many other families of woody angiosperms in this stability of chro-



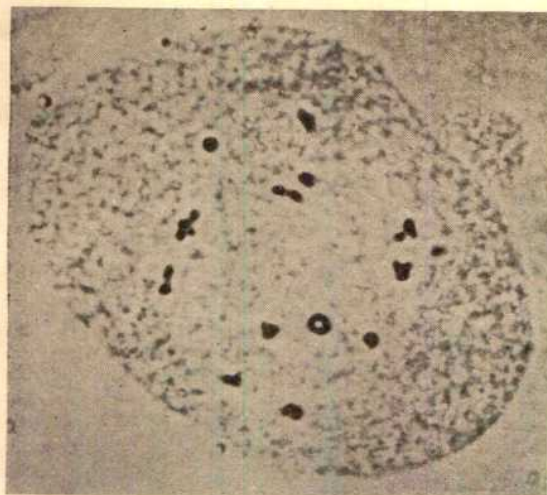


Fig. 1. Diakinesis in *N. solandri* var. *cliffortioides*, with 13 bivalents ( $\times 1,500$ )

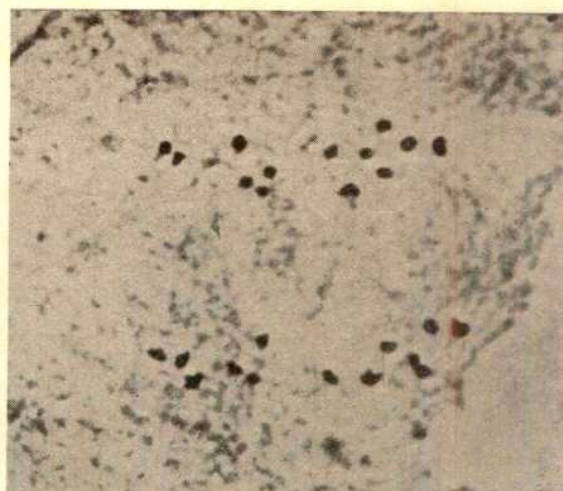


Fig. 2. Anaphase I in *N. menziesii*, 13 chromosomes moving to each pole ( $\times 1,500$ )

mosome number. The southern beech genus, *Nothofagus*, with some 20 species distributed in temperate South America, New Zealand and Australia and extending up into New Guinea and New Caledonia, was at one time included in *Fagus*, and it is more closely allied in features of floral and pollen morphology to *Fagus* than to any of the other genera in the family<sup>4-6</sup>. The four New Zealand species are all endemic and are the principal evergreen hardwoods in much of the indigenous forest. They include representatives of two of the sections into which the genus is divided: in the *fusca* section, *N. fusca*, *N. truncata* and *N. solandri*, all of which hybridize freely in the wild, and in the *menziesii* section, *N. menziesii*, which is not known to cross with any of the other species.

Chromosome numbers have been determined from meiotic divisions in pollen mother cells for the three species growing in Otago: *N. menziesii* (silver beech), *N. fusca* (red beech) and *N. solandri* var. *cliffortioides* (mountain beech)<sup>7</sup>. Meiosis occurs very early in the spring and is completed very rapidly indeed in any one locality. Thirteen bivalents are uniformly present in diakinesis and metaphase I cells (Fig. 1), and cells at anaphase I show 13 chromosomes moving to each pole (Fig. 2). The chromosomes are small (as is usually the case in trees), with median to sub-median centromeres. *N. menziesii* has a significantly lower mean chiasma frequency per cell than the two species in the section *fusca*. Meiosis is normal and leads to the formation of regular tetrads.

This discovery that *Nothofagus* has a haploid number of 13, in contrast to that of 12 in all other members of the family for which information is available, vindicates its generic status. One might even ask whether exclusion from the family Fagaceae is warranted. However, the morphological resemblance to *Fagus* is strong. Both *Fagus* and *Nothofagus* have fossil records going back to the Cretaceous<sup>8</sup>. The basic number of  $x = 13$  could have been derived from the  $x = 12$  condition by the same mechanism, involving unequal translocation, as was postulated by Darlington for *Fritillaria*<sup>8</sup>. *Nothofagus* would thus be a specialized offshoot from a *Fagus*-like ancestor arising early in the evolution of the family. Alternatively,  $x = 13$  could represent an intermediate stage in the origin through stepwise reduction (as in *Crepis*) of  $x = 12$  from  $x = 14$  which is the predominant number in the other two families in the order Fagales, Betulaceae and Corylaceae<sup>1</sup>. *Nothofagus* would then be a conservative ancestral form. If the occurrence of *Nothofagus* pollen in the Eocene of the Isle of Wight<sup>10</sup> is confirmed this will become the more probable alternative. But information on the chromosome numbers of the Australian and South American species of *Nothofagus*

and also of the tropical genera in the family, as yet quite unknown cytologically, may help in deciding this question.

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### Ultrastructural Continuity between Active and Repressed Chromatin

DURING metaphase and anaphase of cell mitosis, mammalian chromosomes are highly condensed, individually distinct, and relatively inert in ribonucleic acid (RNA) synthesis<sup>1-5</sup>. By late telophase, the chromosomes become less condensed, appear less distinct from each other, and begin the synthesis of RNA<sup>1-5</sup>. By early interphase, all distinction between individual chromosomes is lost, and the chromosomal substance is visible either as condensed masses (chromocentres, heterochromatin) of repressed chromatin<sup>6,7</sup>, or as extended microfibrils (euchromatin) of chromatin actively synthesizing RNA<sup>6,7</sup>.

The chromosomal karyotype and gene-linkage pattern within a cell normally remain constant from one mitosis to the next<sup>8</sup>, indicating that the structural integrity of each chromosome is maintained throughout interphase<sup>9</sup>. This implies that the repressed and active portions of a chromosome in favourable material might display structural continuity during cell interphase<sup>10</sup>.

In the course of the isolation of repressed and active chromatin from interphase calf thymus lymphocytes<sup>6</sup>, a stage is reached in which the nuclei swell to twice their normal size, offering a favourable material for the examination of the structural relations between the active chromatin microfibrils and the repressed chromatin masses. Such swollen nuclei were prepared and examined by electron microscopy as described previously<sup>6</sup>.

The condensed masses of repressed chromatin tend to be arrayed at the periphery of the swollen nucleus (Fig.



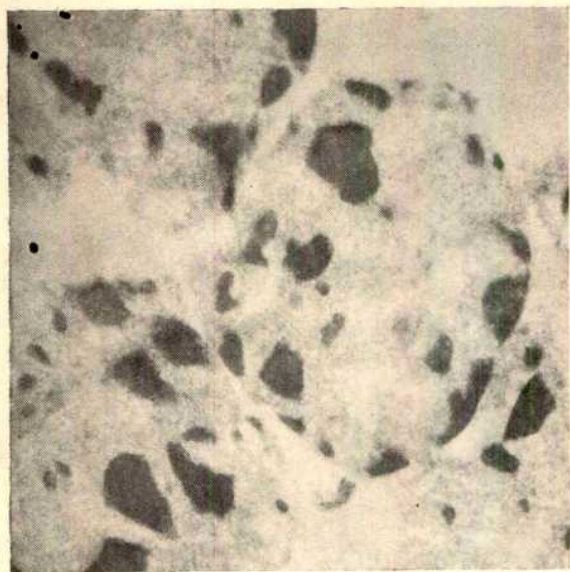


Fig. 1. Swollen nuclei displaying condensed masses of repressed chromatin and extended microfilaments of active chromatin. ( $\times 11,250$ )



Fig. 2. Detail of swollen nucleus displaying the structural continuity of active microfilaments with repressed masses of chromatin. ( $\times 30,000$ )

1), while the extended microfilaments of active chromatin are dispersed between the masses within the interior of the nucleus (Fig. 1). Higher magnifications reveal the active chromatin microfilaments to be of 100 Å diameter (Fig. 2), and these microfilaments can be traced for up to 1.0  $\mu$  of their length (Fig. 2). These extended microfilaments of active chromatin are seen to be structurally continuous with a dense reticulum of fibres within the condensed masses of repressed chromatin (Fig. 2). The zone of transition between the extended microfilaments and the condensed masses is sharp, occurring within less than 100 Å of the length of the microfilaments.

The molecular basis of these structural transitions may lie in the excess of such polyanions as phosphoproteins, RNA, and phospholipids recently found within extended active chromatin as compared to condensed repressed chromatin<sup>11</sup>. These chromatin polyanions can function as de-repressors of RNA synthesis within repressed chromatin by antagonizing the electrostatic interaction between the deoxyribonucleic acid (DNA) and the polycationic repressor histones of repressed chromatin<sup>11</sup>.

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## MATHEMATICS

### Unified Relativity and Quantum Theory

A MATHEMATICAL model has been constructed which reconciles the series of relativity, electro-magnetism and quantum mechanics. It is a deterministic theory, and avoids the rather mystical appeal to fundamental indeterminism which is so prevalent in interpretations of quantum effects.

The 6-vector concomitant of a real basic spinor distribution, which satisfies the fundamental differential equation  $\text{curl } W = 0$ , represents precisely the electromagnetic field of light waves. The purpose behind this investigation was to express the general electromagnetic field as a concomitant of a spinor distribution. The following steps become necessary. First, the spinors are made complex. It is then necessary to interpret the phase as a periodic fifth dimension, as in Kaluza's unified theory of gravitation and electromagnetism, and to assume a topological singularity, which is later identified with spin. The Kaluza theory is then incorporated in the model, the curvature invariants being determined by the concomitants of the spinor.

To extend the differential equation  $\text{curl } W = 0$  to 5 dimensions, the complex nature of the spinors must be reintroduced, since spinors in 5-space of signature 3 must be complex. The differential equation led to wave packets without singularities, which can be interpreted as particles. Dirac's relativistic electron equations follow, the 'negative energy' terms corresponding to waves propagated in the reverse direction round the fifth dimensional cylinder. Stationary orbits and the energy frequency rules for spectra follow.

The final model consists of a 5-dimensional space-time with a distribution of spinors which satisfy the fundamental differential equation. The curvature components of the space-time are determined by the concomitants of the spinors. This model would seem to yield all the principal quantum effects and equations, which fit in with the gravitational and electromagnetic effects via the Kaluza model.

The interaction of two particles corresponding to spinor waves  $W = W_1 + W_2$  is through the effects on the geometry which depend on concomitants of the form:

$$\tilde{W}\psi W = \tilde{W}_1\psi W_1 + \tilde{W}_2\psi W_1 + \tilde{W}_1\psi W_2 + \tilde{W}_2\psi W_2$$

This introduces naturally the interaction terms of the form  $W_1\psi W_2$  so frequently used in quantum mechanics.

A paper on space-time distributions of spinors has been submitted to the Royal Society for publication.

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## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, March 29

INSTITUTION OF ELECTRICAL ENGINEERS, SCIENCE AND GENERAL DIVISION (at Savoy Place, London, W.C.2), at 10 a.m., 2.30 p.m. and 5.30 p.m.—Colloquium on "Earth Loop Impedance Testing".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. Knight, Mr. R. E. Davies and Dr. R. G. Manton: "Vertical Radiation Patterns of h.f. Curtain Arrays on Plateau Sites".

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Eccleston Hotel, London, S.W.1), at 7.30 p.m.—Mr. J. W. Davidson: "Plastics in Building".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. Michael P. Ward: "In Unknown Bhutan".

## Tuesday, March 30

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Mr. E. H. Vick, Mr. J. G. Paterson, Mr. L. B. Escrib, Mr. V. F. Cornish and Mr. W. C. Andrews: "The Southern Outfall Works of the London County Council".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. J. Harrison: "Characteristics of Man". (Afternoon lecture for Sixth Form Boys and Girls in Schools from London and the Home Counties. To be repeated on March 31, April 6 and 7.)

## Tuesday, March 30—Wednesday, March 31

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (joint meeting with the Colloid and Surface Chemistry Group (at the School of Pharmacy, Brunswick Square, London, W.C.1)—Symposium on "Formulation of Pesticides".

## Wednesday, March 31

SYSTEMATICS ASSOCIATION (in the Lecture Hall of the British Museum (Natural History), London, S.W.7), 10.15 a.m.—Demonstration Meeting on "Methods in Taxonomy".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINES GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Methods of Rating Diesel Engines in Mass Production".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—Discussion Meeting on "The Difficulties and Advantages of Super-sonic Civil Transport" opened by Dr. A. E. Russell and Mr. B. S. Shenstone.

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Willis Jackson, F.R.S.: "New Materials in Engineering" (Trueman Wood Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (Annual Joint Meeting with the Graduate and Students Sections of the Institutions of Civil and Mechanical Engineers, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. B. Z. De Ferranti: "Computers".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Meeting on "Food Analysis".

## Thursday, April 1

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. R. R. Race, F.R.S.: "Contributions of Blood Groups to Human Genetics". (Review Lecture.)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. P. Miller: "Ranger 8 Spacecraft, with special reference to Moon Shot Camera Systems".

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Mr. A. K. Alcock: "Trends in Hermetic Unit Design and Applications".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Economics of Automatic Control in Shipping".

## Thursday, April 1—Friday, April 2

BIOCHEMICAL SOCIETY (at University College, Gower Street, London, W.C.1)—Annual General Meeting. Thursday, April 1—Symposium on "The Biochemistry of Insects", followed by Prof. A. Szent-Györgyi: "Growth and Cellular Organization" (Fifth Hopkins Memorial Lecture). Friday, April 2—Scientific Papers followed by the Annual General Business Meeting.

CLAY MINERALS GROUP OF THE MINERALOGICAL SOCIETY (in the Geology Department, The University, Liverpool)—Spring Meeting: "Clay Minerals in Chemical Industry".

## Friday, April 2

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 10.30 a.m. and 2.30 p.m.—Mr. H. Mundy and Mr. P. Spear: "Motor Racing—a Challenge to the Engineer" (Leonardo da Vinci Lecture).

BRITISH MYCOLOGICAL SOCIETY, PLANT PATHOLOGY COMMITTEE (in the Large Physics Lecture Theatre, Imperial College, Prince Consort Road, London, S.W.7), at 10.45 a.m.—Symposium on "Plant Disease Control by Antibiotics, Cultural, and Other Methods".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. C. W. Earp: "Features of a New Doppler VOR Beacon".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Testing of Power Semiconductor Devices and Standardization", opened by Mr. W. D. Sinclair.

## Saturday, April 3

BRITISH MYCOLOGICAL SOCIETY (at Imperial College, London, S.W.7), from 11 a.m. to 5 p.m.—Conversazione.

## Monday, April 5

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the I.E.R.E. Computer Group, at Savoy Place, London, W.C.2), at 2.30 p.m.—Colloquium on "The Design of Real Computer Systems".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. Robert T. Clough: "The Old Lead Smelting Mills of the Yorkshire Dales".

## Monday, April 5—Wednesday, April 7

BRITISH NATIONAL SECTION OF THE INTERNATIONAL ASSOCIATION FOR EARTHQUAKE ENGINEERING (at Imperial College of Science and Technology, London, S.W.7)—Symposium on "Vibration in Civil Engineering".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (either a theoretical reactor physicist with experience in modern computational techniques or a candidate well qualified in the fields of fluid dynamics and heat transfer) IN THE DEPARTMENT OF NUCLEAR ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (April 1).

LECTURER/ASSISTANT LECTURER (preferably with experience in magnetic resonance or gas discharges or mechanical properties of materials or astronomy) IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Keele, Staffordshire (April 2).

ASSISTANT LECTURER or UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF ZOOLOGY—The Secretary, University of Edinburgh, The Old College, South Bridge, Edinburgh, 8 (April 3).

LECTURER (science graduate) IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/10/N (April 3).

LECTURER (with a good honours degree or equivalent and appropriate experience in teaching, research or industry) IN ZOOLOGY IN THE DEPARTMENT OF BIOLOGY—The Academic Registrar, Brunel College, Woodlands Avenue, London, W.3 (April 3).

HEAD (with high academic qualifications and research or industrial experience in biology or related fields of study, and preferably some administrative experience) OF THE DEPARTMENT OF BIOLOGICAL SCIENCES—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth (April 5).

LECTURER IN PURE MATHEMATICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (April 5).

PRINCIPAL LECTURER (with high academic qualifications and preferably some industrial or research experience) IN MATHEMATICS—The Principal, Lanchester College of Technology, Coventry (April 5).

RESEARCH STUDENTS (2) IN THE DEPARTMENT OF ZOOLOGY, to pursue research on various aspects of nutrition in the Platyhelminthes, Rhynchocoela and Nematoda, as part of a research project financed by the United States Public Health Service—Dr. J. B. Jennings, Department of Zoology, The University, Leeds, 2 (April 5).

RESEARCH ASSISTANT IN THE SUB-DEPARTMENT OF COMPUTATION, DEPARTMENT OF MATHEMATICS—The Registrar, The University, Hull (April 9).

EXPERIMENTAL OFFICER (with a university degree or its equivalent) IN THE DEPARTMENT OF ZOOLOGY for technical duties in connexion with research in the field of comparative endocrinology—The Registrar, The University, Sheffield (April 10).

RESEARCH ASSISTANT (graduate with research experience) IN THE DEPARTMENT OF PHYSICS to work on the application of electron microscopy to the study of ferromagnetic domains—The Registrar, The University, Sheffield (April 10).

SENIOR LECTURER and a LECTURER (graduate of medicine or science) IN THE DEPARTMENT OF PHYSIOLOGY—The Assistant Registrar, The Medical School (University of Birmingham), Birmingham, 15 (April 10).

LECTURER IN PHYSICS—The Secretary, The Queen's University, Belfast, Northern Ireland (April 11).

ASSISTANT LECTURER (with a special interest in social and economic geography) IN GEOGRAPHY—The Registrar, The University, Sheffield (April 12).

LECTURER IN PHYSICAL CHEMISTRY—The Very Reverend the Dean, Christ Church, Oxford (April 12).

READER IN HISTOLOGY at Guy's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 12).

ASSISTANT (with an honours degree in an appropriate science or a post-graduate qualification in agricultural engineering or farm mechanization) IN RESEARCH IN AGRICULTURAL ENGINEERING—The Secretary, School of Agriculture, University of Cambridge, Cambridge (April 14).

CHAIR OF COMPUTER SCIENCE—The Secretary, The Queen's University, Belfast, Northern Ireland (April 14).

LECTURER/ASSISTANT LECTURER (with at least a good honours degree with suitable teaching and research experience, and special qualifications in one or more of the following fields: analytical chemistry, chemical technology, inorganic chemistry or physical chemistry) IN THE DEPARTMENT OF CHEMISTRY, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, April 15).

READER IN THEORETICAL PHYSICS, and a LECTURER IN THEORETICAL PHYSICS (preferably interested in physics of the solid state)—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 15).

CHAIR OF INORGANIC CHEMISTRY—The Secretary, The University, Aberdeen (April 17).

CHAIR OF PHYSICAL GEOGRAPHY—The Secretary, The University, Aberdeen (April 17).

SENIOR LECTURER, a LECTURER and an ASSISTANT LECTURER IN THE DEPARTMENT OF PSYCHOLOGY—The Secretary, The University, Aberdeen (April 17).

LECTURER or ASSISTANT LECTURER (with an interest and preferably research experience in plant virology) IN THE DEPARTMENT OF AGRICULTURAL BOTANY—The Registrar, University College of Wales, Aberystwyth (April 21).

SENIOR LECTURER and a LECTURER (preferably with experience in the fields of entomology and fresh-water biology) IN THE DEPARTMENT OF ZOOLOGY, University of Ghana—The Assistant Registrar, Higher Education

Section, Ghana High Commission, 15 Gordon Square, London, W.C.1; or The Registrar, University of Ghana, P.O. Box No. 25, Legon, Accra, Ghana (Ghana, April 22).

LECTURER or ASSISTANT LECTURER (preferably with an interest in the teaching of Greek philosophy) in PHILOSOPHY—The Secretary, The Queen's University, Belfast, Northern Ireland (April 25).

ASSISTANTS IN CHEMISTRY—The Secretary of the University Court, The University, Glasgow (April 30).

CHAIR OF ZOOLOGY—The Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, 2 (April 30).

LECTURER (with a higher degree in botany, some experience in teaching and research, and preferably an interest in ecology, anatomy or systematics) in BOTANY at the University College of Townsville (University of Queensland), Australia—Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, April 30).

LECTURER (with a special interest and research experience in electrochemistry) in PHYSICAL CHEMISTRY—The Secretary of the University Court, The University, Glasgow (April 30).

LECTURER (with either a medical degree or a degree in pharmacology) in PHARMACOLOGY at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (April 30).

SOIL SURVEYORS (with a good honours degree) FOR THE SOIL SURVEY OF ENGLAND AND WALES—The Secretary, Rothamsted Experimental Station, Harpenden, Hertfordshire (April 30).

TUTORIAL FELLOW (preferably under 40 years of age) in ENGINEERING SCIENCE at University College, Oxford, which will be held in conjunction with a post in the Department of Engineering Science—The Senior Tutor, University College, Oxford (April 30).

UNIVERSITY DEMONSTRATOR IN ORGANIC CHEMISTRY—Dr. P. Maitland, University Chemical Laboratory, Lensfield Road, Cambridge (April 30).

LECTURER (preferably with research interests in the field of invertebrate physiology, particularly insect physiology) in ZOOLOGY at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 3).

SENIOR LECTURER/LECTURER IN MATHEMATICAL STATISTICS IN THE FACULTY OF ECONOMICS, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 3).

SENIOR LECTURER or LECTURER IN PHYSICAL or THEORETICAL CHEMISTRY at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 3).

SENIOR LECTURERS or LECTURERS (2) IN PURE MATHEMATICS, at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 3).

CHAIR IN NUCLEAR PHYSICS at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, May 7).

LECTURER (Grade II) in ENTOMOLOGY WITHIN THE DEPARTMENT OF ZOOLOGY—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (May 31).

ASSISTANT or ASSOCIATE PROFESSOR (with a Ph.D. in soil science from a recognized university, and preferably a minor in physical chemistry) of SOIL SCIENCE—D. A. Rennie, Head, Department of Soil Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

HORTICULTURIST (national of the United Kingdom or the Republic of Ireland, with a degree in horticulture, agriculture or natural science, plus experience in tropical countries with fruit crops) in Bolivia, to initiate experiments with tropical fruit crops, sub-tropical crops and temperate crops under sub-tropical conditions, and to organize extension work and train local staff—Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/22/04.

HOSPITAL PHYSICIST (with a science degree in physics or equivalent qualification) in the RADIOTHERAPY CENTRE, Canterbury, for work on medical treatment with radio-isotopes, cobalt beam and other forms of ionizing radiation—The Hospital Secretary, Kent and Canterbury Hospital, Canterbury, Kent.

MASTER (graduate) to teach physics to advanced level—The Headmaster, Allyn's School, London, S.E.22.

MANAGER (with a first degree, or its equivalent, in mathematics and considerable experience in the operation of computers in, and for, industry) of THE COMPUTER CENTRE, to take charge of the operation of the existing IBM 1620 computer, and of additional computers that may be installed in the Centre—The Registrar, Loughborough College of Technology, Loughborough, Leicestershire.

MICRO-ANALYST in the CHEMISTRY DEPARTMENT to conduct routine analysis of organic compounds—The Secretary, Department of Chemistry, University of British Columbia, Vancouver, 8, Canada.

PRINCIPAL LECTURER IN MATHEMATICS to teach one branch of mathematics up to the standard of the B.Sc. (Special Mathematics) degree—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

PRINCIPAL/SENIOR RESEARCH OFFICERS (nationals of the United Kingdom or the Republic of Ireland, with a good honours degree in botany or chemistry with appropriate postgraduate training and experience) in Uganda, to be responsible for detailed supervision of research work which may involve taking full charge of a main research station—The Appointments Officer, Room 301, Ministry of Overseas Development, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 213/183/05.

RESEARCH FELLOWS in the CHEMISTRY DEPARTMENT, for work under Prof. R. M. Barrer, on (1) non-stoichiometric inclusion and clathration complexes, and (2) high pressure adsorption of gases by porous crystals and molecular sieves—Prof. R. M. Barrer, F.R.S., Chemistry Department, Imperial College, London, S.W.7.

Bonds, Part 2 (Covalent Bond). 29 frames+booklet. 5: Chemical Bond Part 3 (Conclusion). 21 frames+booklet. (Surbiton: Marian Bay, Villiers Avenue, 1964.)

British Museum (Natural History). The John Murray Expedition, 1933-4 Scientific Reports, Vol. 10. No. 8: Crustacea: Chirostyliidae (Galatheidæ) By Dr. Nasima M. Tirmizi. Pp. 385-415. (London: British Museum (Natural History), 1964.) 15s.

The University of Newcastle upon Tyne. Report of the Dove Marine Laboratory, Third Series, No. 14: The Marine Fauna of the Culterco District, 2. Pp. 32. (Cullercoats, North Shields: University of Newcastle upon Tyne, Dove Marine Laboratory, 1964.) 7s. 6d.

London and Home Counties Regional Advisory Council for Technological Education. Bulletin of Special Courses in Higher Technology, Management Studies and Commerce, 1964-65. Part 2: Spring and Summer Term Pp. 105. (London: London and Home Counties Regional Advisory Council for Technological Education, 1964.) 5s.

The Night Sky 1965: Stars Month by Month from *The Times*. Pp. 28 (London: The Times Publishing Co., Ltd., 1964.) 4s. net.

Department of Scientific and Industrial Research: Torrey Research Station. The National Collection of Industrial Bacteria—Catalogue of Strains. Second edition. Pp. iv+130. (London and Edinburgh: H.M. Stationery Office, 1964.) 9s.

Department of Scientific and Industrial Research and Fire Offices' Committee Joint Fire Research Organization. Fire Research Technical Paper No. 11: Fire Hazards of Caravans. By Jennifer E. Gaunt. Pp. vii+20. (London: H.M. Stationery Office, 1964.) 2s. 3d. net.

Review of Coal Tar Technology, Vol. 16, Part 1 (January-June, 1964) Pp. 1-84. (Gomersal, Leeds: The Coal Tar Research Association, 1964.) 30s. per volume.

Planning, Vol. 30, No. 484 (December, 1964): Steel Pricing Policies: A Comparative Study of the ECSC and the British and American Systems Pp. 317-376. (London: Political and Economic Planning, 1964.) 10s.

The Medical Research Council of Ireland. Annual Report for the year ended December 31, 1963. Pp. v+80. (Dublin: Medical Research Council of Ireland, 1964.) 5s.

Privy Council, Medical Research Council Memorandum No. 42: The Industrial Rehabilitation of Long-Stay Schizophrenic Patients—a Study of 45 Patients at an Industrial Rehabilitation Unit. By J. K. Wing, D. H. Bennett and John Denham. Pp. vi+42. (London: H.M. Stationery Office, 1964.) 4s. 6d. net.

Tweed River Purification Board. Annual Report for the year ended 15th May, 1964. Pp. 47. (Newton St. Boswells: Tweed River Purification Board, 1964.)

Ministry of Aviation. Programming in ALGOL. Booklet prepared by the Superintendent and Staff of the Mathematics Laboratory, Royal Radar Establishment. Pp. 31. (London: H.M. Stationery Office, 1964.) 4s. 6d. net.

#### Other Countries

Queen Victoria Museum and Art Gallery, Launceston. Annual Report 1961-1962. Pp. 8. Annual Report 1962-1963. Pp. 12. Records of the Queen Victoria Museum, New Series. No. 16: Notes on the Petrology and Structure of the Precambrian Metamorphic Rocks of the Upper Mersey-Forth Area. By Alan Spry. Pp. 11. No. 17: The Tick Fauna of Tasmania. By F. H. S. Roberts. Pp. 8. (Launceston: Queen Victoria Museum and Art Gallery, 1963 and 1964.)

Australia: Commonwealth Scientific and Industrial Research Organization Land Research Series, No. 11: General Report on Lands of the Leichhardt-Gilbert Area, Queensland, 1953-54. By R. A. Perry, J. R. Twidale, C. E. Pritchard, R. O. Slatyer, M. Lazarides and F. H. Collins. Pp. 224+12 plates+map. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1964.)

Anuario del Observatorio Astronómico de Madrid para 1965. Pp. 407. (Madrid: Observatorio Astronómico, 1964.)

Australia: Commonwealth Scientific and Industrial Research Organization. CSIRO Water Research Bibliography, 1923-1963. Compiled by Lois Davey. Pp. ii+98. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1964.)

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 104: Méthodologie et Interprétation des Essais sur l'Alimentation Minérale des Plantes Annuelles. Par E. Van Hoek, C. Myttenaere et Dr. A. Riepoet. Pp. 134. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1964.) 170 francs.

New Zealand. Report of the Board of Trustees of the National Art Gallery and Dominion Museum for the year ended 31 March 1964. Pp. 27. (H. 21.) (Wellington: Government Printer, 1964.) 1s. 6d.

Politechnika Lodzka. Skład Osobowy i Program Wykładów na Rok Akademicki 1964/65. Pp. 335. (Lodz: Nakładem Politechniki Lodzkiej, 1964.)

Indian Forest Records (New Series)—Entomology. Vol. 10, No. 10: Studies on the Morphology and Taxonomy of Indian Bostrychidae. V-A Revision of the Indian Species of *Heterobostrychus* Lesne (Coleoptera: Bostrychidae). By Kuldeep Rai and P. N. Chatterjee. Pp. 205-218 (4 plates). Rs. 1.00; 2s. 4d. Vol. 10, No. 11: Revision of the Termite Genus *Microtermes* Wasmann (Isoptera: Termitidae: Macrotermittinae) from the Indian Region. By P. N. Chatterjee and M. L. Thakur. Pp. 219-260. Rs. 2.85; 6s. 8d. (Delhi: Manager of Publications, 1964.)

Annals of the New York Academy of Sciences. Vol. 105, Article 16: Life-Forms in Meteorites and the Problem of Terrestrial Contamination—a Study of Methodology. By Paul Tasch. Pp. 927-950. (New York: New York Academy of Sciences, 1964.) 2 dollars.

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